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ERRATA

Page 23, line 4, delete "(1940)"

Page 28, line 9, delete "(1940)"

Page 133, Table IV, last column, "70" should read "90"

Page 340, line 31, "2,4,6-Tribromochlorophenoxyacetic" should read
"2,4,6-Tribromophenoxyacetic"

ALSO NOTE ERRATA TO VOLUME II AS FOLLOWS

Page 255, Table II, Cage No. 4, Column 8, "*" should read "***"

Page 498, right column, line 30 should read "Gentiobioside, Heptaacetyl
and heptapro-"

ADDENDUM TO VOLUME II

Page 465, line 15, add "1" to "Aerosol OT" with footnote "Aerosol OT is
identical with Vatsol OT, private communication from American
Cyanamid Company, 30 Rockefeller Plaza, New York, N. Y."

FORMATIVE EFFECTS INDUCED WITH β -NAPHTHOXYACETIC ACID¹

P. W. ZIMMERMAN AND A. E. HITCHCOCK

Mention has frequently been made of the varied responses induced by many growth substances though all have in common the capacity to initiate root primordia. The naphthalene group includes a number of the most active substances. Two of these are α -naphthaleneacetic acid and β -naphthoxyacetic acid and their derivatives. The former was first described as a physiologically active substance in 1935 (10). β -Naphthoxyacetic acid was first mentioned as an active substance by Irvine (7) in 1938. In 1939 it was again mentioned in two papers by the present authors (8, 9) and in two by Bausor (1, 2). Bausor, Reinhart, and Tice (3) reported in 1940 on histological peculiarities induced by this chemical.

The purpose of the present paper is to report some interesting modifications of plant organs induced by β -naphthoxyacetic acid and its derivatives applied as a spray, as a vapor, or added to the soil.

MATERIALS AND METHODS

β -Naphthoxyacetic acid (NOA) was synthesized in our laboratory. Directions for preparing it are as follows: Dissolve 48 grams of β -naphthol and 25 grams of potassium hydroxide in 450 cc. of water and mix with 30 grams of chloroacetic acid and 25 grams of potassium hydroxide dissolved in 250 cc. of water. Pour the mixture into a flask and reflux for one to two hours on steam bath. Upon cooling, potassium naphthoxyacetate precipitates. The salt is only slightly soluble in alcohol or water. Filter the mixture and wash with water or alcohol. After the salt has been dried, add 5 grams to 100 cc. of 50 per cent acid (5 per cent sulphuric or hydrochloric) alcohol. The precipitate will dissolve. Next add 250 cc. of water, and β -naphthoxyacetic acid precipitates out. Collect on a Büchner filter. The acid can be improved and purified by redissolving in alcohol and again precipitating with distilled water. This process can be repeated until the acid has a melting point of 154° to 156° C.

α -Naphthoxyacetic acid is synthesized by using α -naphthol instead of the beta form. It was not used in the present experiments because it is only slightly active.

A convenient stock solution of 10 mg./cc. in 95 per cent alcohol served for making dilutions. The concentrations used ranged from 100 mg./l. to 1000 mg./l. The substance was applied as a spray with an atomizer.

¹ This article was preprinted March 18, 1941.

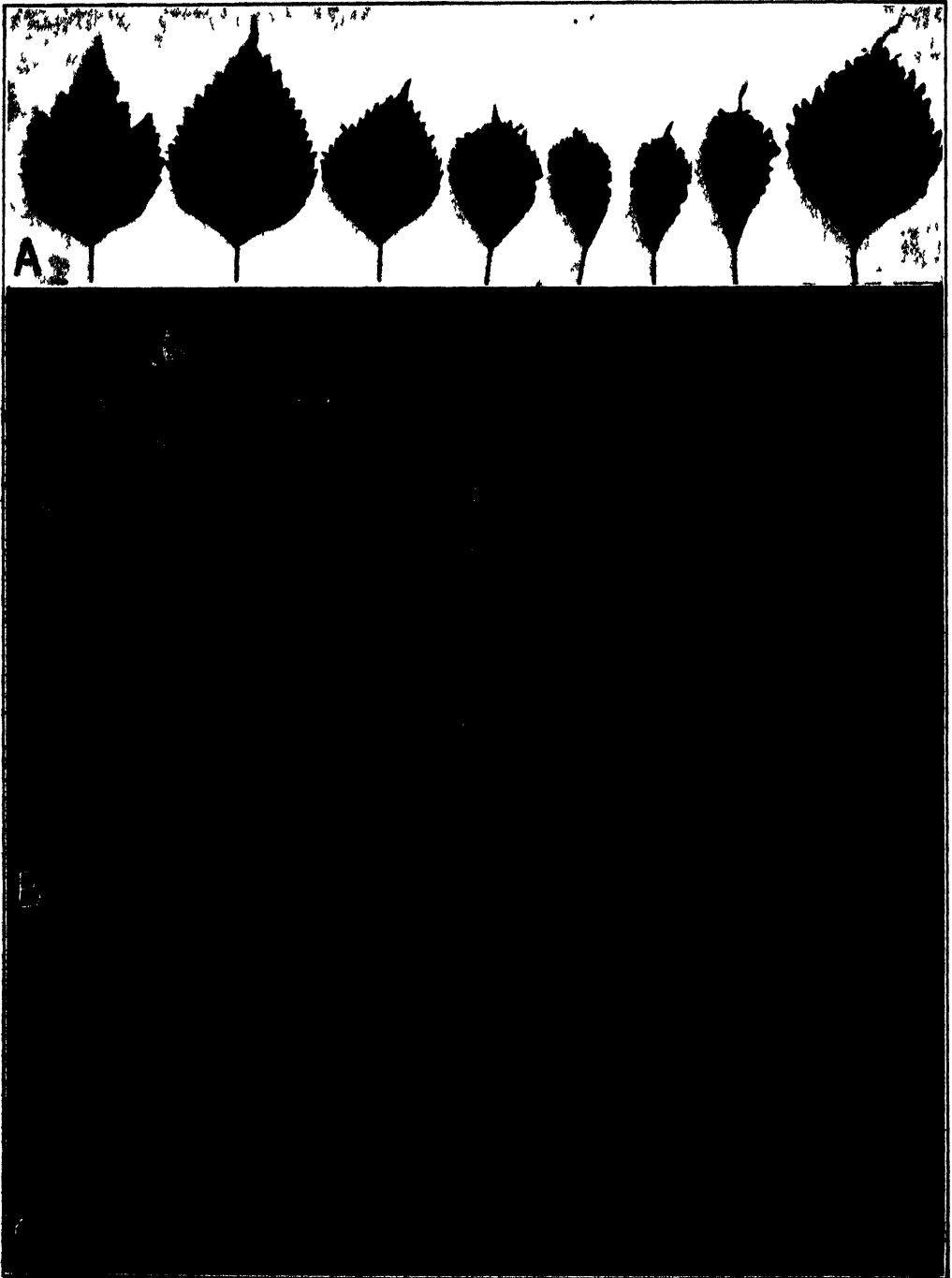


FIGURE 1. Modifications of leaves induced by naphthoxyacetic acid sprayed on the tip of the stem. A. Hibiscus leaves which grew after tip had been sprayed with 300 mg./l. Normal leaf on left was near the tip when experiment started. Note clearing of veins and difference in size and pattern of the leaves. B. Tomato leaves from one plant sprayed twice with 300 mg./l. Normal leaf on upper left. C. Tomato leaves from one plant in series from normal (left) toward tip, 31 days after tip was sprayed with 250 mg./l. Note slight modifications and recovery.

Spreaders such as casein, Aerosol, or Penetrol and lanolin emulsions were sometimes used but these were not essential. The higher concentrations were used with alcoholic solutions or emulsions.

When used as a vapor the crystals were heated under a bell jar with the plants as described in earlier publications (8, 9). The esters are more volatile than the acid or the acetamide and can be applied without heat. Soil treatments usually consisted of 50 cc. of the solution (concentration 100 to 1000 mg./l.) applied to the soil in a four-inch pot. Only one treatment was necessary for the most sensitive species.

The plants responding most readily were tomato (*Lycopersicon esculentum* Mill.), hibiscus (*Hibiscus rosa-sinensis* L.), Turkish tobacco (*Nicotiana tabacum* L.), sensitive plant (*Mimosa pudica* L.), fuchsia (*Fuchsia hybrida* Voss.), Jerusalem artichoke (*Helianthus tuberosus* L.), marigold (*Tagetes erecta* L.), and yellow Paris daisy (*Chrysanthemum frutescens* L.).

EXPERIMENTAL RESULTS

Formative effects on leaves. β -Naphthoxyacetic acid has in common with many other growth substances the capacity to induce epinasty of leaves, curvature of stems, swelling and proliferation of tissue, inhibition of buds, retardation of roots, etc. It differs from other hormone-like compounds in its effects on leaves and flowers.

Leaves of hibiscus which grew after the tip of the plant was sprayed with 100 mg./l. to 1000 mg./l. of naphthoxyacetic acid in water were modified in form and venation. Several modifications produced by one plant are illustrated in Figure 1 A. The plant had been sprayed at the growing point three different times at seven-day intervals with 300 mg./l. of naphthoxyacetic acid in water. The leaves were modified in size, shape, veins, and serrate projections at the edge and tip. The picture shows the progressive stages to the most pronounced modification and finally signs of recovery.

The peculiar clearing of the veins is notable. When viewed with transmitted light the modified leaves appeared to have nearly transparent veins, a characteristic which is often associated with virus diseases (4). The leaf on the right in Figure 1 A shows transparent veins near the midrib though the outer part is nearly normal. The effect of the chemical was nearly lost when the younger (outer) part of the leaf developed. The next leaf to grow was apparently normal. The shape of old leaves was not affected by the substance. To bring about modification either new leaves or parts of leaves had to grow under the influence of the chemical.

Tomato leaves were modified in size, shape, type, number of leaflets, pattern, and venation where the growing point was sprayed with solutions or emulsions containing any concentration of naphthoxyacetic acid from 100 mg./l. to 1000 mg./l. To bring about pronounced modifications with low concentrations it was necessary to repeat the application two or more

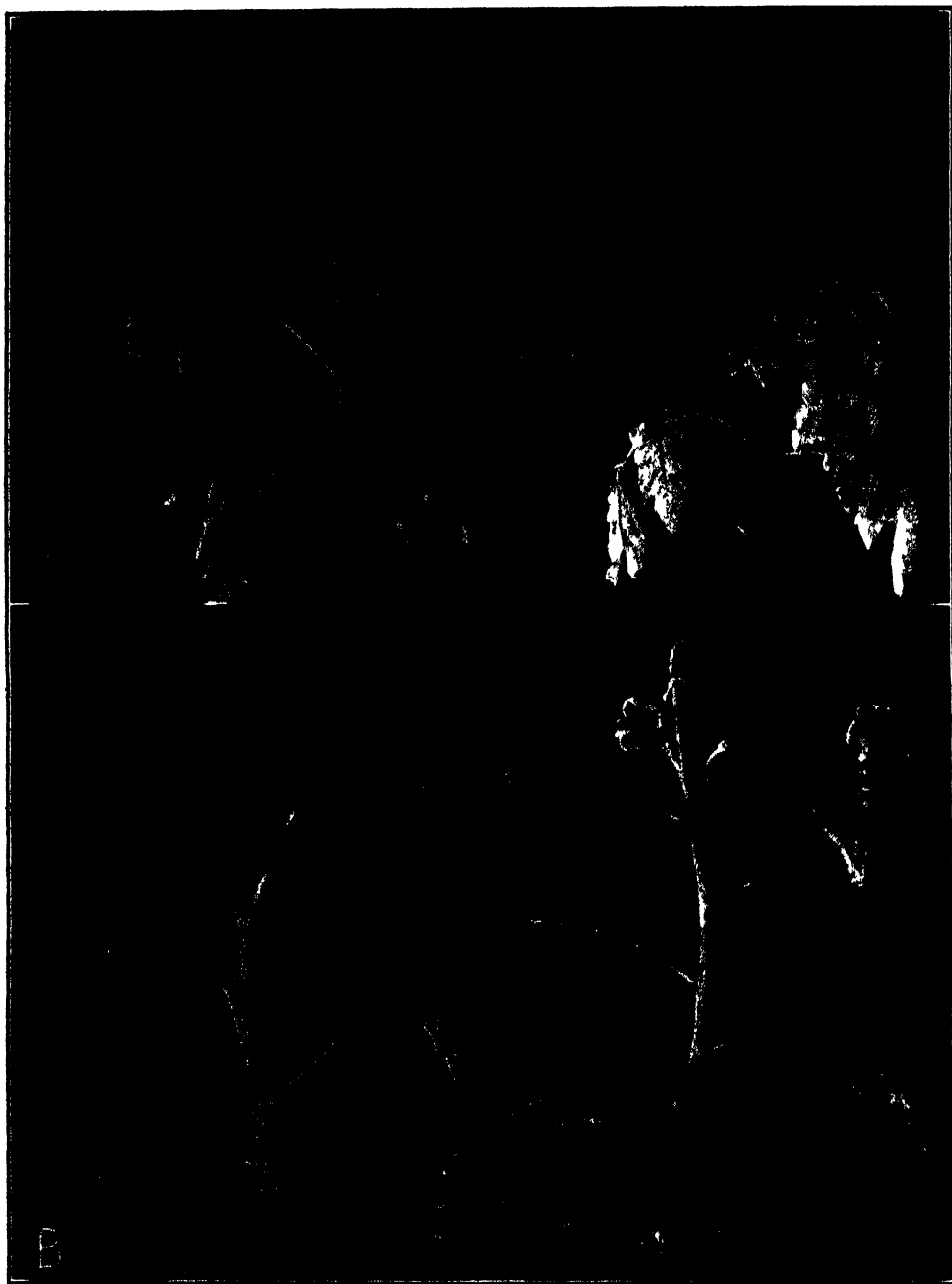


FIGURE 2. Tomato plants treated with naphthoxyacetic acid. A. Left, control. Right, plant exposed to vapors under bell jar for 24 hours. New leaves which grew after treatment showed modification of pattern, shape, size of leaflets, and clearing of veins. B. Left, control. Right, plant treated on soil with 50 cc. of solution containing 300 mg./l. The insert is an enlargement of the tip. Note how leaflets grew together, causing the midrib to push through the opening: The curvature of the older part of the stem occurred within a few hours after treatment. One leaf is only partly modified because it was partially formed when experiment started.

times. Figure 1 B illustrates the several peculiar types of leaves taken from one plant 30 days after the plant had been sprayed with 300 mg./l. of the substance. The leaf on the left of the upper row was near the growing tip when the plant was treated. It grew to maturity with no noticeable modification in shape. All other leaves produced thereafter were greatly modified in size and shape. The veins showed clearing which, together with the modified forms, resembled leaves of plants affected with tobacco mosaic virus or mite infestation. Some types of virus diseases cause clearing of the veins and frenching or fern-like tomato leaves. Some of the treated plants produced several fern-like leaves in succession and then reverted to normal after the influence of the chemical disappeared. In contrast to frenching, some leaves showed failure of leaflets to separate, occasionally forming a nearly simple leaf instead of compound. Frequently the leaflets appeared on one side of the midrib but not on the other. Curling and twisting of growing leaves was striking. One such leaf is shown in Figure 1 B. The light color of the tip is due to twisting so that the under side shows on top. Many other modifications not easily described were evident. In general the response of the plants to naphthoxyacetic acid and its derivatives is one of the best examples of chemical influence of a formative nature.

Recovery toward normal usually started within a month after treatment, though there was much variation especially with the higher dosages. When the growing tip of plants treated with high dosages was removed to force axillary buds, the new shoots produced modified leaves. Nearly normal leaves grew on axillary shoots of plants treated with 100 mg./l.

Soil treatments involving 50 cc. of solution (250 mg./l.) brought out many of the modifications described for sprays. The highest concentrations caused even more modification and more lasting effects than sprays. The leaves that grew after treatment were badly distorted. The tips of leaflets often grew together, forcing the midrib to curl and grow through the opening (Fig. 2 B). The treated plants produced new leaves showing a series of modifications up to maximum distortion and then reverting toward the normal as the chemical influence subsided.

Flowers on plants which were modified by soil treatment or repeated applications of emulsions developed long, tubular calyxes with divisions near the tip. The buds resembled those of the genus *Geranium*, known as cranesbill.

Tomato plants were placed under bell jars and exposed to vapors of naphthoxyacetic acid, the acetamide, and esters for a period of 24 hours. The first effects were epinasty of all leaves and then inhibition of growth without killing the growing points. Within a few days, however, growth was resumed and all new leaves produced were considerably modified, as shown in Figure 2 A. The modifying influence persisted for a considerable

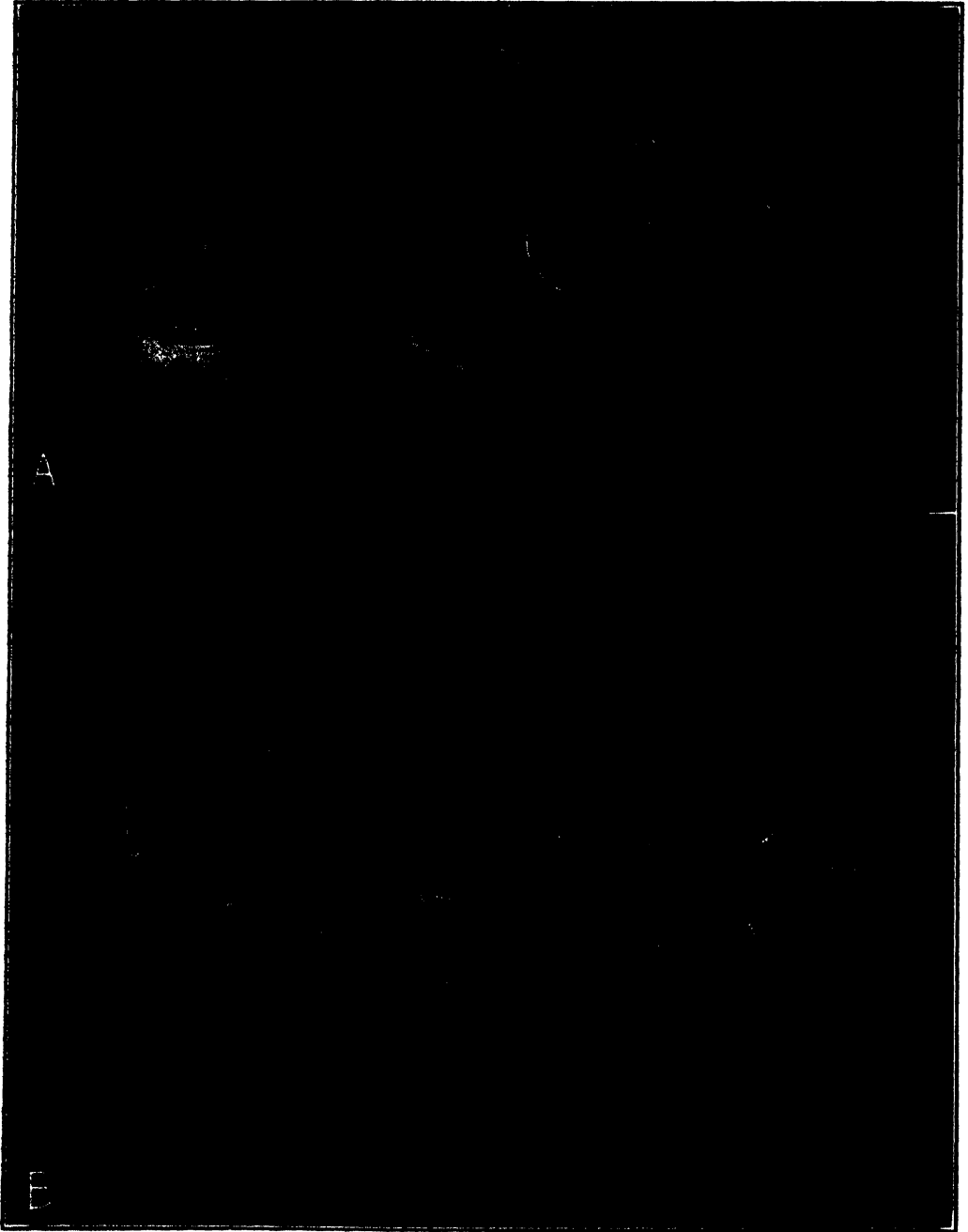


FIGURE 3. Modifications induced with naphthoxyacetic acid. A. *Mimosa* plants showing one control on the left and two plants sprayed at growing point with 300 mg./l. The insert is an enlargement of a modified leaf to show the regular projections on leaflets. The middle plant shows two fasciated leaves, the leaflets failing to separate from each other. The plant on the right has a badly fasciated leaf that resembles a bloom. B. Paris daisy showing control on left, and on right modification of leaves induced after being sprayed at the growing tip with an emulsion containing 300 mg./l. of naphthoxyacetic acid. Photograph taken 18 days after treatment.

period of time but eventually the plants recovered and again produced normal leaves. The effects in general were not as lasting as when the substance was applied to the soil. Also, the buds and flowers produced on the plants treated with the vapors were approximately normal in contrast to long, slender buds on plants modified through soil treatments.

Tobacco plants were sprayed at the growing point with emulsions and water solutions of naphthoxyacetic acid. The leaves developing thereafter curled downward and were modified in several ways. They were curled and narrow and the veins showed clearing as if the plants had been attacked by mites. The plants treated with 300 mg./l. of naphthoxyacetic acid grew more rapidly in height and flowered considerably ahead of controls. The leaves became nearly normal as the effect of the chemical disappeared.

Marigold plants treated with 300 and 500 mg./l. produced skeleton-like leaves, the midribs becoming prominent with practically no blades. The stems of treated plants became swollen and developed adventitious roots.

The Paris daisy proved to be a very sensitive species. When treated at the tip with concentrations of 100 to 300 mg./l. of naphthoxyacetic acid, the young leaves curled downward within two hours and continued to show pronounced epinasty for several days. New leaves which grew after the chemical was applied were modified in size, shape, width of segments, and size of the lamina, as shown in Figure 3 B. Clearing of veins was also evident as described for hibiscus. The influence of the chemical was lost within 30 to 40 days and new leaves which grew thereafter appeared normal.

Rapidly growing artichoke plants were sprayed at the tips with 100 to 500 mg./l. of naphthoxyacetic acid solution. Within two hours the young leaves showed epinasty and curling. Within 48 hours the stem tips became swollen and soon thereafter produced adventitious roots. Stem elongation was inhibited for several days, varying with the concentration of the chemical. Many modifications appeared as the axillary buds grew or the terminal bud resumed growth. Fasciation of leaves, lobing of leaves, clearing of veins, growth of the midribs with small blades or lamina on one side only, and opposite leaves grown together to make a tube around the terminal bud, were some of the most evident results.

Mimosa plants were sprayed at the growing tip with 100, 300, and 500 mg./l. of naphthoxyacetic acid. The new leaves produced were modified in various ways. The partially formed leaves at the time the plant was treated developed with narrow leaflets resembling fern leaves and having projections near the base of the leaflet. The regularity of these projections indicated some morphological peculiarity which was not evident on the normal leaf, but was greatly pronounced from the influence of the chemical. Many leaves which were formed after the treatment showed considerable

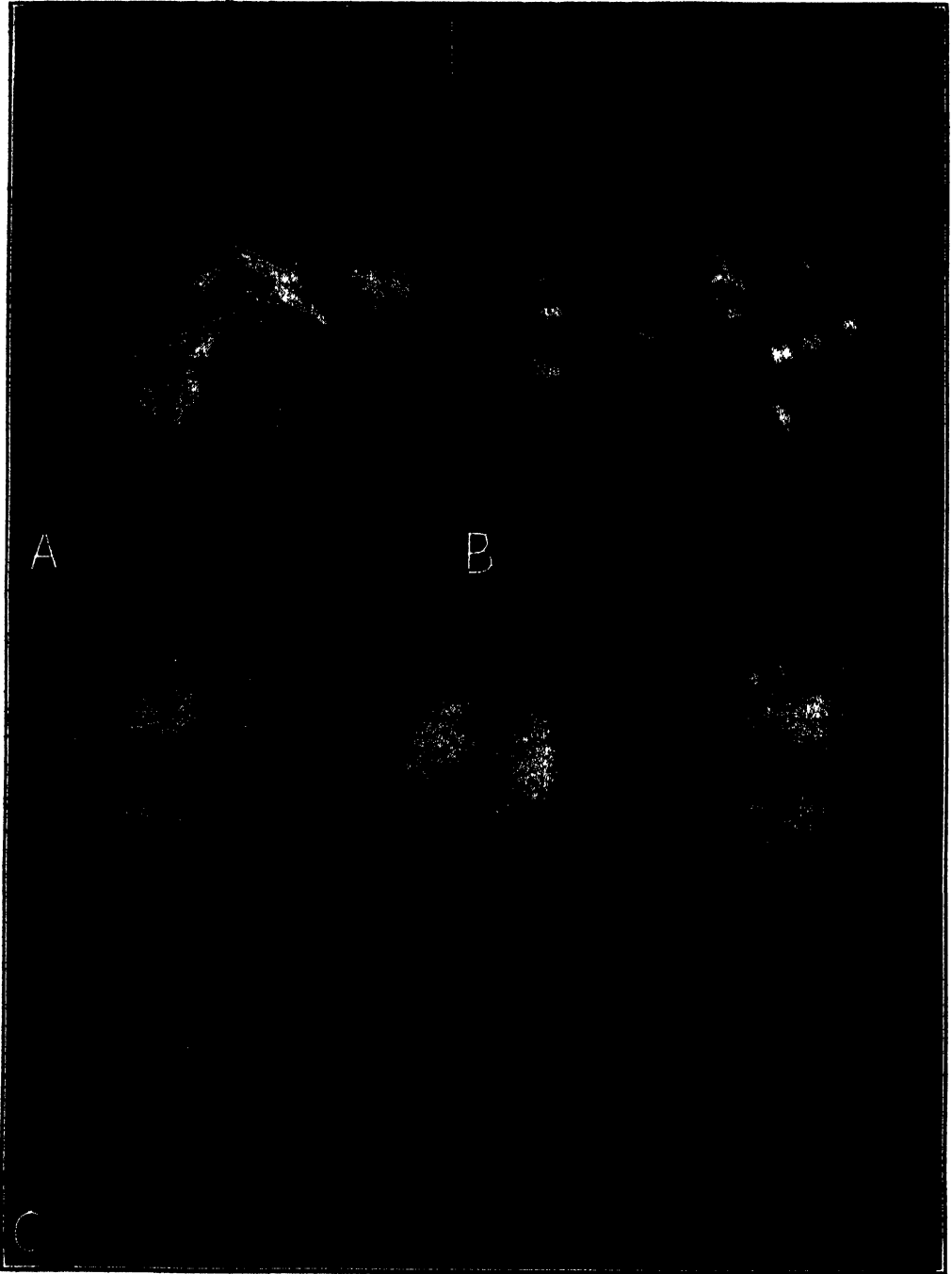


FIGURE 4. The modification of tomato fruit and flowers sprayed with 300 mg./l. of naphthoxyacetic acid. A. Early parthenocarpic development and persistence of perianth parts ten days after treatment. When the oldest flower (right) was fully open, the one on left was just opening, and the two in middle (back) were closed buds. B. The same fruit as shown in "A" 30 days after treatment. The perianth of the fruit on left was removed for examination. C. Cross sections of normal fruit (above) with seeds, and seedless fruit (below) induced with naphthoxyacetic acid.

fasciation, leaflets failing to separate. In some cases two of the midribs were fasciated, causing three rows of leaflets, one pointing upward. Some of these formative effects are shown in Figure 3 A. The plants treated with naphthoxyacetic acid were partially desensitized for a few days.

Formative effects on flowers and fruit. As mentioned above, the greatest modification of flowers occurred on plants treated through the soil with 50 cc. of 250 to 1000 mg./l. of naphthoxyacetic acid. All organs of the plant growing thereafter were considerably modified. Flower buds developed with long tubular calyxes, splitting only near the tip. They varied from this pronounced modification to nearly normal and finally normal flower buds after the influence of the chemical disappeared.

When normal flower buds just beginning to open were sprayed with any of the concentrations mentioned above, the ovaries were induced to enlarge rapidly and the perianth parts persisted for 10 to 30 days. This peculiarity is shown in Figure 4 A. Also, the styles persisted and the stigmas appeared to be in a receptive condition for pollination for a much longer period than normal. During December to February, while these experiments were under way, fruit set occurred only rarely on control plants. However, clusters of flowers and buds sprayed with naphthoxyacetic acid set seedless fruit readily during this period. If the spray was applied when the first bud opened, the oldest flower and some of the others developed, producing parthenocarpic (seedless) fruit. The youngest buds usually withered and dropped. When the petals showed much withering, the flower was too old to respond to the chemical treatment. Such flowers usually withered within a few days after being sprayed and abscised. The number of fruit set varied from two to five. Table I shows the number of fruit induced to set when flower clusters were sprayed. In no case were all of the flowers induced to set fruit with one spraying. Either the youngest or the oldest abscised. Flowers in the bud stage at the time the spray was applied frequently set fruit without opening. Emasculated flowers set fruit poorly. Perhaps in this case a lower concentration of the substance might have been more favorable. For practical purposes it would be best to spray the clusters at two or more different stages of development, that is, when the first two flowers are open and then at some later periods when the younger flowers show considerable development.

When the seedless tomato fruit ripened, the taste was considerably sweeter than normal fruit with seeds.

Normally the hibiscus does not develop seed pods in the Institute greenhouses. Parthenocarpic development of hibiscus ovaries was induced by spraying the open flower with various concentrations of naphthoxyacetic acid. Though the ovary was induced to grow, the seed did not set even though the flowers were pollinated before and after chemical treatment. When tomato flowers were pollinated after early parthenocarpic develop-

ment a few seeds were formed. These were tested, however, for viability and found viable.

Fuchsia flowers persisted for a long time and parthenocarpic develop-

TABLE I

PARTHENOCARPIC DEVELOPMENT OF TOMATO OVARIES INDUCED BY SPRAYING THE FLOWER CLUSTERS WITH NAPHTHOXYACETIC ACID IN CONTRAST TO NATURAL FRUIT SET OF CONTROLS

Conc. of NOA spray	Date of treatment	No. of flowers and buds when treated	Stage of development when sprayed	No. fruit set on Jan. 29	Remarks
400 mg./l.	Jan. 15	5	Only one flower open entirely	4	Youngest bud still remains
"	"	6	2 open	4	2 buds starting to turn yellow
"	"	9	1 starting to open	5	2 buds in good condition
"	"	4	4 open		
500 mg./l.	Jan. 20	5	1 starting	3	
"	"	6	3 open	3	2 dead
"	"	6	1 old flower	4	Old one abscised
"	"	5	3 open		
"	"	5	All in bud stage	2	3 buds remaining
"	Jan. 24	5	3 open	3	2 buds in good condition
"	"	4	1 opening	2	2 buds in good condition
"	"	4	1 starting to open	3	1 bud small
"	"	4	1 starting to open	2	2 buds in good condition
"	"	5	1 old flower	3	Old flower abscised
"	"	4	2 buds opening		
"	"	4	2 open	3	1 bud still in good condition
"	"	4	2 buds	2	1 dead, 1 bud remaining
"	"	4	1 open		
Check	Jan. 15	4	1 starting	0	All dead
Check	"	5	1 flower		
shaken	"	5	3 buds	2	3 dead
Check	Jan. 20	4	2 flowers	0	All dead
Check	"	6	3 buds		
shaken	"	6	2 flowers	2	4 dead
Check	"	5	4 buds		
shaken	"	5	1 flower	1	4 dead
Check	"	5	4 buds		
shaken	"	5	2 flowers	0	All dead
Check	"	5	2 buds		
shaken	"	5	2 flowers	0	4 dead
Check	"	5	3 buds		
"	"	4	1 flower	1	
"	"	6	1 flower	2	4 dead
"	"	6	5 buds		

ment occurred when treated with vapors of naphthoxyacetic and naphthaleneacetic acids (8) and when sprayed with solutions of these substances. The sepals often developed chlorophyll and appeared leaf-like.

β-Naphthoxyacetic acid derivatives and other growth substances. β-Naph-

thoxyacetamide, ethyl, methyl, and toluol naphthoxyacetate, and the potassium salt, were all active and induced the same formative effects as the acid itself. Indoleacetic acid, indolebutyric acid, and naphthaleneacetic acid are very active substances and have many influences in common with β -naphthoxyacetic acid. They lack, however, the unique capacity to modify leaf types as shown for the naphthoxyacetic acid and its derivatives.

DISCUSSION

The persistence of floral parts when tomato ovaries are induced to grow by spraying with naphthoxyacetic acid recalls the reports (5, 8) that holly fruit was induced to set when the flower buds were sprayed or exposed to vapors of methyl naphthaleneacetate. In the latter case the petals did not open fully but remained in good condition for more than a month whereas control flowers dropped within a short time (8). Both cases indicate regulating influences by hormone-like chemicals. In fact, all the hormone-like responses to growth substances indicate the regulatory effects of physiologically active compounds.

There is a close resemblance in appearance of modified leaves through the influence of naphthoxyacetic acid and virus-diseased leaves (4). An inexperienced person would be in danger of mistaking the formative effects of the chemical for virus symptoms. In the case of the latter, however, the active agent is usually multiplied in the growing tissue while the influence of applied naphthoxyacetic acid is lost by the growing plant.

In nature many modifications in leaf patterns are known to occur. The variations of submerged and aerial parts of amphibious plants are good examples. The literature on this subject is voluminous. A review of the published results and a discussion of possible relationships between naturally occurring and chemically induced modifications will be considered in a later publication.

Irvine (7), who was the first to report that β -naphthoxyacetic acid is physiologically active, was interested in the comparative effects on primordial tissue of X-radiation and several growth substances. She reported that plants treated with X-radiation and lanolin preparations of β -indoleacetic acid, colchicine, α - and β -naphthoxyacetic acid showed disarranged phyllotaxy, deformation and joining of leaf primordia, and splitting of the apex into several primordial regions. Strangely enough all of the treatments and substances had the same effects. We have not found α -naphthoxyacetic acid as active as the beta form and in our experience indoleacetic acid did not modify leaves of treated plants, as described for β -naphthoxyacetic acid.

Bausor *et al.* (3) in studying histological responses to lanolin preparations of naphthoxyacetic acid found that the substance caused cell en-

largement, cell division, differentiation, and organ formation. The response varied with the kind and age of the tissue. They did not mention modification of leaves produced after the tips were treated but stated that multiple roots developed from different centers of organization and new centers of growth in the primordium.

We have shown that naphthaleneacetic acid modifies the roots which are initiated, causing fasciated or new organs of large diameter. This substance, however, does not modify the leaves as described for naphthoxyacetic acid.

The ease with which naphthoxyacetic acid is synthesized and the low cost of the necessary ingredients should make this growth substance of special interest in experimental and college laboratories. Its possibilities for practical applications are evident. One of these is its use in large scale production of seedless tomato fruit. It has the advantage over indolebutyric acid in being the more effective on buds and flowers without emasculation. Gustafson (6) has shown the possibilities for producing seedless fruit with several substances but the preparation of the flowers for the treatment with lanolin mixtures is more difficult than spraying the cluster of buds and flowers. It is understood that some large scale commercial growers of greenhouse tomatoes spray the flowers with an emulsion containing indolebutyric acid. However, the flowers are first emasculated before being sprayed and this occupies the time of several employees. Perhaps the practice of emasculating is not necessary. Naphthoxyacetic acid can be sprayed effectively on the cluster without this tedious operation. The possibility of producing seedless fruit on field grown tomatoes and other species of plants should be further investigated. Our preliminary results indicate that parthenocarpic development can be induced when buds of sweet cherry are sprayed with naphthoxyacetic acid.

In applying the solution to flowers care must be taken not to spray the growing stem tips or modification of new leaves will occur. This would not seriously damage the plants but the chemical might slightly inhibit growth for a short time and also reduce the total leaf area. Methods can most likely be improved to the point where these difficulties will be overcome.

Several solvents, carriers, emulsions, and spreaders have been used with naphthoxyacetic acid, including ether, alcohol, glycerine, soaps, lanolin, lanolin emulsions, olive oil, paraffin oils, etc. Lanolin or lanolin emulsions are more effective carriers than alcohol and water or water alone. The acid is much more active and the effect more lasting in lanolin preparations than in water. Plants sprayed with 1000 mg./l. in alcoholic solution resume growth quickly. The same concentration in lanolin emulsions had a pronounced inhibiting and lasting effect. The difference in effectiveness might have been associated with the ease of penetration or the length of time the substance was held in contact with the treated tissue.

SUMMARY

Several species of plants were treated with solutions and vapors of β -naphthoxyacetic acid and their regulatory and formative effects were compared and contrasted with those induced by other growth substances. The principal differences concerned modifications of new organs formed after the plants were treated with naphthoxyacetic acid.

The venation pattern and form of the leaves of tomato, hibiscus, mimosa, artichoke, Paris daisy, tobacco, and marigold were modified when solutions of naphthoxyacetic acid and its derivatives were sprayed on the growing tip of the plants. This change occurred only in new leaves or parts of leaves that were formed after the plants were treated. Fully formed leaves showed the characteristic epinasty and swelling of the tissue which are well known responses to physiologically active compounds.

The pattern of modified leaves of tomatoes varied from frenched or fern-leaf types to simple leaves. The leaflets often failed to separate on one side of the midrib, giving a different appearance to the two sides of the leaf. In many respects the leaves resembled those of virus-diseased or mite-infested plants. Clearing of the veins was pronounced in hibiscus, tomato, artichoke, Paris daisy, and tobacco leaves. Mimosa leaves were fasciated or fern-like with peculiar projections on the leaflets.

Plants exposed to vapors of naphthoxyacetic acid first showed pronounced epinasty of leaves and growth was inhibited for a few days. The new leaves which developed thereafter showed the characteristic modifications.

Solutions of naphthoxyacetic acid applied to the soil brought out all the characteristics described for the sprays and vapors but the effects were even more pronounced and more lasting. The flower buds of plants treated by way of the soil were abnormally long with a decided calyx tube, separating only at the tip.

Parthenocarpic development of tomato fruit was induced when flowers or well developed buds were sprayed with emulsions or solutions of naphthoxyacetic acid. The ovaries often enlarged before the flowers opened, eventually breaking through on one side of the calyx tube. The floral parts—petals, stamens, and styles—persisted for an abnormally long time during parthenocarpic development. In some cases both petals and stamens remained in good condition for 21 days whereas controls withered within 3 days after the flowers opened.

Compared with other growth substances naphthoxyacetic acid has several advantages for practical production of seedless tomatoes and possibly of other fruits.



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FORMATION OF β -2,2,2-TRICHLOROETHYL-GENTIOBIOSIDE
IN TOMATO PLANTS GROWN IN MEDIA CONTAINING
CHLORAL HYDRATE, TRICHLOROETHYL AL-
COHOL, OR CHLORAL CYANOHYDRIN

LAWRENCE P. MILLER

Tomato plants, growing in sand cultures, have been found to be able to withstand the addition of considerable quantities of various chemicals which are absorbed by the growing plants and which can later be recovered combined with a sugar as β -glycosides (5). In experiments with chloral hydrate ($\text{CCl}_3\text{CH}(\text{OH})_2$) amounts up to a gram or more were added to individual cultures in 5-inch pots, over a period of 7 to 15 days, without the plants showing serious injury. Plants from these cultures had absorbed considerable quantities of the added chloral hydrate and contained a chlorine-containing β -glycoside which was obtained in crystalline form as both the acetyl and the propionyl derivative. Since chloral hydrate is an aldehyde, it was suspected that reduction to the corresponding alcohol took place before glycoside formation. Such a reduction occurs in the animal body as the first step in the detoxication of chloral hydrate (9, p. 763). Accordingly, some plants were grown in cultures with added trichloroethyl alcohol ($\text{CCl}_3\text{CH}_2\text{OH}$) instead of chloral hydrate. The acetyl glycoside obtained from these plants was identical with that obtained from the chloral hydrate treated plants. The chlorine content of this acetyl glycoside corresponded to that required for the heptaacetate of a trichloroethyl glycoside with a C_{12} disaccharide. Since tomato roots form β -*o*-chlorophenyl-gentiobioside from absorbed *o*-chlorophenol (7), it was thought probable that the new glycoside would also be a gentiobioside. β -2,2,2-Trichloroethyl-gentiobioside heptaacetate and heptapropionate were therefore synthesized and found to be identical with the corresponding derivatives from the tomato plants. It thus appears that the tomato readily reduces chloral hydrate to the corresponding alcohol, unless in these experiments, reduction took place in the culture medium before absorption. This latter possibility seems unlikely. These results have been referred to in a previous note (6).

Some experiments were also carried out with chloral cyanohydrin [$\text{CCl}_3\text{CH}(\text{OH})(\text{CN})$] since naturally occurring glycosides of cyanohydrins are rather widely distributed in plants. Chloral cyanohydrin proved to be considerably more toxic to the tomato than chloral hydrate or trichloroethyl alcohol, but sufficient quantities could be added to the cultures to induce the formation of moderate amounts of β -glycoside. The acetylation of partially purified preparations of this glycoside yielded only β -trichloro-

ethyl-gentiobioside heptaacetate. The possibility of the formation of small amounts of a cyanophoric glycoside as well is, of course, not necessarily excluded by this result.

EXPERIMENTAL

METHODS

Treatment of Growing Tomato Plants with Chemicals

Tomato (*Lycopersicon esculentum* Mill.) plants were started in good greenhouse soil and transferred to sand cultures when three to six inches tall. The nutrient solution used and the method of handling the cultures were the same as in previous experiments (7). The addition of the chemical was not started until the plants were well established and in vigorous condition and before they had begun to set fruit. Usually 0.63 millimol (with chloral cyanohydrin 0.2 millimol) of the chemical in 25 cc. distilled water was added six times weekly for 10 to 15 applications. The culture vessels were supplied with saucers so that unabsorbed chemical would accumulate in the cultures from day to day. The chemical was added in these relatively small amounts in order that the growing plants would not have to handle large quantities of the chemical at one time, but could convert it gradually to the presumably less toxic glycoside.

Determination of Amount of Chemical Absorbed and Quantity of β -Glycoside Formed

Within a few days after the end of the last treatment the plants were sampled. The tops and roots were ground separately through a food chopper and the juice extracted by squeezing through cheesecloth. The residue remaining was mixed with water with a little toluene and again squeezed through cheesecloth. These extracts were centrifuged, heated to 80° to inactivate enzymes, and again centrifuged. In order to determine the content of absorbed chemical, samples of the expressed juices were analyzed for chloride before and after previous heating with N KOH. The heating with KOH served to convert non-ionic chlorine to the ionic form and the difference between the two values obtained was a measure of the non-ionic chlorine in the expressed juice. This method possibly yielded somewhat low results in the case of the trichloro-compounds used in these tests, but was sufficiently accurate for the purposes for which it was used; that is, to serve as a measure of the amount of non-ionic chlorine absorbed and to serve as a guide in the isolation procedures employed. Determinations carried out with chloral hydrate solutions indicated that over 90 per cent of the non-ionic chlorine was recovered.

Portions of the expressed juices were subjected to the action of emulsin (50 mg. per 100 cc. in a M/20 acetate buffer at pH 4.75) after which the non-ionic chlorine recovered on distillation was determined. The values obtained with the samples carried through without added emulsin were a

measure of the uncombined chemical remaining in the treated tissues, and the increase after emulsin hydrolysis gave an indication of the quantity combined as a β -glycoside.

Procedure for Obtaining Preparations of the β -Glycoside Suitable for Acetylation

Partially purified preparations of the glycoside suitable for the preparation of the crystalline acetyl or propionyl derivatives were obtained as follows. The expressed juices and aqueous extracts were treated with an excess of lead acetate, and the lead remaining in the filtrate removed with H_2S . The precipitated lead sulphide was separated by filtration and the excess H_2S in the filtrate driven off by a stream of N. The filtrate, after neutralization to about pH 6.5 with NH_4OH , was concentrated *in vacuo* to a thin syrup and extracted with hot acetone. The water content of the syrup must be so regulated that the addition of acetone results in a separation into two layers without the formation of a precipitate in the aqueous layer. If such a precipitate is formed, extraction with acetone is not efficient, and this can be remedied by adding a little water. If the syrup contains too much water, so that the added acetone dissolves, it is necessary to concentrate the syrup further before extraction with acetone can be attempted. The extraction is conveniently carried out by shaking the flask containing the syrup and the acetone under a stream of hot water until the acetone boils, then setting the flask aside to permit clear-cut separation into two layers, and pouring off the acetone layer while it is still hot. It is necessary to make a series of extractions, if much glycoside is present in the syrup, and to add a little water to later portions of acetone since the acetone removes some water from the syrup. It is desirable to analyze samples from these extracts as they are being made to follow the course of the extraction.

Acetylation

The acetone extracts were concentrated under diminished pressure to remove the acetone and the aqueous solutions remaining were shaken a number of times with ethyl ether to take out fatty substances. The solutions were then evaporated to dryness and acetylated by adding dry pyridine and acetic anhydride. After the reaction mixture had stood overnight at room temperature it was poured into five to seven times its volume of ice water. The product was separated from the ice water by centrifugation, dissolved in acetone and absolute alcohol from which it crystallized after standing for a little time at room temperature. The acetate crystallizes rather readily from relatively impure mixtures and was obtained without difficulty from both the top and roots with all three chemicals included in these studies. The product was purified with the use of Norite and several recrystallizations from absolute alcohol.

RESULTS

Experiments with Chloral Hydrate

After preliminary tests had shown that tomato plants form a β -glycoside from absorbed chloral hydrate and some information had been obtained as to the amount of chloral hydrate that could be added safely to the sand cultures, three series involving 48 to 100 plants each were run in order to obtain material for the isolation and characterization of the glycoside formed. Data as to the amount of chloral hydrate added to each culture and the content of a trichloro-compound per 100 cc. of the expressed juice of the tops and roots at sampling time, as well as the quantity freed as a result of emulsin hydrolysis, are summarized in the first part of Table I. It is seen that a large proportion of the non-ionic chlorine compound in the root samples is present in glycosidal combination as shown by the fact that after being subjected to the action of emulsin it is recoverable on distillation. In the juice from the tops a smaller proportion is freed on emulsin hydrolysis. Whether this indicates the formation of another compound, not hydrolyzable by emulsin, or merely less complete hydrolysis, because of the presence of a substance or substances which retard the action of emulsin, is not known. Calculated as β -trichloroethyl-gentiobioside on the basis of the non-ionic chlorine content, the expressed juices contained as much as one gram of gentiobioside per 100 cc.

TABLE I

FORMATION OF β -GLYCOSIDES BY TOMATO PLANTS GROWN IN A NUTRIENT MEDIUM CONTAINING CHLORAL HYDRATE, TRICHLOROETHYL ALCOHOL, OR CHLORAL CYANOHYDRIN

Substance used	Quantity added, millimols per culture	Amount present per 100 cc. expressed juice		Amount recovered on distillation			
				Tops		Roots	
		Tops	Roots	Emulsin hydrolysis	Without emulsin	Emulsin hydrolysis	Without emulsin
Chloral hydrate	4.38	0.98	1.12	0.14	0.05	0.95	0.03
	5.63	1.67	1.40	0.40	0.02	1.03	0.02
	9.10	2.10	2.30	0.99	0.10	2.05	0.07
Trichloroethyl alcohol	4.80	0.75	1.25	0.27	0.01	0.95	0.07
	5.00	0.88	1.08	0.21	0.02	0.73	0.01
Chloral cyano-hydrin	1.60	0.46	0.50	0.07	0.02	0.19	0.03

The addition of these amounts of chloral hydrate to the culture medium (0.63 millimol 6 times weekly until the quantities shown in Table I had been added) produced definite but not very marked retardation in growth as compared to control plants. Injury, if present, involved browning of the edges of the leaflets, especially near the tip of the plants.

Isolation of β -trichloroethyl-gentiobioside as the heptaacetate from treated plants. Following the procedure described in a previous section the chlorine-containing compound formed in the tomato was obtained as the crystalline acetyl derivative from both tops and roots. A total of about 10 grams was prepared from the several series. After a number of recrystallizations from absolute alcohol the substance melted at 184.5° to 185.0° C. (corr.) and had a specific rotation of $[\alpha]_D^{25} = -28.8^{\circ}$ (concn., 2.57 g. in 100 cc., CHCl_3). In a mixed melting point determination with synthetic β -trichloroethyl-gentiobioside heptaacetate no depression in melting point was observed.

Analysis: Calculated for β -trichloroethyl-gentiobioside heptaacetate, $\text{C}_{28}\text{H}_{37}\text{O}_{18}\text{Cl}_3$: Cl, 13.85. Found: Cl, 13.71, 13.76.

Isolation of β -trichloroethyl-gentiobioside as the heptapropionate. Using propionic anhydride instead of acetic anhydride the propionyl derivative was obtained from a preparation from the root juice which contained non-ionic chlorine equivalent to 5.8 millimols of a trichloro-compound. Yield of crude product was 3.19 g. After three recrystallizations from absolute alcohol the compound (1.87 g.) showed partial melting at 134.0° to 136.0° C. and on further heating melted completely at 149.5° to 150.5° . Specific rotation was $[\alpha]_D^{24} = -26.0^{\circ}$ (concn., 2.56 g., CHCl_3). No depression was observed in a mixed melting point determination with synthetic β -trichloroethyl-gentiobioside heptapropionate.

Analysis: Theory for β -trichloroethyl-gentiobioside heptapropionate, $\text{C}_{35}\text{H}_{51}\text{O}_{18}\text{Cl}_3$: Cl, 12.28. Found: Cl, 12.33, 12.30.

Experiments with Trichloroethyl Alcohol

Trichloroethyl alcohol was prepared according to the method of Chalmers given in Organic Syntheses (2). The product was purified by fractional distillation under reduced pressure, and the fraction boiling between 59° and 62° at 12 mm. pressure was used. Data showing the amount of trichloroethyl alcohol absorbed in two series of plants and hydrolysis by emulsin of the β -glycoside formed are shown in Table I.

Isolation of β -trichloroethyl-gentiobioside as the heptaacetate. A preparation obtained from the juice expressed from the tops of the treated plants from the first series and containing non-ionic chlorine equivalent to 1.24 millimols of a trichloro-compound was acetylated in 30 cc. dry pyridine and 15 cc. of acetic anhydride. Yield, 0.30 g. After one recrystallization from absolute alcohol 0.21 g., melting at 184.5° to 185.0° , was obtained. Specific rotation was $[\alpha]_D^{25} = -28.5^{\circ}$ (concn., 2.23 g., CHCl_3).

Analysis: Calculated for β -trichloroethyl-gentiobioside heptaacetate, $\text{C}_{28}\text{H}_{37}\text{O}_{18}\text{Cl}_3$: Cl, 13.85. Found: Cl, 13.75.

Similarly, acetylation of a preparation from the root juice of the plants in the second series containing 1.78 millimols of a trichloro-compound yielded 0.54 g. of an acetyl derivative. Two recrystallizations from abso-

lute alcohol gave 0.34 g., m. p. 184.5° to 185.0° ; $[\alpha]_D^{25} = -28.8^{\circ}$ (concn., 3.115 g., CHCl_3); Cl, 13.89 per cent.

Experiments with Chloral Cyanohydrin

Since chloral cyanohydrin was considerably more toxic to the tomato than chloral hydrate or trichloroethyl alcohol, only 0.2 millimol was added to the cultures at each application and the plants were sampled after 1.6 millimols had been added to each culture. The data showing the presence of a β -glycoside hydrolyzable by emulsin in both tops and roots in an experiment involving 48 plants are given in Table I. The expressed juice from the tops and roots when carried through the same purification procedure as was used with chloral hydrate and trichloroethyl alcohol treated plants yielded β -trichloroethyl-gentiobioside heptaacetate. It thus appears that trichloroacetaldehyde was split off from the cyanohydrin and reduced to the alcohol before glycoside formation took place.

Isolation of β -trichloroethyl-gentiobioside as the heptaacetate. A preparation from the expressed juice from the tops, containing non-ionic chlorine equivalent to 2.1 millimols of a trichloro-compound, yielded, on acetylation, 0.49 g. of product. This was recrystallized three times from absolute alcohol. Yield, 0.30 g.; m. p. 184.5° to 185.0° ; $[\alpha]_D^{26} = -29.3^{\circ}$ (concn., 2.105 g., CHCl_3); Cl, 13.67 per cent. No depression in melting point resulted in a mixed melting point determination with synthetic β -trichloroethyl-gentiobioside heptaacetate.

The acetyl derivative (0.37 g.) obtained from the root sample was identical with the foregoing. M. p. 184.5° to 185.0° ; $[\alpha]_D^{25} = -28.3^{\circ}$ (concn., 2.035 g., CHCl_3); Cl, 13.83 per cent.

Synthesis of β -2,2,2-Trichloroethyl-Gentiobioside Heptaacetate and Heptapropionate

β -Trichloroethyl-gentiobioside heptaacetate, which has not been synthesized previously, was obtained by condensing trichloroethyl alcohol with acetobromogentiobiose. The acetobromogentiobiose was prepared from β -gentiobiose octaacetate which was obtained from β -*d*-glucose-1,2,3,4-tetraacetate and acetobromoglucose as described by Reynolds and Evans (8). The methods of Reynolds and Evans were also used for the preparation of 6-trityl- β -*d*-glucose-1,2,3,4-tetraacetate and the β -*d*-glucose-1,2,3,4-tetraacetate required for the synthesis of β -gentiobiose octaacetate.

Acetobromogentiobiose. Acetobromogentiobiose was prepared from β -gentiobiose octaacetate by a procedure differing somewhat from that used by previous workers (8, 10). Five-gram portions of the octaacetate were dissolved in 50 cc. of alcohol-free, dry CHCl_3 in a 250 cc. distilling flask and the solution cooled to 0° in an ice-salt bath. Fourteen cc. of a saturated solution of HBr in acetic acid (also cooled to 0°) were added and the mix-

ture kept at 0° for 2.5 hours. The flask was then connected to a vacuum pump and some of the HBr removed while the flask was still in the ice-salt bath. Toluene (90 cc.) was then added and the mixture distilled in a water bath, with the temperature of the bath below 20° at the start of the distillation and later not over 35° . The syrup remaining was crystallized by adding dry ether and petroleum ether. Yield of crude product usually about 65 per cent. The substance was recrystallized from alcohol-free CHCl_3 and ether after which it melted at 141° to 142° ; yield 57 per cent. This was of sufficient purity for subsequent use even though the melting point was still a little lower than that of the pure substance (1).

β -Trichloroethyl-gentiobioside heptaacetate. In the first preparation 8.94 g. of trichloroethyl alcohol (.06 mol.) were added to 4.19 g. (.006 mol.) of acetobromogentiobiose dissolved in 15 cc. dry benzene cooled in an ice bath and 2.0 g. of Ag_2CO_3 added in small portions over a period of one hour. The mixture was then kept at room temperature overnight, after which the precipitated AgBr was filtered off and washed with benzene. The benzene was evaporated off *in vacuo* and the evaporation continued after the addition of several portions of water to remove the excess trichloroethyl alcohol. The residue was taken up in absolute alcohol from which the product crystallized. The product was rather difficult to purify, but after six recrystallizations from absolute alcohol, 0.40 g. of the pure substance was obtained. Yield, 9 per cent.

A better yield was obtained when Drierite was used as an internal desiccant. A mixture of 12 cc. alcohol-free, dry CHCl_3 , 1.28 g. (.0086 mol.) trichloroethyl alcohol, 5.0 g. Drierite, and 1.1 g. Ag_2O was allowed to stand with occasional shaking for one-half hour. Two-tenths of a gram of iodine (3) was added followed by the addition of 3.0 g. (.0043 mol.) of acetobromogentiobiose in small portions during the course of one hour. The mixture stood at room temperature overnight and was then filtered through Celite and the precipitated AgBr and Drierite washed well with CHCl_3 . The CHCl_3 solution was extracted three times with water and concentrated under reduced pressure. Twenty cc. of water were added and the evaporation continued to remove excess trichloroethyl alcohol. The product was crystallized from absolute alcohol. Yield of pure compound, after four recrystallizations from absolute alcohol, 0.71 g. or 21 per cent.

Synthetic β -trichloroethyl-gentiobioside heptaacetate was found to have a melting point of 184.5° to 185.0° and a specific rotation $[\alpha]_D^{25} = -28.7^{\circ}$ (concn., 2.833, CHCl_3).

Analysis: Calculated for $\text{C}_{28}\text{H}_{37}\text{O}_{18}\text{Cl}_3$: Cl, 13.85. Found: Cl, 13.78, 13.84.

β -Trichloroethyl-gentiobioside heptapropionate. Synthetic β -trichloroethyl-gentiobioside heptaacetate (350 mg.) was suspended in 15 cc. of dry methanol and the solution cooled in an ice-salt bath. The equivalent of

2 cc. of a half-normal solution of barium methylate in methyl alcohol was added, and the mixture allowed to stand at 5° overnight (4). The barium methylate was neutralized exactly by $N/2$ H_2SO_4 and the solution evaporated to dryness *in vacuo*, with the addition of several portions of absolute alcohol to aid in driving off the last traces of moisture. Five cc. dry pyridine and 3 cc. propionic anhydride were then added and after standing overnight the reaction mixture was poured into 100 cc. of ice water. The crystalline product was obtained from the ice water by centrifugation. It was dissolved in acetone, the solution filtered and the compound crystallized after the addition of absolute alcohol. Yield, 0.31 g. After a further recrystallization from absolute alcohol 0.22 g. of the pure compound was obtained. It showed partial melting at 136° to 136.5° and on further heating melted sharply at 150° to 150.5°. If cooled after the partial melting at 136°, the substance solidified and on reheating showed no change at the lower temperature, but melted at 150° to 150.5°. Specific rotation was $[\alpha]_D^{25} = -25.6^\circ$ (concn., 3.335, $CHCl_3$).

Analysis: Calculated for $C_{35}H_{51}O_{18}Cl_3$: Cl, 12.28. Found: Cl, 12.15.

SUMMARY

The tops and roots of tomato plants, grown in a medium to which chloral hydrate had been added, were found to contain a chlorine-containing β -glycoside, which was obtained in crystalline form as the acetyl and as the propionyl derivative. The same β -glycoside was obtained when trichloroethyl alcohol instead of chloral hydrate was added to the nutrient medium. Through the synthesis of β -2,2,2-trichloroethyl-gentiobioside heptaacetate and heptapropionate, which were prepared for the first time, this glycoside has been shown to be β -trichloroethyl-gentiobioside. β -Trichloroethyl-gentiobioside is also formed from absorbed chloral cyanohydrin. It thus appears that the tomato readily reduces trichloroacetaldehyde to the corresponding alcohol. With chloral cyanohydrin, hydrolysis apparently precedes the reduction. Quantities of β -trichloroethyl-gentiobioside up to one gram per 100 cc. of expressed juice were present in the plants grown with added chloral hydrate.

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SYNTHESIS OF β -2-CHLOROETHYL-*d*-GLUCOSIDE BY WHEAT PLANTS GROWN WITH ETHYLENE CHLOROHYDRIN ADDED TO THE NUTRIENT MEDIUM

LAWRENCE P. MILLER

Biochemical investigations in connection with the use of ethylene chlorohydrin in breaking the rest period (2, 3, 4) have shown that both potato tubers (*Solanum tuberosum* L.) and *Gladiolus* corms form β -2-chloroethyl-*d*-glucoside from the absorbed ethylene chlorohydrin (5, 6). Further studies have indicated that many species of higher plants will form β -glycosides when they absorb various chemicals which can serve as aglycons. Experimental formation of glycosides in growing plants which have absorbed chemicals which can unite with sugars in glycosidal combination has previously received little attention from phytochemists, although Ciamician and Ravenna [as quoted by Armstrong and Armstrong (1, p. 86)] showed, thirty years ago, that certain plants will form glucosides when inoculated with aromatic products of glucoside hydrolysis. For example, they were able to isolate salicin from corn plants which had absorbed saligenin.

The experiments in this laboratory have been confined to compounds which are not known to occur naturally in plants and have shown that the artificially produced plant glycosides may involve sugars other than *d*-glucose. Thus, although gentiobiose has not previously been known to occur in *gladiolus* corms or in tomato plants (*Lycopersicon esculentum* Mill.), and perhaps is not normally found in these species, a β -gentiobioside is formed if these plants are treated with *o*-chlorophenol (7, 8). Such investigations may thus yield information on the occurrence or formation of sugars in various species and on differences or similarities between metabolic processes in plants. It therefore seemed desirable to characterize artificially induced glycosides in additional species and the present report deals with experiments with wheat (*Triticum aestivum* L.) grown in sand cultures to which ethylene chlorohydrin had been added. Qualitative tests showed the presence of a chlorine-containing β -glycoside in both the tops and roots of the treated plants. This β -glycoside was shown to be β -2-chloroethyl-*d*-glucoside through its isolation from the expressed juice of the tops as the tetraacetate.

EXPERIMENTAL

In the first experiment 100 seeds of Marquis Beardless spring wheat were planted in pure quartz sand in each of six 10-inch shallow earthenware pots supplied with saucers and watered with tap water. After the

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seed had germinated a nutrient solution containing the equivalent of 25 cc. of each of the four main stock solutions (2 per cent KNO_3 ; 4 per cent $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 2 per cent KH_2PO_4 and 2 per cent $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) and 2 cc. of a 5 per cent ferric tartrate solution was added to each culture. The same quantity of nutrient was again added 22 days later. Thirty-five days after the start of the experiment 10 millimols of ethylene chlorohydrin were added to half the cultures and this was repeated six days later. After a further two days the material was sampled. The tops and roots were ground separately through a food chopper and the juice expressed by squeezing through cheesecloth. The treated tops and roots were found to contain 3.25 and 1.45 millimols, respectively, of non-ionic chlorine per 100 cc. of expressed juice (as determined by the difference in chloride content before and after heating with an equal volume of N KOH). As would be expected the control plants did not contain non-ionic chlorine. After digestion with emulsin (50 mg. per 100 cc.) in an acetate buffer at pH 4.75 for 18 hours and subsequent distillation, 2.04 and 0.95 millimols of non-ionic chlorine per 100 cc. were recovered from the top and root juice, respectively. The samples carried through without emulsin yielded only a small amount of non-ionic chlorine on distillation, thus showing the absence of an appreciable quantity of free chlorohydrin in the treated plants.

To obtain sufficient material for the isolation of the glycoside an experiment involving a larger number of plants was carried out. The wheat was grown in sand culture in 50 5-inch pots with 25 seeds planted in each pot. The same nutrient solution was used except that each culture received only two-fifths of the quantity added to the larger cultures previously employed. When the plants were 10 to 14 inches tall, 0.5 millimol of ethylene chlorohydrin in a volume of 50 cc. was added to each culture six times weekly until each culture had received 3.5 millimols. The plants were then sampled and the expressed juice obtained. A further extract was made by mixing the residues with water and a little toluene and again squeezing through cheesecloth. The expressed juice and the extract were heated to 80° C. to inactivate enzymes, and centrifuged. The expressed juice of the tops and roots was found to contain 2.5 and 0.9 millimols of non-ionic chlorine, respectively, and hydrolysis with emulsin resulted in release of about half of the non-ionic chlorine. As in the previous experiment little free chlorohydrin remained in the treated plants.

The juice expressed from the tops and the further extract obtained on mixing the residue with water and again squeezing through cheesecloth were combined and an excess of lead acetate added. After filtering off the lead precipitate the excess lead in the filtrate was removed by precipitation with H_2S . The lead sulphide was filtered off and the solution concentrated under reduced pressure to a thin syrup. This syrup was extracted with three successive 100-cc. portions of hot acetone. The combined ace-

tone extracts, which contained 5.42 millimols of non-ionic chlorine, were concentrated *in vacuo* to remove the acetone and the aqueous solution remaining extracted a number of times with ethyl ether to remove fatty material. The solution was again evaporated to a thin syrup and extracted first with 50 cc. of hot acetone and then with two 50-cc. portions of hot 90 per cent acetone. The combined extracts, containing 4.8 millimols of non-ionic chlorine, were evaporated under vacuum to dryness, with the addition of several portions of absolute alcohol to aid in driving off the last traces of moisture. The residue was acetylated at room temperature by adding 60 cc. of dry pyridine and 35 cc. of acetic anhydride and permitting the reaction to proceed overnight. The reaction mixture was poured into 750 cc. of ice water and the product extracted by shaking with chloroform. The chloroform solution was washed with cold 10 per cent H_2SO_4 , followed by cold saturated sodium bicarbonate and water. After evaporation under vacuum to remove the chloroform the residue was taken up in hot absolute alcohol from which it crystallized. The crystals were filtered off and washed with absolute alcohol. Yield, first crop, 0.73 g., m. p. 114.5° to 115.0° C. (corr.). On concentration of the filtrate a further 0.43 g. with about the same melting point was obtained. After three recrystallizations from absolute alcohol the substance (0.54 g.) melted at 117° to 118° and had a specific rotation $[\alpha]_D^{20} = -13.1^\circ$ (concn., 4.26 g. in 100 cc., $CHCl_3$). These constants compare with a melting point of 118.5° to 119.0° and a specific rotation of -13.4° found for synthetic β -2-chloroethyl-*D*-glucoside tetraacetate (5). In a mixed melting point determination the melting point was 118° to 119° .

Analysis: Calculated for β -2-chloroethyl-*D*-glucoside tetraacetate $C_{16}H_{23}O_{10}Cl$: Cl, 8.64; acetyl, CH_3CO , 41.91. Found: Cl, 8.76, 8.52; acetyl, 42.33, 41.90.

SUMMARY

A chlorine-containing β -glycoside is formed in both roots and tops of wheat plants grown in a medium containing ethylene chlorohydrin. This glycoside has been shown to be β -2-chloroethyl-*D*-glucoside through the preparation of the acetyl derivative which was found to be identical with synthetic β -2-chloroethyl-*D*-glucoside tetraacetate.

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SYNTHESIS OF β -2,2,2-TRICHLOROETHYL-GENTIOBIOSIDE BY GLADIOLUS CORMS TREATED WITH TRICHLORO- ETHYL ALCOHOL

LAWRENCE P. MILLER

Experiments on the formation of β -glycosides in plants as a result of the absorption of chemicals which serve as aglycons have shown that *Gladiolus* corms form β -2-chloroethyl-*d*-glucoside if treated with ethylene chlorohydrin (1), but if *o*-chlorophenol is the foreign aglycon furnished, a β -gentiobioside is formed (2). It is thus seen that, in the same plant organ, the sugar component of artificially induced β -glycosides may vary with the nature of the aglycon used. It therefore seemed of interest to include other aglycons in studies with gladiolus corms and the present paper reports results showing that a β -gentiobioside is formed if trichloroethyl alcohol is the added aglycon. It should be pointed out, however, that, even though β -2,2,2-trichloroethyl-gentiobioside, as the heptaacetate and as the heptapropionate, was the only glycoside isolated, this does not necessarily mean that it was the only glycoside formed in the treated corms.

EXPERIMENTAL

Corms of the Giant Nymph variety (2704 g.) were placed in two 7-liter desiccators and treated with trichloroethyl alcohol by drawing through the desiccators a stream of air which had first been bubbled through two tubes containing trichloroethyl alcohol. The air was passed through at the rate of about 12 liters per hour, and the treatment was continued for one week. After another week the corms were ground through a food chopper and the juice expressed through cheesecloth. The expressed juice was centrifuged, heated to 80° C., and again centrifuged. The expressed juice was found to contain non-ionic chlorine equivalent to 3.04 millimols of a trichloro-compound per 100 cc. Distillation of a portion of the expressed juice showed that 1.8 millimols was still present in the free state since it was recovered by distillation, but hydrolysis with emulsin resulted in the recovery of an additional 0.6 millimol, indicating that some of the absorbed trichloroethyl alcohol had been fixed as a β -glycoside.

A partially purified preparation of the glycoside, suitable for acetylation, and containing 4.4 millimols as indicated by the non-ionic chlorine content, was obtained by the same procedure previously used with tomato extracts (3). Acetylation yielded 0.44 g. of crystalline product. On treating with Norite and recrystallizing twice from absolute alcohol 0.28 g. melting at 184.5° to 185.0° C. was obtained. Specific rotation was $[\alpha]_D^{25} = -28.5^\circ$

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(concn., 1.905 g., CHCl_3). These constants compare with a melting point of 184.5° to 185.0° and a specific rotation of -28.7° found for synthetic β -2,2,2-trichloroethyl-gentiobioside heptaacetate (3). No depression was observed in a mixed melting point determination.

Analysis: Calculated for β -trichloroethyl-gentiobioside heptaacetate, $\text{C}_{28}\text{H}_{37}\text{O}_{18}\text{Cl}_3$: Cl, 13.85. Found: Cl, 13.64.

The propionyl derivative was prepared from 180 mg. of the acetyl derivative by the method used for the preparation of the heptapropionate from synthetic β -trichloroethyl-gentiobioside heptaacetate (3). Yield after one recrystallization from absolute alcohol, 100 mg. The substance melted partially at 135° to 136.5° C. and on further heating melted completely at 150° to 150.5° . Specific rotation was $[\alpha]_D^{25} = -25.5^\circ$ (concn., 2.16 g., CHCl_3). Synthetic β -trichloroethyl-gentiobioside heptapropionate has been found to melt partially at 136° to 136.5° with complete melting at 150° to 150.5° ; $[\alpha]_D^{26} = -25.6^\circ$ (3).

Analysis: Calculated for β -trichloroethyl-gentiobioside heptapropionate, $\text{C}_{38}\text{H}_{51}\text{O}_{18}\text{Cl}_3$: Cl, 12.28. Found: Cl, 12.13.

SUMMARY

Gladiolus corms have been shown to form β -2,2,2-trichloroethyl-gentiobioside from absorbed trichloroethyl alcohol.

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SOME X-RAY OBSERVATIONS REGARDING THE MEMBRANE STRUCTURE OF HALICYSTIS¹

WAYNE A. SISSON

Halicystis is a large oval, single-celled, marine alga which often grows to a diameter of three centimeters. Owing to its large size, it is especially adapted to X-ray diffraction studies of membrane structure. In contrast to the closely related alga, *Valonia*, which has been the subject of considerable X-ray work (2, 13, 19, 20) no X-ray studies had been reported on *Halicystis* previous to 1937, when a preliminary examination (16) gave a most unexpected result. The X-ray diffraction pattern showed the presence of mercerized cellulose which was oriented in a manner similar to that in the synthetic membrane, Cellophane. A more detailed investigation, which is reported in this paper, has confirmed this preliminary observation (16).

There are two polymeric crystalline forms of cellulose (native and mercerized) which may be distinguished by their X-ray diagrams, as shown in Figure 1. In the native form (Fig. 1 A), cellulose gives three principal X-ray diffraction rings corresponding to interplanar spacings of 6.1, 5.4, and 3.95 Å. If native cellulose is dispersed and coagulated from solutions (e.g. cuprammonium hydroxide) or swollen with certain swelling agents (e.g., 18 per cent sodium hydroxide), then a product is obtained which gives a new diffraction pattern after the swelling agent is washed out (10). In this form it is known as "hydrate" or "mercerized" cellulose, although it has the same analytical composition as the native form. In addition to being more reactive chemically, it differs crystallographically from the native form in that the glucose units of the cellulose chain are rotated 30° to give a new unit cell arrangement (1). The difference between the native and mercerized unit cell structure is illustrated in Figure 1 C and D. Owing to this crystallographic difference, the X-ray diffraction method is the most satisfactory and only definite method for distinguishing between native and mercerized cellulose.

In all samples of plant cellulosic membranes heretofore subjected to X-ray diffraction analysis the cellulose is found to exist in the native form. The native form is usually considered to be the unstable form, since cellulose always changes to the mercerized form when treated with strong swelling agents, and after once in the mercerized form it is extremely difficult to revert to the native form. For this reason the existence of mercerized cellulose in *Halicystis* is of unusual chemical, as well as biological, interest.

¹ A contribution from the former Cellulose Department of the Chemical Foundation, Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

In addition to the 7.4, 4.45, and 4.0 Å cellulose lines, there is also present in the composite X-ray diagram of *Halicystis* two other patterns: (a) an amorphous pattern and (b) a diffraction line, not due to cellulose, which corresponds to the approximate spacing of 12.5 Å. This 12.5 Å line is comparatively intense and has the same orientation as the 7.4 Å cellulose line. Most other cellulosic membranes which have been examined with X-rays show only the presence of an amorphous non-cellulosic pattern having a random orientation.

In the present investigation the structure and orientation of the cellulose is studied in the untreated membrane, and in membranes which have been subjected to various purification treatments. The investigation also includes a study of the non-cellulosic constituents and their relation to the cellulose in building up the membrane structure. The X-ray studies are supplemented by microscopic examination and physical tests and the results on *Halicystis* are compared with those on *Valonia* and Cellophane. The mercerized structure of the cellulose is further confirmed by separating the cellulose from the non-cellulosic constituents, by conversion to the native form, and by the formation of known derivatives and swelling compounds.

MATERIALS AND METHODS

Three species of *Halicystis* were examined: *grandis* Blinks, *ovalis* (Lyngb.) Aersch., and *osterhoutii* Blinks and Blinks. *H. osterhoutii* was collected in Bermuda by Dr. L. R. Blinks; *H. grandis* in the Bahama Islands by the New York Botanical Garden; and *H. ovalis* in Bermuda by Dr. J. F. G. Wheeler in 1938 and by Dr. R. E. Reeves in 1939. Unless otherwise noted, all studies were carried out on the species *ovalis* collected in Bermuda.

The samples for examination with the beam parallel to the membrane surface consisted of several strips one to two millimeters wide, the ends of which were fastened across a U-shaped cork support while being exposed to the X-ray beam. For the perpendicular exposures these same samples were removed from the U support and placed flat against the slit system. For the membranes disintegrated by the purification process and for the fractions recovered from the extracting reagents, the samples were packed into small celluloid holders made by boring a hole 2 mm. in diameter through a flat section of celluloid 2 mm. thick.

X-ray diagrams were made with unfiltered copper radiation ($K\alpha = 1.54$ Å) produced in a Philips Metalix tube operating at 28 kilovolts and 25 milliamperes. The X-ray beam was defined through a pinhole system using 0.6 mm. pinholes placed 10 cm. apart. In referring to the X-ray results the term "diagram" will be used to refer to the composite diffraction rings obtained from the sample, while "pattern" will refer to the diffraction rings characteristic of a particular component or material of the sample.

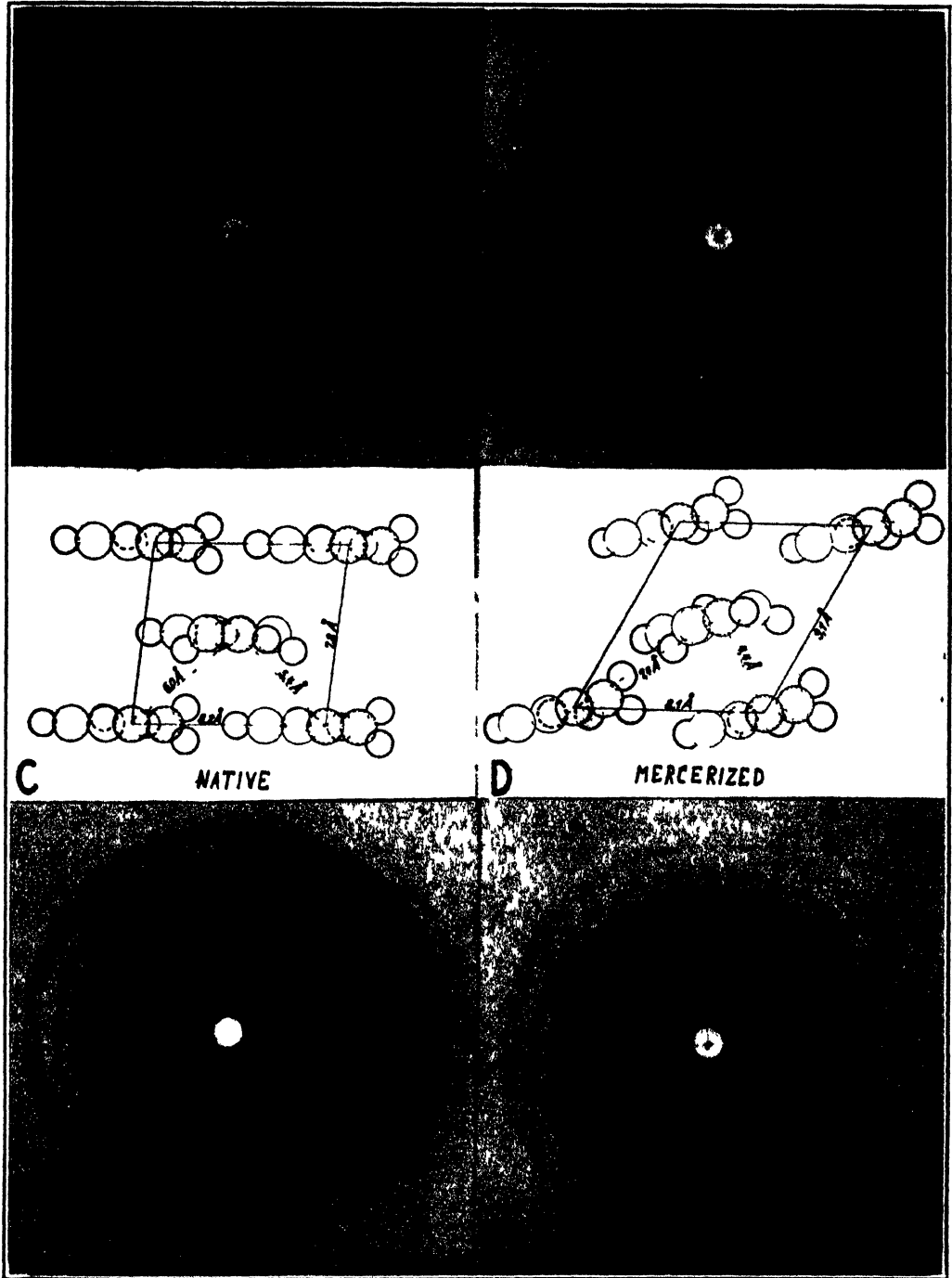


FIGURE 1. Representative X-ray diffraction diagrams of (A) native, and (B) mercerized cellulose from cotton fibers. The unit cell arrangement of glucose units in (C) native and (D) mercerized cellulose. The X-ray patterns of cellobiose actaacetate obtained from (E) *Halicystis* and (F) cotton.

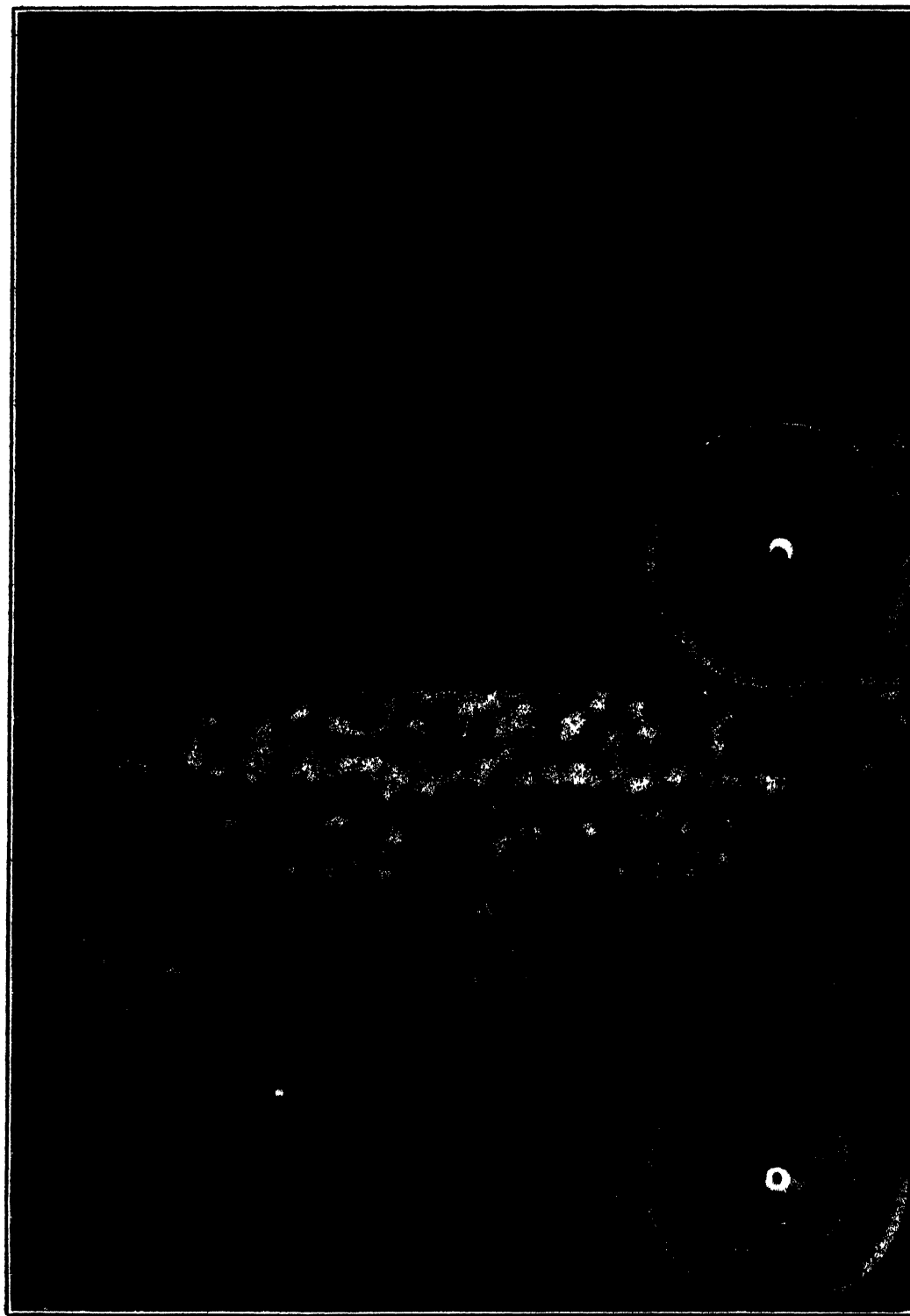


FIGURE 2. Photomicrographs and X-ray diagrams of *Halicystis* membrane taken (A) perpendicular and (B) parallel to the membrane surface.

RESULTS

The experimental results are described in the chronological order in which they were obtained: (a) untreated membranes, (b) effect of purification, (c) reversion to the native form, and (d) formation of derivatives.

UNTREATED MEMBRANES

A representative X-ray diagram of an untreated *Halicystis* membrane is shown in Figure 3 A. This composite diagram shows diffraction effects which apparently arise from three different components of the membrane: First, there is present the 7.4, 4.45, and 4.0 Å diffraction lines characteristic of mercerized crystalline cellulose. Second, there is superimposed upon these lines an amorphous pattern which tends to obscure the crystalline cellulose pattern. Owing to its amorphous nature, it is impossible to identify this material from the X-ray diagram. Third, close to the primary beam, there is a definite diffraction ring corresponding to the interatomic spacing of 12.5 Å. As yet, no substance has been found which is known to give a diffraction ring corresponding to this spacing. As will be discussed later, there is also present in the stretched diagram a 6.0 Å line.

Observations made on two X-ray diagrams of each variety indicate that the relative intensity of these three diffraction patterns differs in the three species. *H. grandis* has a relatively strong amorphous pattern and a pronounced inner diffraction ring (12.5 Å), while *H. ovalis* shows a weak amorphous pattern and a faint inner ring. *H. osterhoutii* is similar to *H. grandis*. In the *grandis* and *osterhoutii* species the intensity of the amorphous diagram makes it difficult to distinguish the two separate 4.45 and 4.0 Å cellulose diffraction rings, which are quite clearly resolved in the *ovalis* species. Several patterns of the *ovalis* species indicate considerable variation from membrane to membrane.

Orientation. The cellulose in *Halicystis* has a random orientation in the plane of the membrane, but a selective orientation with reference to the surface. If the X-ray beam, therefore, is passed perpendicular (see Fig. 2 A) to the membrane surface, the 7.4 Å cellulose line is missing and the 4.45 and 4.0 Å lines show a random orientation. With the beam parallel (see Fig. 2 B), the 7.4 Å line is present as two arcs while the 4.45 and 4.0 Å lines still show a random orientation. This indicates that the 7.4 Å crystallographic planes are oriented parallel to the membrane surface, while the *b* axes of the crystallites (i.e., the direction of cellulose chains) have a random orientation in the plane of the membrane. This type of orientation (selective uniplanar orientation) is similar to that observed for Cellophane (17). In this connection it is interesting to note that *Halicystis* is also similar to Cellophane in that the cellulose in both possesses a mercerized crystalline structure.

The non-cellulosic material which gives the 12.5 Å line also possesses

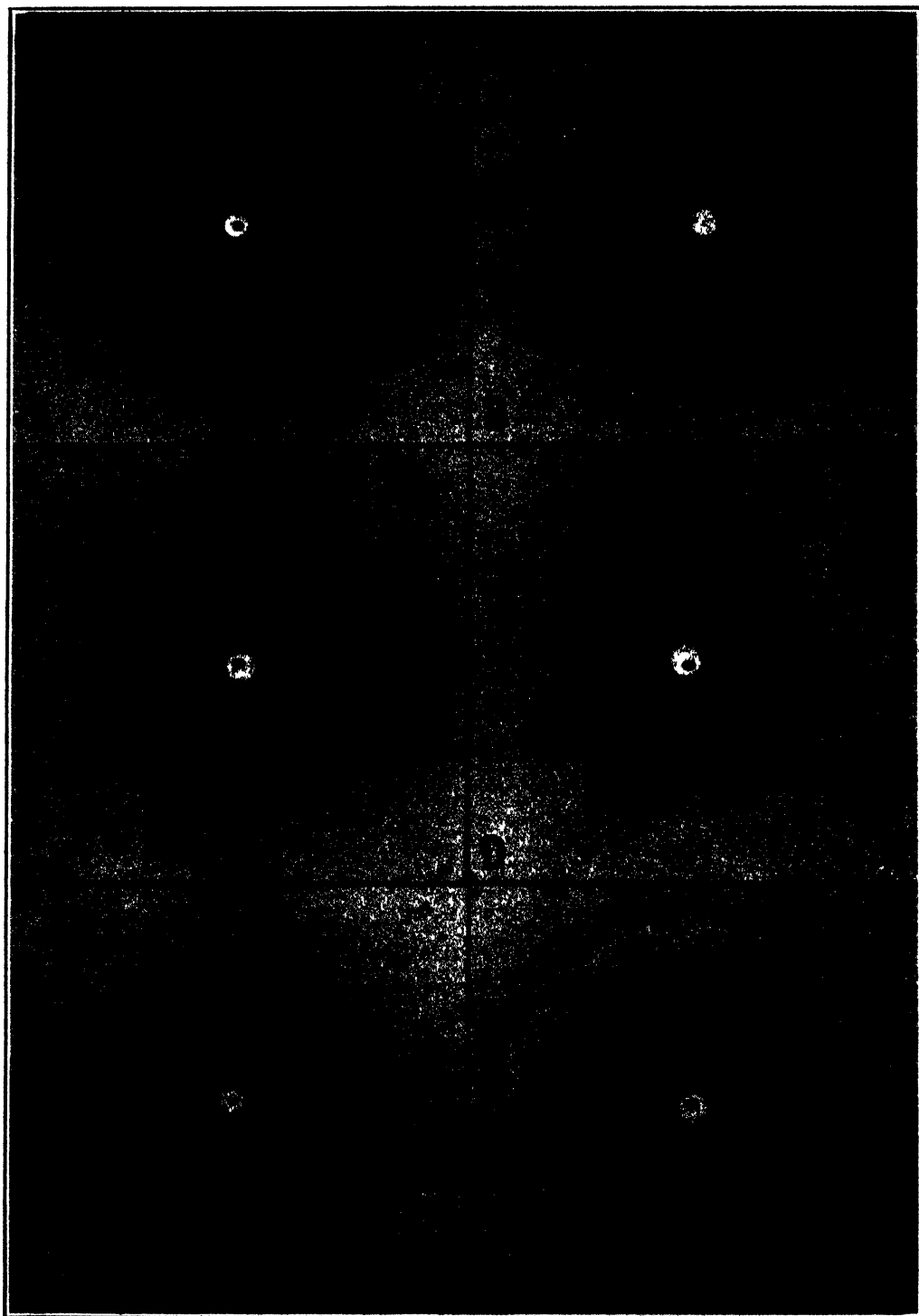


FIGURE 3. X-ray diffraction diagrams of *Halicystis* (A) untreated, (B) stretched 75 per cent (fiber axis vertical), (C) after purification treatments, (D) after treatment with hot glycerin, (E) after treatment with hot 0.1 N hydrochloric acid and 1 per cent sodium hydroxide, and (F) for comparison, the X-ray diagram of mercerized cotton treated as in (E).

a selective orientation, as indicated by the fact that the 12.5 Å line is absent with the X-ray beam perpendicular, and present as two arcs when the beam is parallel to the membrane. It will be noticed that this orientation is similar to that of the 7.4 Å cellulose line (compare A and B, Fig. 2). There is no indication of orientation in the amorphous diagram with the X-ray beam either perpendicular or parallel to the membrane surface.

Stretching. When *Halicystis* is stretched the crystalline cellulose behaves in a manner similar to that in other cellulosic membranes. The result of stretching is shown in Figure 3 B, which is the X-ray diagram of several narrow strips of *Halicystis* which were slightly twisted, stretched 75 per cent while moist, and allowed to dry. Twisting the strip permits the X-ray diagram to represent the equivalent of a strip turned through 360°, and gives an average rather than a perpendicular or parallel exposure. In the stretched X-ray diagram, the 7.4, 4.45, and 4.0 lines all form arcs on the equator. This means that the original selective uniplanar orientation has been displaced by a uniaxial orientation of the crystallites parallel to the direction of stretch. Other native (15) and mercerized (17) cellulosic membranes behave in a similar manner when stretched.

The 12.5 Å plane also orients itself parallel to the direction of stretching as indicated by two arcs on the equator. It should be noticed that these lines show a degree of orientation higher than that of the cellulose as indicated by the smaller angle of the arcs. The concentration of the rings into arcs now makes apparent in the diagram of *Halicystis* one other diffraction ring corresponding to a spacing of approximately 6.0 Å which was not observed in the diagram of the unstretched material. This line also exists as two arcs on the equator indicating an orientation parallel to the direction of stretching. This 6.0 Å arc too, like the 12.5 Å arc, shows a higher degree of orientation than the cellulose, and it is possible that the 12.5 and 6.0 Å diffraction lines arise from the same constituent of the membrane.

Upon stretching, part of the *Halicystis* diagram, which was previously referred to as amorphous, now shows a preferred orientation as indicated by four arcs which appear, each at 45° to the equator. Since these arcs are superimposed upon the 4.45 and 4.0 Å cellulose lines, it is impossible to determine their corresponding interatomic spacings or whether they arise from the same constituent as 12.5 and 6.0 Å non-cellulosic lines.

Comparison of Halicystis with Valonia. The marine alga, *Valonia ventricosa* Agardh, is somewhat similar to *Halicystis* in outward appearance, but very dissimilar in membrane structure. The X-ray diagram of *Valonia* is unlike *Halicystis* in that *Valonia* shows only the X-ray pattern of native cellulose, and there is no evidence of an amorphous or non-cellulosic constituent present. The orientation in *Valonia* also differs from that in *Halicystis*. In *Halicystis* the mercerized cellulose is oriented at random in the plane of the membrane, while in *Valonia* the native cellulose

is oriented parallel in two directions which make an angle of approximately 80° to each other (2, 13). The two membranes are similar, however, in that they both possess a selective orientation. In *Halicystis* the $7.4 \text{ \AA}$ plane is parallel to the surface, and in *Valonia* the $6.1 \text{ \AA}$ plane. Thus, *Halicystis* may be described as having a selective uniplanar orientation and *Valonia* as having a selective biaxial orientation (15).

Comparison of X-ray and microscopic structure. The X-ray and microscopic results on *Halicystis* and *Valonia* show a close correlation, as may be seen by comparing Figure 2, which contains X-ray diagrams and photomicrographs of *Halicystis* taken perpendicular and parallel to the membrane surface, with similar photographs of *Valonia* published elsewhere (6). The photomicrographs show the presence of cellulose particles in both *Halicystis* and *Valonia*. They differ, however, in that the particles exist separately in random arrangement in *Halicystis*, while in *Valonia* they are united end to end to form fibrils which are oriented in two directions at approximately 80° to each other. This is in agreement with the random X-ray diagram of *Halicystis* and the two 80° arcs in *Valonia*.

The selective orientation shown in the X-ray diagram of *Halicystis* and *Valonia* cannot be detected in the microscope because of the ellipsoidal shape of the cellulose particle (18). The microscopic results, however, do not belie its existence since cross sections (6) show a laminated structure for both *Halicystis* and *Valonia*.

The presence of non-cellulosic constituents shown in the X-ray diagram of *Halicystis* is also corroborated by microscopic studies (5, 6) which show the presence of a large amount of non-cellulosic material between the cellulose particles in *Halicystis*.

Physical properties. The physical properties of *Halicystis* are compared with those of Cellophane and *Valonia* in Table I. All the data are listed relative to dry Cellophane which is given a value of 1. The values for Cellophane are the average of transverse and longitudinal strips. The Cellophane contained a plasticiser and was of approximately the same thickness (0.02 mm.) as the average *Halicystis* membrane. One of the most remarkable properties of *Halicystis* is the fact that it has a higher tensile strength in the wet than in the dry condition. This is in direct contrast to Cellophane which is weaker when wet. *Valonia* is also stronger when wet, but this is to be expected since *Valonia* possesses a fibril structure like cotton, which is also stronger wet than dry. Another interesting property of *Halicystis* is its high elongation and elastic recovery. It is not unreasonable to assume that this unusual physical property is due to the large amount and nature of the non-cellulosic constituents which separate the cellulose particles. The low elongation and high elastic recovery of *Valonia* in the dry condition is probably due to the fibril structure. Owing to the

difficulty of making measurements on the small pieces of membrane, the values given in Table I are only approximate and of a relative nature, but they serve to emphasize the influence of membrane structure on physical properties.

TABLE I
PHYSICAL PROPERTIES OF CELLOPHANE, HALICYSTIS, AND VALONIA

	Cellophane	<i>Halicystis</i>	<i>Valonia</i>
Light transmission	1	1.1	1.0
Vapor permeability (H ₂ O)	1	1.3	1.5
Moisture absorption	1	1.9	0.9
Tensile strength {Dry	1	1.0	1.5
Wet	0.8	1.3	1.8
Elongation {Dry	1	1.3	0.2
Wet	3	5.0	1.0
Elastic recovery {Dry	1	1.0	2.0
Wet	1.2	1.5	0.7

PURIFICATION

When applied to *Halicystis*, the usual purification treatments for cellulose fail to produce a product which gives only the X-ray diagram of cellulose. Extraction with organic solvents in a Soxhlet extractor, heating with one per cent sodium hydroxide at 100° C. for 24 hours, and bleaching with two per cent sodium hypochlorite for 15 minutes remove part of the amorphous pattern, but the 12.5 Å line is still present in the diffraction pattern, as shown in Figure 3 C.

Successful purifications have been carried out by Dr. Reeves of this laboratory (14) by extracting first with 1 per cent alcoholic solution of potassium hydroxide, treating with 0.1 normal hydrochloric acid, and then extracting with aqueous 1 per cent sodium hydroxide. In each case the extractions were at 75° C. for 24 hours. This process disintegrates the membrane and leaves a product which now gives a clear pattern of mercerized cellulose, as shown in Figure 3 E. Chemical tests, however, show that this residue still contains non-cellulosic materials, and that part of the cellulose was removed with the soluble fraction. In this connection it should be emphasized that the above treatments in no way affect the X-ray diagram of either native or mercerized cellulose, as shown by Figure 3 F.

The present evidence would indicate that the chemical nature of the *Halicystis* membrane is changed by treatment with hydrochloric acid. The untreated membrane is not swollen by strong sodium hydroxide or quaternary ammonium hydroxide or dispersed in cuprammonium hydroxide solutions. If the membrane, however, is first treated with normal hydrochloric

acid at 75° C. for 15 minutes it swells readily in strong bases and disperses in cuprammonium hydroxide. The X-ray diagram of the product coagulated from cuprammonium solution, however, still contains the 12.5 Å line. Treatment with hydrochloric acid alone does not change the X-ray diagram, but if the acid treatment is followed by extraction with dilute alkali, then the X-ray pattern of the non-cellulosic constituents is decreased in intensity and that of the mercerized cellulose increased.

REVERSION TO NATIVE FORM

Additional proof for the existence of mercerized cellulose in *Halicystis* comes from the fact that the mercerized structure may be partially converted to the native form. It has been shown (11, 12) that when mercerized cellulose from cotton or rayons is heated above 200° C. in formamide or glycerine the mercerized cellulose is converted directly into native cellulose. With *Halicystis*, attempts at conversion were successful with glycerine but not with formamide.

When *Halicystis* was treated with boiling glycerine (250° C.) for 15 minutes the membrane partially dissolved. The undissolved portion gave an X-ray diagram showing the presence of both mercerized and native cellulose (Fig. 3 D). Mercerized cotton heated in glycerine under similar conditions also gave a mixed diagram. In addition to partially reverting the cellulose to the native form, the treatment with glycerine also removed the 12.5 Å line and most of the amorphous pattern, leaving only the mixed diagram of mercerized and native cellulose. When the *Halicystis* and cotton samples giving the mixed diagrams were treated with 18 per cent sodium hydroxide and washed with water, the cellulose was again completely changed back to the mercerized form.

When a *Halicystis* membrane was placed in boiling formamide (210° C.) it dissolved in about 15 minutes. Upon cooling and dilution with water a brown precipitate formed which gave an amorphous pattern. When the liquid was added to an equal volume of cold acidified alcohol a white precipitate formed which gave only the X-ray pattern of mercerized cellulose identical with that shown in Figure 3 E. This precipitate also gave the sulphuric acid-iodine test for cellulose. Although treatment with hot formamide failed to change the cellulose to the native form, the treatment did serve as a convenient method of isolating a product from *Halicystis* which gave only the mercerized cellulose diagram.

It is also known that mercerized cotton may be partially changed to the native form by treating with hot 18 per cent sodium hydroxide and washing in boiling water (8). *Halicystis* treated with hot 18 per cent sodium hydroxide and washed in boiling water gave a partial reversion to the native form. Unlike glycerine, the sodium hydroxide did not remove the 12.5 Å line.

FORMATION OF CELLULOSE DERIVATIVES

Additional evidence for the existence of cellulose in the mercerized form in *Halicystis* comes from the formation of a definite swelling compound with ethylenediamine which can be detected by its X-ray pattern. Trogus and Hess have shown (21) that when ethylenediamine swells cellulose, a definite swelling compound is formed, and that the extension of the 101 plane is greater when the compound is formed from mercerized than when formed from native cellulose. Ethylenediamine has no effect on the untreated membrane, but if it is first treated with normal hydrochloric acid at 75° C. for 15 minutes, then a cellulose swelling compound is formed, the spacings of which correspond to the compound formed from mercerized cotton cellulose.

Further proof for the existence of cellulose in *Halicystis* comes from the work of Compton of this laboratory (3) which shows that the definite cellulose derivative, cellobiose octaacetate, may be formed. The similarity of cellobiose octaacetate obtained from *Halicystis* and cotton is indicated by their identical X-ray diagrams (shown in Fig. 1 E and F), melting points (222° C.), and optical rotations ($[\alpha] = +41.6^\circ$).

Since cellobiose octaacetate may be obtained from either native or mercerized cellulose, the similarity of the product obtained from *Halicystis* and cotton does not prove that mercerized cellulose is present in *Halicystis*, but it does prove that the cellulose molecule is present, and this eliminates the possibility of some other unknown carbohydrate being present in *Halicystis* which would give the same diffraction pattern as mercerized cellulose.

DISCUSSION

The X-ray identification of mercerized cellulose in *Halicystis* is further supported by microchemical tests by Farr (7) which show that the cellulose particles in *Halicystis* turn blue when treated with iodine-potassium iodide solution. Van Iterson (9) observed that the *Halicystis* membrane turned blue when treated with iodine, and since he believed the blue coloration to be due to amyloid, he questioned the cellulose nature of the crystalline material which was doubly-refractive in polarized light. The X-ray identification of mercerized cellulose now explains van Iterson's results, since it is well known (4) that mercerized cellulose stains a deeper color than native cellulose when treated with iodine.

Although all the chemical, X-ray, and microscopic data point to the existence of cellulose which has a mercerized crystalline structure, there is other evidence such as its solubility in sodium hydroxide, viscosity, and chemical reactivity which indicates that the cellulose isolated from *Halicystis*, especially in its colloidal behavior, is not identical with mercerized cotton cellulose. In its isolated form, it cannot be classified as *alpha*-cellulose, but rather as a *beta*- or *gamma*-cellulose, depending upon the

method of isolation from the *Halicystis* membrane. If compared with cotton cellulose, the present data indicate that the cellulose from *Halicystis* is more like cellulose from cotton which has been treated with hydrochloric acid and then mercerized with sodium hydroxide.

The "degraded" nature of the cellulose in *Halicystis* is also indicated by the relative intensity of the X-ray diffraction lines. In the pattern of mercerized raw cotton, the 4.45 and 4.0 Å lines are of about equal intensity. If the cotton, however, is treated with hydrochloric acid to the point where it will disperse in cold 10 per cent sodium hydroxide, then the 4.45 Å line of the mercerized diagram is much more intense than the 4.0 Å line. From Figure 3 E it may be seen that the 4.45 Å diffraction line of *Halicystis* is more intense than the 4.0 Å line.

The non-cellulosic material which gives the 12.5 Å line appears to be closely bound with the cellulose as indicated by the fact that their diffraction lines have the same orientation in both the stretched and unstretched membranes and by the fact that they are not separated by the usual cellulose purification treatments. The amorphous X-ray pattern is greatly decreased by extraction with organic solvents and dilute sodium hydroxide and bleaching with sodium hypochlorite, but the membrane must be broken down before the cellulose can be separated from the material which gives the 12.5 Å line. This would indicate that this material serves as a matrix or cementing material between the cellulose in building up the membrane structure. Whether the bond between this material and the cellulose is of a physical or chemical nature is not indicated by the X-ray data, since it is difficult to determine whether the lack of resolution of the cellulose lines (especially the 4.45 and 4.0 Å lines) is due to a masking produced by the superimposition of other patterns on the cellulose lines, or whether it is due to a broadening of the cellulose lines resulting from a mixed crystallization of the cellulose and non-cellulosic materials.

The close association of cellulose with the non-cellulosic constituents makes it difficult to determine the amount of cellulose present in *Halicystis*. Rough estimates, however, based upon the weight of cellulose that can be separated, indicate approximately 50 per cent cellulose. Furfuraldehyde determinations by Reeves of this Laboratory (14) indicate the presence of over 35 per cent pentosans. The lack of sufficient material prohibits further work regarding the amount and chemical nature of the various constituents of the *Halicystis* membrane.

The exact rôle which the non-cellulosic constituents play in contributing to the physical and chemical properties of the *Halicystis* membrane is not clear. It seems reasonable to assume, however, that the unusual wet strength and the elongation and elastic regain of the membrane is due to the non-cellulosic constituents. It also appears probable that the inertness of the membrane to strong alkaline swelling agents which ordinarily swell

cellulose, is due to the protective effect of the non-cellulosic materials.

The biological significance of mercerized cellulose in *Halicystis* also is not clear, since it is not known whether the mercerized condition is specific for *Halicystis*. Comparatively few of the lower plant membranes, or membrane constituents other than cellulose, have been subjected to X-ray analysis. It is generally believed that native cellulose is only a metastable form, because it is extremely difficult, once it has been transformed into mercerized cellulose by treatment with strong swelling agents, to get it back again to the native form. The question as to why cellulose should be deposited in the stable mercerized form in *Halicystis* and in the unstable native form in the closely related cell, *Valonia*, is yet to be answered.

SUMMARY

1. X-ray examination of three species of untreated *Halicystis* shows the presence of diffraction lines corresponding to mercerized crystalline cellulose. There is also present a crystalline non-cellulosic pattern and an amorphous pattern.

2. Both the cellulosic and non-cellulosic constituents have a random orientation in the plane of the membrane but a selective orientation with reference to the surface. When the membrane is stretched a new orientation is produced parallel to the direction of stretching.

3. X-ray and microscopic data on *Halicystis* are compared and discussed in relation to similar data on *Valonia*.

4. Some physical properties of *Halicystis*, *Valonia*, and Cellophane are measured and discussed in relation to membrane structure.

5. The non-cellulosic constituents are not removed by the usual cellulose purification treatments. After pretreatment with warm dilute hydrochloric acid the purification treatment leaves a residue which gives only the diagram of mercerized cellulose.

6. The orientation and purification studies indicate a close bond between the cellulosic and non-cellulosic constituents, the exact nature of which is not indicated.

7. When *Halicystis* is treated with hot glycerine the mercerized cellulose is partially converted to the native form.

8. The cellulose in *Halicystis* forms swelling compounds with ethylenediamine and yields the definite derivative cellobiose octaacetate.

9. The mercerized cellulose in *Halicystis* has the same crystalline structure as mercerized cotton cellulose, but exhibits different colloidal and chemical reactions.

10. The identification of mercerized cellulose in *Halicystis* is of both biological and chemical interest since it is the first example of a plant membrane subjected to X-ray diffraction analysis which shows the cellulose to exist in the mercerized form.

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SPRAYS THAT BREAK THE REST PERIOD OF PEACH BUDS

JOHN D. GUTHRIE

For the proper growth of buds of peach trees in the spring it is necessary that they receive a certain amount of cold weather. In southern parts of this country peach trees do not foliate properly following warm winters and this appears to determine the southern limit of peach culture. An important contribution toward the solution of this problem made by Boyce, Chandler, and Kimball (1), who used 2,4-dinitro-6-cyclohexylphenol, suggested that it would be of interest to try other substances.

MATERIALS AND METHODS

One-year-old peach trees (*Prunus persica* Sieb. & Zucc.) of the Elberta variety were planted in the spring in 10-inch pots. They were pruned to give a head of three to six twigs. After growing outside until November they were moved to the greenhouse at 65° to 75° F. The sprays were applied to individual twigs with an atomizer. After three weeks the number of buds growing on each twig was recorded. Observations one or two weeks later did not differ much from the three-week observations and will not be presented. In general, very few buds grew on the unsprayed twigs even after the trees were placed out-of-doors in the spring. An exception to this was twigs infested with aphids. It appeared that aphids feeding on the twigs terminated the rest period of the buds. Frequent nicotine fumigation was necessary to keep these under control. The conditions in the greenhouse were not favorable to the survival of flower buds. The data presented in the tables are for leaf buds only.

EXPERIMENTS

In preliminary experiments about 40 substances were tried. The solvent used was 10 g. paraffin oil dissolved in 490 cc. of acetone. One per cent solutions of the substances in this solvent were used except in cases where a lower concentration was necessary to avoid injury. Of the substances tried, three showed sufficient stimulative action to warrant further trial. These were *p*-thiocresol ($\text{CH}_3\text{C}_6\text{H}_4\text{SH}$), 4-chloro-*o*-phenylphenol ($\text{C}_6\text{H}_5\cdot\text{ClC}_6\text{H}_3\text{OH}$), and α -nitronaphthalene ($\text{C}_{10}\text{H}_7\text{NO}_2$). Weinberger (2) has reported chloro-*o*-phenylphenol ineffective in concentrations of 0.06 and 0.12 per cent. The results of further experiments with these substances follow.

Experiment 1. In this experiment the sprays were applied on February 21, 1940. The solvent used was 10 g. of paraffin oil in 490 cc. acetone. As a

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positive control a 0.2 per cent solution of 2,4-dinitro-6-cyclohexylphenol was used. Each spray was applied to nine twigs. After three weeks the number of leaf buds growing was recorded for each twig. Since the size of the twigs varied, the total number of buds sprayed was also recorded. In each case the values presented in the first experiment of Table I are the totals for nine twigs. It will be seen that all of the substances tested induced two or three times as many buds to grow as grew on the controls sprayed with the paraffin oil-acetone mixture alone. It appears that after having been in the greenhouse for several months, the buds respond to some extent to the carrier alone. In the next experiment on the next season's lot of trees the sprays were applied soon after removal of the trees to the greenhouse.

TABLE I

BREAKING THE REST PERIOD OF LEAF BUDS OF PEACH WITH SPRAYS CONTAINING VARIOUS SUBSTANCES

Substance	Per cent substance in spray	Experiment 1			Experiment 2		
		No. buds sprayed	No. buds growing	Per cent growing	No. buds sprayed	No. buds growing	Per cent growing
<i>p</i> -Thiocresol	1.00	110	58	53	187	63	34
<i>p</i> -Thiocresol	0.25	84	41	49	164	17	10
Control—carrier only	0.00	104	24	23	183	2	1
4-Chloro- <i>o</i> -phenylphenol	1.00	98	53	54	171	11	6
4-Chloro- <i>o</i> -phenylphenol	0.25	92	41	45	174	3	2
Control—carrier only	0.00	91	14	15	177	0	0
α -Nitronaphthalene	1.00	113	64	57	137	36	26
α -Nitronaphthalene	0.25	97	49	51	137	5	4
Control—carrier only	0.00	119	22	18	143	8	6
2,4-Dinitro-6-cyclohexylphenol	0.20	98	30	31	154	29	19
2,4-Dinitro-6-cyclohexylphenol	0.09*				146	25	17
Control—unsprayed	0.00				142	0	0

* Spray prepared according to U. S. Patent No. 2,166,123 (1).

Experiment 2. In this experiment the sprays were applied on November 23, 1940, about three weeks after the trees were moved to the greenhouse. The carrier used was the same as that used in Experiment 1, with the exception of the inclusion of a spray containing 2,4-dinitro-6-cyclohexylphenol in a light petroleum oil emulsified with blood albumen according to Boyce, Chandler, and Kimball (1). It will be noted that, with the exception of 4-chloro-*o*-phenylphenol, some response to the substances tried was observed even at this stage of the rest period. The paraffin oil-acetone carrier had little effect at this stage of the rest period.

Experiment 3. In order to see if *p*-thiocresol, 4-chloro-*o*-phenylphenol, and α -nitronaphthalene could be used in an oil emulsified in water, an experiment was made in which a light petroleum oil of the dormant type emulsified with Penetrol was used. Penetrol alone was also tried as a car-

rier. These sprays which were applied December 27, 1940 were prepared by warming 500 mg. of the substance in 5 cc. of the oil until dissolved, adding 1 cc. of Penetrol and then shaking with 45 cc. of water. The sprays in which Penetrol alone was used were prepared by dissolving 500 mg. of the substance in 5 cc. of warm Penetrol and shaking with 45 cc. of water. Each spray was applied to six twigs. The results are shown in Table II. It will be noted that *p*-thiocresol and 4-chloro-*o*-phenylphenol were effective in inducing the growth of buds when applied in an oil emulsion. α -Nitronaphthalene appears to be ineffective in such a carrier at this stage of the rest period. Little or no effect was observed for any of the three substances in Penetrol alone.

TABLE II
INFLUENCE OF THE CARRIER ON THE EFFECTIVENESS OF SUBSTANCES THAT BREAK
THE REST PERIOD OF LEAF BUDS OF PEACH

Substance	Per cent substance in spray	Substance in light oil emulsified with Penetrol			Substance in Penetrol alone		
		No. buds sprayed	No. buds growing	Per cent growing	No. buds sprayed	No. buds growing	Per cent growing
<i>p</i> -Thiocresol	1.0	90	36	40	75	6	8
4-Chloro- <i>o</i> -phenylphenol	1.0	86	25	28	89	11	12
α -Nitronaphthalene	1.0	101	5	5	105	1	1
Control—carrier only	0.0	85	3	4	85	3	4
Control—unsprayed	0.0	99	0	0			

SUMMARY

p-Thiocresol, 4-chloro-*o*-phenylphenol, and α -nitronaphthalene break the rest period of peach buds when applied in sprays.

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AN ANALYSIS OF FACTORS CAUSING VARIATION IN SPORE GERMINATION TESTS OF FUNGICIDES. III. SLOPE OF TOXICITY CURVES, REPLICATE TESTS, AND FUNGI¹

S. E. A. MCCALLAN, R. H. WELLMAN,² AND FRANK WILCOXON

The present paper constitutes a further study of the factors causing variation in spore germination tests of fungicides. Particular reference has been paid to variations introduced by differences in the slope of the toxicity curves, by replicate tests, and by the use of different fungi.

An attempt has been made to answer three main questions: Do all toxicity curves give straight lines when plotted as probits against logarithm of dose? Do tests replicated at different times using different lots of spores vary more than is to be expected from their internal error, or from tests replicated at the same time using the same lot of spores? How do some commonly used fungi compare as laboratory test organisms? Some other questions arising from these concern the differences in steepness of slope, the suitability of the LD₅₀ for all comparisons, the conditions under which a standard fungicide may be used effectively to reduce day-to-day variation, and the question of whether different fungi rate compounds in like order.

On the basis of this analysis a laboratory design is outlined to give maximum efficiency with regard to the number of spores, doses, fungi, and replicate tests. The choice of an appropriate LD level and error term is discussed with reference to the method of evaluating results.

METHODS

A general study was first made on six fungi and twenty compounds replicated six different times. This constituted a total of 600 individual toxicity curves. Later a more detailed examination was made of copper sulphate and "Standard Laboratory Bordeaux" mixture (1) on four fungi replicated at the same and different times. These two materials were chosen because each has been commonly used as a standard for the two techniques of application.

Fungi. The species selected were *Sclerotinia fructicola* (Wint.) Rehm.,³ *Glomerella cingulata* (St.) Sp. & von S.,⁴ *Alternaria solani* (Ell. & Mart.)

¹ A preliminary report on this paper was presented before the American Phytopathological Society, Philadelphia, Pa., December, 1940 (14).

² Investigator, Crop Protection Institute.

³ Field isolate V38 (15).

⁴ Obtained through the courtesy of Dr. J. F. Adams, formerly of Delaware Agricultural Experiment Station, Newark, Delaware, 1935.

Jones & Grout,^{4,5} *Macrosporium sarcinaeforme* (Cav.),⁶ *Botrytis* sp. (*cinerea* type),⁷ and *Rhizopus nigricans* Ehr., + strain.⁸ The first four fungi are of particular interest since they probably have been the most commonly used species for laboratory tests, while the other two have also been used to a lesser extent.

All species were cultured on potato dextrose agar⁹ slants at $21.5^{\circ} \pm 0.5^{\circ}$ C. and the spores obtained by the rubbing and centrifuging technique (15) from seven-day-old cultures, except *Macrosporium sarcinaeforme* where 21-day-old cultures were employed as suggested by Heuberger and Horsfall (9). The spores were obtained from three replicate transfers for all species except *M. sarcinaeforme* for which six to eight transfers were required. The spore suspension concentration was adjusted to 50,000 spores per cc. and 0.1 per cent ultrafiltered orange juice added. *M. sarcinaeforme* was an exception in that the spore suspension was adjusted to 25,000 for the general study and 15,000 for the special copper sulphate and Bordeaux tests. The spores were examined for germination after 20 to 24 hours in the constant temperature room at $21.5^{\circ} \pm 0.5^{\circ}$ C. and counts made on 100 potentially viable spores per concentration or deposit. Replicate counts were not made since it has been shown that these are binomially distributed (15).

Compounds. The 20 compounds comprised three series fairly representative of (a) the soluble heavy metal salts, (b) "insoluble" copper fungicides, and (c) soluble and "insoluble" organic compounds. There were seven heavy metals consisting of silver and copper sulphates, and mercury, cadmium, nickel, cobalt, and zinc chlorides. The eight "insoluble" coppers comprised "Standard Laboratory Bordeaux" (1), and proprietary samples of copper ammonium silicate, basic sulphate, hydroxide, oxychloride, phosphate, zeolite, and cuprous oxide. The organic compounds comprised five synthetic materials.

The first four fungi were employed in testing all compounds and in the detailed examination of copper sulphate and Bordeaux, while in addition *Rhizopus nigricans* was used in the heavy metal and organic series and *Botrytis* sp. (*cinerea* type) in the "insoluble" copper series.

⁴ This fungus was isolated by Dr. Adams from "collar rot" lesions on young tomato transplants from Georgia. Due to the abundance of spores produced in culture and their small size, there may be some question of its species identity. However, this isolate has become distributed and used as a test organism under the name *A. solani*.

⁵ Isolated by and obtained through the courtesy of Dr. J. G. Horsfall, formerly of New York Agricultural Experiment Station, Geneva, New York, 1939.

⁷ Isolated from peony, Yonkers, New York (13, p. 331).

⁸ Obtained through the courtesy of Dr. A. F. Blakeslee, Carnegie Institution, Cold Spring Harbor, New York, 1939.

⁹ Formula: Distilled water 1000 cc., peeled potatoes 500 g., agar 20 g., dextrose 10 g., NaCl 1 g.

Concentrations or deposits. Two techniques were used in making up the various doses; dose being defined as the amount of chemical to which a given number of spores are exposed. For the heavy metal and organic series the test tube dilution method described in the first paper of this series (15) was employed. In brief, 2 cc. of solution were placed in a test tube, 0.5 cc. of spore suspension plus orange juice added, and drops pipetted onto the glass slides contained in the moist chambers.

The settling tower method of applying spray deposits discussed in the second paper of this series (16) was employed for the insoluble coppers. Stock suspensions containing 0.01, 0.025, or 0.10 per cent copper were required for the different materials, and exposure periods of 1, 2, 4, and 8 were used. These exposure periods constituted a dose ratio of 2; this ratio was also used for the heavy metals, except silver and mercury, where a ratio of 1.5 was employed. The dose ratio for the organic compounds was 1.33. These dose ratios permitted from 2 to 8 finite values per curve. For the special study of copper sulphate and Bordeaux mixture the dose ratio was 1.414.

Replicate tests. For each series of compounds, e.g. the heavy metals, all members were replicated on six different days, and on each of the days all the different species of fungi were tested. A different series of compounds was tested on each of another six days. In this case the replicate tests are similar to the replicate experiments as defined in the first paper (15), namely, tests replicated at different times and hence with different lots of spores, though from the same isolate. However, in the special study of copper sulphate and Bordeaux two stocks of each, differing by 25 per cent, were prepared and each tested at the same time with the same lot of spores, as well as being replicated at different times with different lots of spores.

SLOPE OF TOXICITY CURVES

TYPES OF SLOPES

The slopes of the 600 toxicity curves were determined graphically by plotting the concentration or dose against the per cent inhibition of spore germination on logarithmic probability paper (21). If the distribution of individual lethal doses is symmetrical against the logarithm of the dose this form of plotting will give a straight line as has been illustrated in an earlier article (21). It was found, however, that in addition to the simple straight line several types of compound slopes were obtained. In all there were four types which are illustrated in Figures 1 and 2. The first type is the orthodox straight line, while the second type has a left hand "break" in the lower values, giving a curve concave upwards. The third type is the reverse of the second in that the break is toward the right and in the upper or middle values producing a curve convex upwards. Finally, the fourth type is a combination of the second and third resulting in a triple slope or sig-

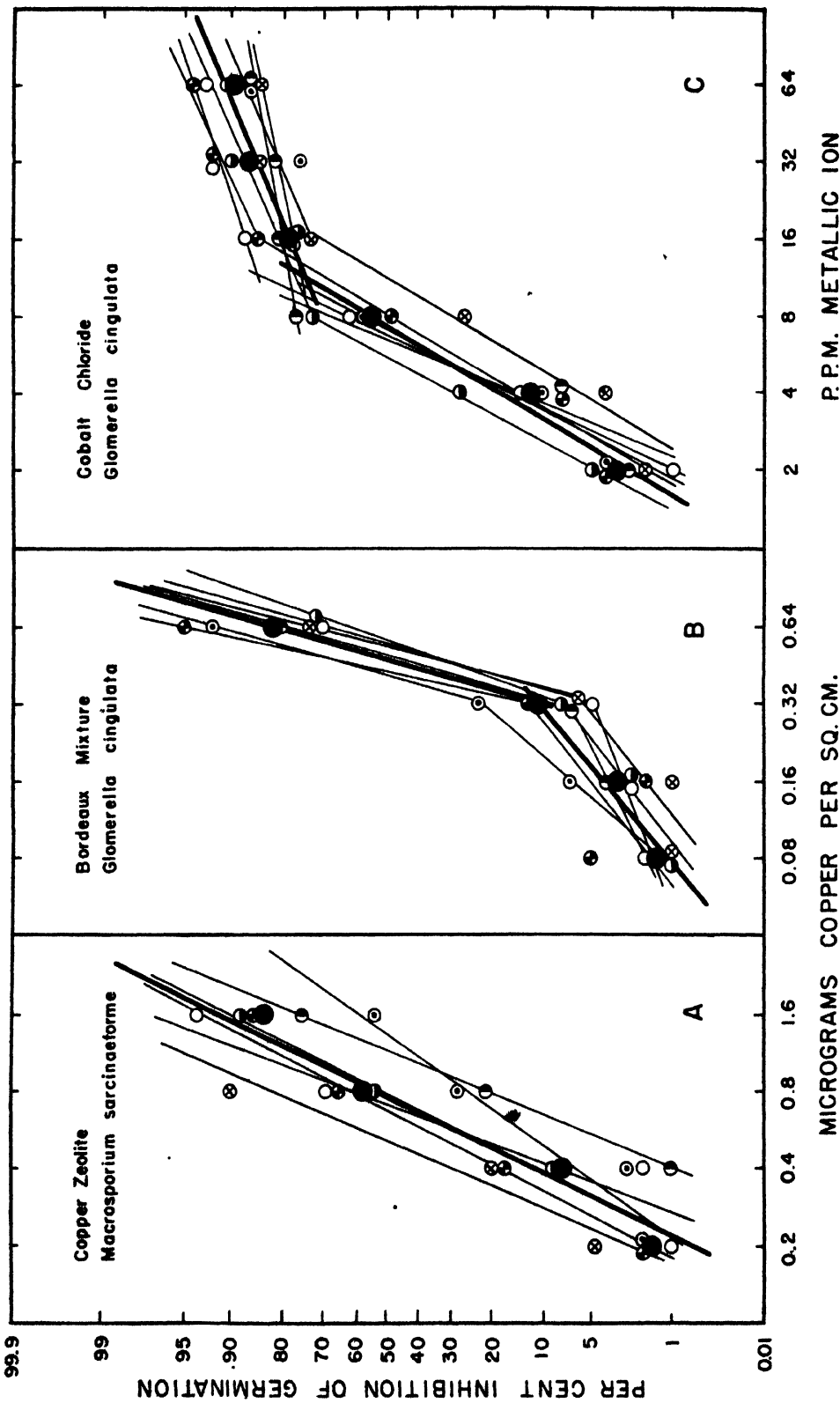


FIGURE 1. Types of toxicity curves plotted on logarithmic probability paper. A. Straight line. B. Double slope, curvature concave upwards. C. Double slope, curvature convex upwards. Different kinds of small circles and light lines indicate different replicate tests, large black circle and heavy line mean of all tests.

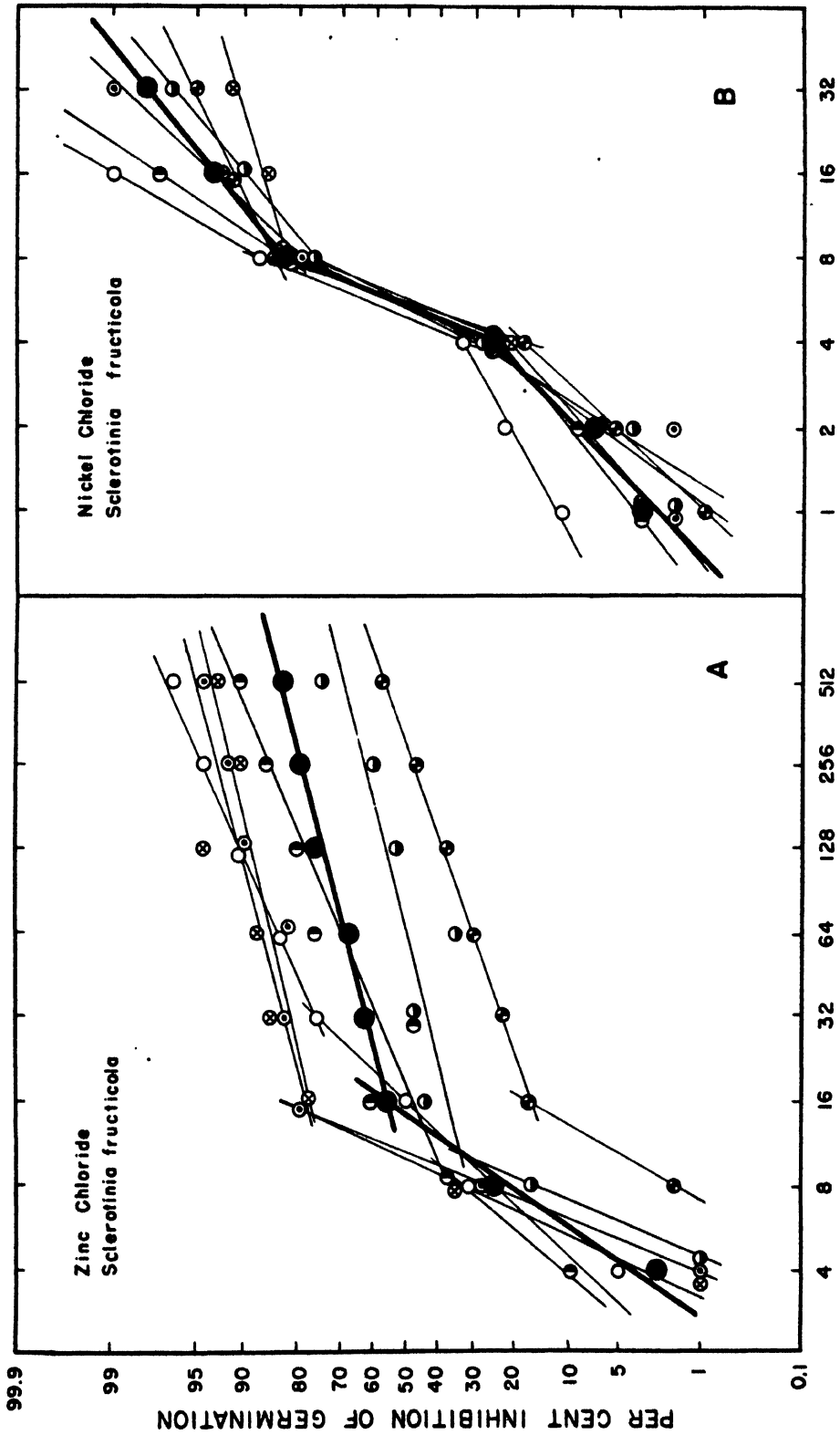


FIGURE 2. Types of toxicity curves plotted on logarithmic probability paper. A. Double slope, curvature convex upwards. B. Triple slope or sigmoid curve. Different kinds of small circles and light lines indicate different replicate tests, large black circle and heavy line mean of all tests.

moid curve. Replicated tests of a given compound and fungus gave rise to the same type of slope.

The distribution of the types for the 100 compound-fungus combinations are shown in Table I, where it may be seen that a particular type appears to be associated more with the compound than with the fungus. For example, copper zeolite and the organic compounds studied give straight lines, copper sulphate and Bordeaux tend to give concave slopes, and cobalt and zinc chlorides convex slopes. The sigmoid type was found only in the case of nickel chloride on *Sclerotinia fructicola* and *Glomerella cingulata*. In no case did both concave and convex types result from the action of the same compound on different fungi. It is possible that further study of some curves now defined as straight lines would show them to be of one of the other types.

TABLE I

TYPES OF SLOPES* FOR TOXICITY CURVES OF DIFFERENT COMPOUNDS AND FUNGI WHEN PROBITS ARE PLOTTED AGAINST LOGARITHM OF DOSE

Compounds	<i>Alternaria solani</i>	<i>Glomerella cingulata</i>	<i>Macrosporium sarcinaeforme</i>	<i>Sclerotinia fructicola</i>	<i>Botrytis cinerea</i>	<i>Rhizopus nigricans</i>
Silver sulphate	Concave	Straight	Straight	Concave		Straight
Copper sulphate	Concave	Concave	Concave	Concave		Concave
Mercuric chloride	Concave	Concave	Straight	Concave		Concave
Cadmium chloride	Straight	Straight	Straight	Straight		Straight
Nickel chloride	Convex	Sigmoid	Convex	Sigmoid		Straight
Cobalt chloride	Convex	Convex	Convex	Convex		Straight
Zinc chloride	Convex	Convex	Convex	Convex		Straight
Copper oxychloride	Straight	Straight	Straight	Straight	Straight	
Copper zeolite	Straight	Straight	Straight	Straight	Straight	
Cuprous oxide	Concave	Straight	Straight	Concave	Straight	
Bordeaux mixture	Concave	Concave	Straight	Concave	Concave	
Copper hydroxide	Straight	Straight	Convex	Straight	Straight	
Copper amm. silicate	Straight	Convex	Convex	Straight	Straight	
Basic copper sulphate	Straight	Straight	Straight	Convex	Straight	
Copper phosphate	Straight	Straight	Straight	Convex	Straight	
All 5 organic compounds	Straight	Straight	Straight	Straight		Straight

* Concave or convex upwards.

The use of the methods of Bliss and associates (2, 3, 4, 17) for calculating LD₅₀ values, assumes that the toxicity curve can be fitted best by a straight line, at least over the greater part of its length. However, Bliss (2) has given examples in which the lower portion of the toxicity curve is on another flatter slope, i.e. concave type. In the data examined, 83 per cent of the curves were of the straight line or concave type. Such curves present no theoretical difficulties and can be evaluated reasonably in terms of the LD₅₀ and reciprocal of the slope. However, the remaining 17 per

cent with well defined "breaks" in the upper dosages present particular difficulties. In these cases the LD₅₀ is based on a range of doses which are of no practical interest in the evaluation of fungicides. In order to rate fungicides with slopes flattened at the top it will be necessary to compare at the higher values, such as the LD₉₅.

The toxicity curve for zinc chloride on *Sclerotinia fructicola* is especially noteworthy for the break is in the range of the 50 per cent point. In some tests the LD₅₀ is on one slope and in others on the second as may be seen in Figure 2 A. Preliminary results indicate that zinc nitrate and zinc sulphate will produce the same type of slope on *S. fructicola*, but the slope with zinc acetate is a simple straight line. It is possible that in the case of zinc salts of strong acids there is an antagonistic action between the zinc and hydrogen ions resulting in a changed distribution of resistances above a certain concentration. An adequate explanation for these double and triple slopes cannot be given at this time, but their presence is evidence that all toxicity curves cannot be fitted by a single straight line on logarithmic probability paper.

STEEPNESS OF SLOPE

Since it is probable that differences in slope of the toxicity curves indicate difference in mode of action it was deemed of interest to compare the reciprocals of the logarithms of the slope of the straight line and concave curves. However, it is necessary, first, to establish that each compound-fungus can be characterized by a single slope. The detailed data on copper sulphate and Bordeaux mixture were analyzed by the methods of Miller, Bliss, and Braun (17) as regards homogeneity of slopes replicated at the same time with the same lot of spores and at different times with different lots of spores. For all four fungi 29 pairs of curves were available for the "within day" or same spores comparison, and only four of these gave a probability less than 0.05. The total n and χ^2 for copper sulphate was 13 and 16.347, a very reasonable probability, while for Bordeaux, the corresponding values were 16 and 31.732. If the one outstandingly high value is omitted ($P < 0.01$) n becomes 15 and χ^2 22.083, giving a reasonable probability. Thus it is concluded that in tests replicated with the same lot of spores, the variation of slopes are no greater than would be expected from the deviations of the individual points from the line. The χ^2 test applied to the slopes of curves replicated with different lots of spores indicates, however, as may be seen in Table II, some lack of homogeneity. This apparent inconsistency may be attributed at least in part to the fact that copper sulphate and Bordeaux mixture give concave curves. When comparing replicates with different lots of spores it is not always possible to work at exactly the same upper level on the curve.

The frequency distribution of the mean λ values (reciprocals of loga-

rhythms of slope) for the six replications of the straight line and concave type slopes obtained graphically is given in Figure 3. The distribution is seen to be definitely skew. The organic compounds and silver sulphate and mercuric chloride have the lowest λ values or steepest slopes, while Bordeaux mixture, cuprous oxide, copper zeolite, copper sulphate, cadmium and nickel chlorides are intermediate in slope. The remaining copper fungicides are distributed over a very wide range and account for much of the skewness.

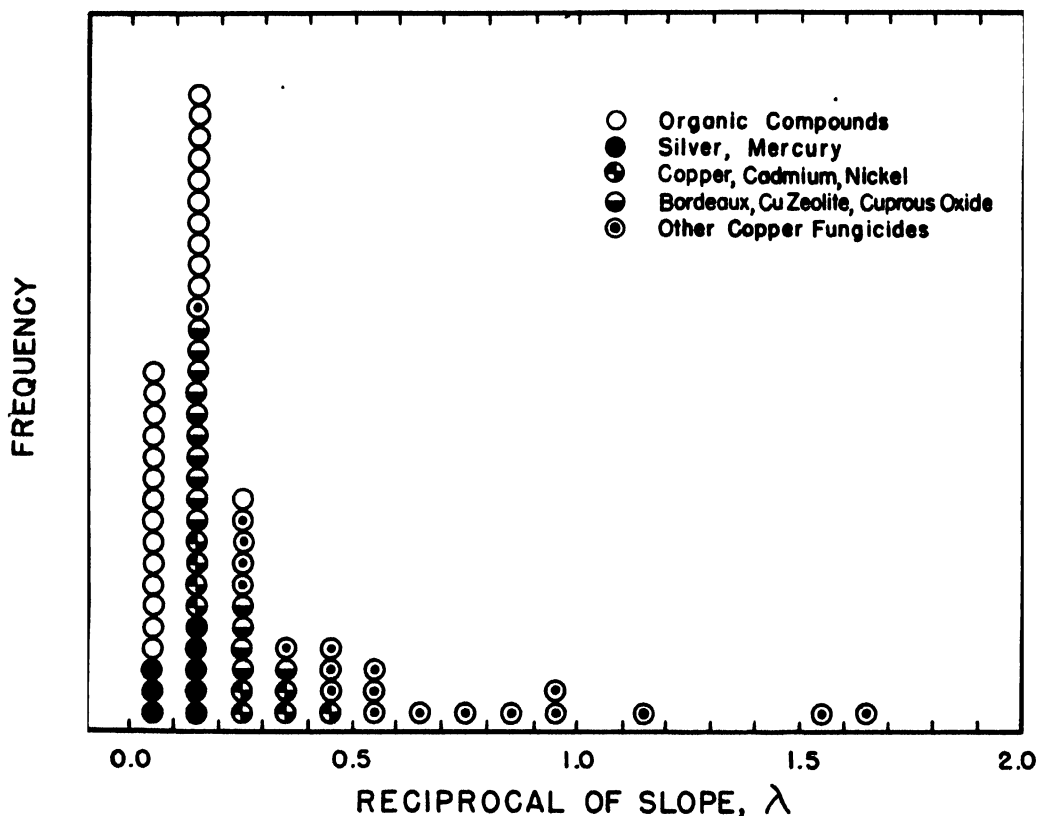


FIGURE 3. Frequency distribution of reciprocals of slope (λ), of toxicity curves for various compounds. Each circle represents mean of six replicate tests for a given fungus and compound.

The two highest values are the curves of copper oxychloride on *Botrytis cinerea* and *Alternaria solani*. Although the slope of their toxicity curves indicates that the copper fungicides may be classified into two groups, namely, Bordeaux mixture, cuprous oxide, and copper zeolite on the one hand, and the basic copper sulphate, hydroxide, oxychloride, phosphate, and silicate on the other, their chemical composition does not suggest any such division.

In order to determine whether the difference in steepness of slope of the toxicity curves was associated mainly with the compounds or with the fungi, a χ^2 test for homogeneity was performed on the λ^2 values (20, p.

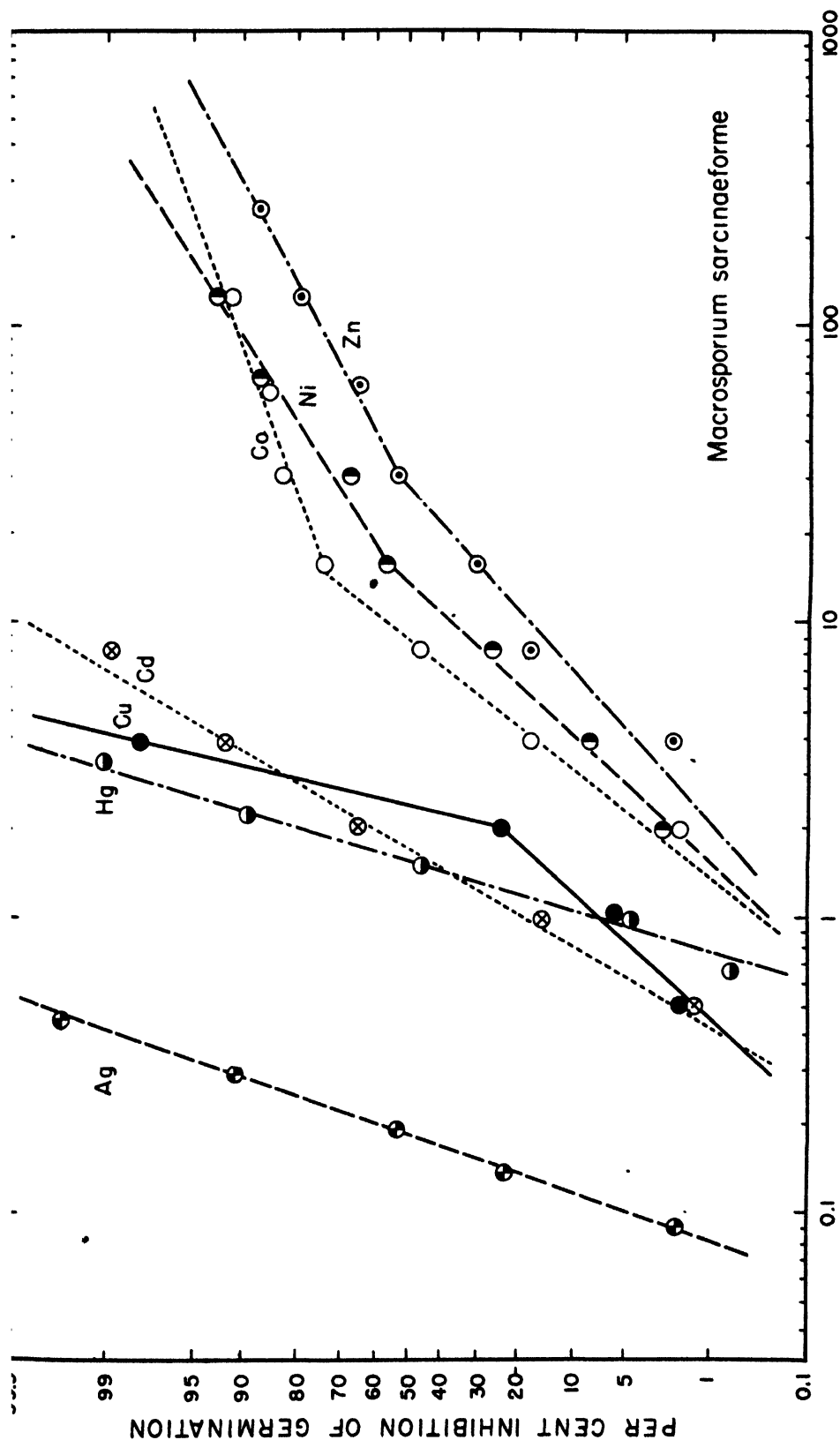


FIGURE 4. Toxicity curves of heavy metals on *Macrosporium sarcinaeforme*. Note that relative toxicity of the metals varies at the different inhibition levels due to changes in slope of the curves. Each point mean of six replicate tests.

196). Tests made within a given fungus for all compounds gave a total χ^2 of 450.681 with n equal to 74, while tests within a given compound for all fungi with a total n of 63 gave a χ^2 of 75.376. This shows that the slope of the toxicity curve is determined mainly by the compound, and that the fungi do not differ significantly in steepness of slope of toxicity curves.

CORRELATION OF SLOPE AND LD₅₀

Correlation coefficients were determined for the λ and logarithmic LD₅₀ values of the toxicity curves of the straight line and concave types. The calculations were based on the mean of the six replications as determined graphically. *Rhizopus nigricans* was omitted from the heavy metal data because, as will be shown later, it is more sensitive than the other fungi and has a lower level of LD₅₀ values. The coefficient r for the heavy metals and copper fungicides was respectively 0.6195 and 0.6746; both of these are highly significant. The organic compounds studied differ little in slope and no significant correlation could be obtained. Individual curves for a given fungus, *Macrosporium sarcinaeforme*, are illustrated in Figure 4. The convex type slopes were not included in the above correlation, but as shown in Figure 4, they would substantiate this correlation.

VARIATION OF REPLICATE TESTS

COMPARISON WITH INTERNAL ERROR

It has been shown (15) that there exists between tests replicated on different days, that is with different lots of spores, an error greater than that of random sampling. Thus it is of interest to determine if the between day variation is greater than would be expected from the internal error of the individual curves or LD₅₀s or from tests replicated on the same day, i.e. with the same lot of spores. If the tests on the same day have been completely replicated as regards preparation and application of the fungicide dose, and are found to vary no more than expected from their internal error than the replicate test, the variation probably cannot be ascribed to errors in technique or dose. However, if the between day variation is greater than the within day variation, the former must be due in the main to the use of different lots of spores.

χ^2 test. In order to investigate this question the special tests with copper sulphate and Bordeaux mixture were designed. Two stocks of each material differing by 25 per cent were freshly prepared and applied by the two techniques (test tube method for copper sulphate and settling tower for Bordeaux mixture). The same lot of spores was used to test both stocks. On another day the procedure was repeated and of necessity a different lot of spores was used. The between day tests were replicated four times except for Bordeaux mixture on *Sclerotinia fructicola*, *Alternaria solani*, and *Macrosporium sarcinaeforme* where five replications were obtained.

TABLE II

SUMMARY OF WITHIN AND BETWEEN DAY χ^2 TESTS ON LOGARITHMS OF LD₅₀ AND SLOPE FOR COPPER SULPHATE AND BORDEAUX MIXTURE

	Within day	<i>n</i>	χ^2
LD ₅₀	All fungi—Copper sulphate	13	28.829
	Omit 2 highest values	11	17.198
	All fungi—Bordeaux mixture	17	64.800
	Omit 2 highest values	15	24.144
Slope	All fungi—Copper sulphate	13	16.347
	All fungi—Bordeaux mixture	16	31.732
	Omit highest value	15	22.083
	Between days		
LD ₅₀	<i>Alternaria solani</i> Copper sulphate	3	126.352
	<i>Glomerella cingulata</i> " "	2	14.318
	<i>Macrosporium sarcinaeforme</i> " "	1	12.602
	<i>Sclerotinia fructicola</i> " "	3	4.259
	<i>Alternaria solani</i> Bordeaux mixture	3	42.295
	<i>Glomerella cingulata</i> " "	3	427.058
	<i>Macrosporium sarcinaeforme</i> " "	4	1012.857
	<i>Sclerotinia fructicola</i> " "	3	137.664
	<i>Alternaria solani</i> Copper sulphate	2	10.408
	<i>Glomerella cingulata</i> " "	1	0.532
Slope	<i>Macrosporium sarcinaeforme</i> " "	1	4.522
	<i>Sclerotinia fructicola</i> " "	2	6.387
	<i>Alternaria solani</i> Bordeaux mixture	1	6.527
	<i>Glomerella cingulata</i> " "	2	36.123
	<i>Macrosporium sarcinaeforme</i> " "	4	7.802
	<i>Sclerotinia fructicola</i> " "	3	31.043

LD₅₀ values were calculated by the method of Bliss (3) and the χ^2 test (17) applied as described under the slope discussion.

Due to the fact that copper sulphate and Bordeaux mixture have concave slopes it was not always possible, with a dose ratio of 1.414, to obtain three or more points on the upper slope and hence all the data were not available for the χ^2 test. The results of this test are given in Table II. About 52 per cent of the individual toxicity curves had reasonable χ^2 values, i.e. *P* greater than 0.05, but the total χ^2/n result was 5.3608. This value is in fair agreement with that of 3.440 which may be obtained from Table IV, as well as with a value of approximately 4 indicated in an earlier article (21, p. 337). Thus it is evident that in the laboratory testing of fungicides certain extraneous variables are present which tend to give an internal variance somewhat greater than that of random sampling alone. On the basis of the internal error, 22 out of 30 within day comparisons gave a reasonable χ^2 , i.e. *P* greater than 0.05. The total χ^2 values are unreasonable but if the two highest are omitted from both the copper

sulphate and Bordeaux data, the remainder are reasonable. The between day χ^2 values on the LD₅₀s are in all cases but one unreasonably high. Thus it is to be concluded that tests on the same day with the same lot of spores for the most part do not vary more than is to be expected from their internal error. But tests replicated on different days with different lots of spores depart greatly from the expected variation. Hence, the variation of tests replicated on different days is due in the main to the use of different lots of spores and not to errors in the dose of the fungicide.

TABLE III

ANALYSIS OF VARIANCE ON LOGARITHMIC LD₅₀ VALUES OF COPPER SULPHATE AND BORDEAUX MIXTURE. TWO DIFFERENT STOCKS OF EACH SEPARATELY PREPARED AND APPLIED BUT TESTED WITH SAME LOT OF SPORES

	D.F.	Copper sulphate		Bordeaux mixture	
		Variance	Significance	Variance	Significance
Compounds (stocks)	1	0.00009	No/CF	0.00383	No
Fungi	3	0.01054	No/CF	0.07480	No
Replicate tests	3	0.01274	No/CT	0.07762	No
Compounds×Fungi	3	0.00679	No/CFT	0.00081	No
Compounds×Tests	3	0.00800	No/CFT	0.00932	Sign.
Fungi×Tests	9	0.02698	High/CFT	0.06675	High
Compounds×Fungi×Tests	9	0.00258		0.00183	
Within fungi					
Compounds	4	0.00511	No/CTW	0.00156	No
Replicate tests	12	0.02342	High/CTW	0.06947	High
Compounds×Tests	12	0.00394		0.00370	

Error terms for Tables III, V, and VI: CF = Compounds×Fungi; CT = Compounds×Tests; CFT = Compounds×Fungi×Tests; CTW = Compounds×Tests Within Fungi. Significance: High, odds greater than 100:1; Sign., between 100:1 and 20:1; No, less than 20:1.

Analysis of variance of LD₅₀ values. In order to obtain further information on the magnitude of the internal and within and between day errors, an analysis of variance was performed on the logarithms of the LD₅₀ values for the special copper sulphate and Bordeaux mixture data. A separate analysis was made for each material on a complete block of four replicated "between day" tests on all four fungi. The results are shown in Table III. The "within day" effect appears under the heading compounds or stocks, and "between day" under replicate tests. It will be seen that within fungi the replicate tests are highly significant while the "within day" or stock effect is non-significant. In the general analysis no significant replicate test effect for all the fungi together can be demonstrated over the fungus×replicate test interaction. This is further evidence that the so-called day-to-day variation is associated with a particular lot of spores. On a given day there is no general error of technique affecting all the different fungi so that the sensitivity of all is lowered or raised.

The replicate test variation within fungi for Bordeaux mixture is seen to be significantly greater than that for copper sulphate. This is to be expected in view of the significant test variation demonstrated for the settling tower technique in the second paper of this series (16). The χ^2 test shown in Table II also indicated a similar difference.

Presumably the triple interaction, compounds $\times$ fungi $\times$ replicate tests, should be equivalent to the basic error or internal error of the individual curves. The pooled variance from the two triple interactions is 0.002208, while the weighted mean variance of the LD₅₀s of the individual toxicity curves is 0.002192. This is an excellent agreement, though identical data were not used in the two calculations, the former being based on a complete block of four replicate tests and the latter only on these curves providing three or more finite values on the second slope.

HEAVY METALS, COPPER AND ORGANIC COMPOUNDS

In the laboratory testing of fungicides it is frequently necessary to compare somewhat heterogeneous materials so that an analysis on the replicate tests within the following groups, heavy metals and insoluble copper and organic compounds, is desirable.

Comparison of graphic with calculated method of obtaining LD₅₀ values. The large amount of data involved and the "broken" and steep slopes which in many cases did not provide three or more finite values for calculating the LD₅₀, necessitated a simple method of obtaining these values. A comparison was made of the LD₅₀ values obtained by the method of Bliss (3) with a rapid graphic method on ten curves chosen at random from the heavy metal data and ten from the copper fungicides. The graphic estimates were made prior to the precise calculations and the method was essentially that given in a previous paper (21, p. 333). The arithmetic values of λ and d were averaged and the resulting number was raised to the 0.07443 power.¹⁰ This gives the standard deviation of the LD₅₀ in logarithmic form. The logarithmic variance was obtained by squaring this number. If the value of χ^2 had a probability less than 0.05, the variance was multiplied by χ^2/n (21, p. 335).

¹⁰ Gaddum (8) has shown that for n individuals per dose, the variance of the log. LD₅₀ or $\sigma m^2 = K\lambda d/n$, d and λ being expressed in logarithmic form. If the slope of the characteristic curves is exactly known $K = 1/\{S(w)(d/\lambda)\}$. Irwin (11, p. 27) has calculated from tables of the probability integral for different values of d/λ that K is very nearly constant and is equal to 0.554. Hence, if d and λ are substantially equal and in arithmetic form, for

100 individuals, the $\sigma_{LD_{50}} = \frac{d + \lambda}{2}$ raised to the power of $\sqrt{.554/n}$ or 0.07443. The table of factors (21) for the 19/20 zones of error for the LD₅₀, was prepared by raising the values of various numbers to a power whose exponent is 1.96×0.07443 or 0.146. In the case of the LD₉₅, K was empirically determined to be 1.76, hence the exponent for the 19/20 zone table becomes $\sqrt{1.76/100} \times 1.96$ or 0.260.

TABLE IV
COMPARISON OF GRAPHIC WITH CALCULATED METHOD OF OBTAINING χ^2 , LD₅₀,
AND VARIANCE OF LD₅₀

Fungus	Compound	n	χ^2		Log. LD ₅₀ *		LD ₅₀ variance	
			Calc.	Graph	Calc.	Graph	Calc.	Graph
<i>Alternaria solani</i>	Cadmium chloride	1	0.511	0	0.718	0.716	.00071	.00050
	Nickel chloride	1	0.011	0.1	0.440	0.439	.00047	.00041
	Copper hydroxide	2	3.438	0.8	0.533	0.477	.00215	.00224
	Copper silicate	2	1.195	1.2	0.378	0.322	.01257	.01402
<i>Glomerella cingulata</i>	Silver sulphate	2	7.167	10.5	0.345	0.322	.00072	.00121
	Zinc chloride	1	4.699	7.3	1.013	0.982	.00216	.00314
	Bordeaux	1	11.637	13.0	0.811	0.845	.00630	.00483
	Copper zeolite	1	1.240	1.4	0.305	0.301	.00027	.00028
<i>Macrosporium sarcinaeforme</i>	Cobalt chloride	1	1.364	0.8	0.765	0.771	.00040	.00036
	Nickel chloride	3	6.326	5.1	1.483	1.477	.00060	.00092
	Basic Cu sulphate	2	20.897	38.1	0.552	0.544	.00468	.00647
	Cuprous oxide	1	0.007	0	1.010	1.000**	.00249	.00054
<i>Sclerotinia fructicola</i>	Copper sulphate	1	2.331	1.7	0.296	0.301	.00031	.00059
	Cadmium chloride	1	5.872	5.8	0.441	0.431	.00158	.00186
	Basic Cu sulphate	1	1.370	3.0	0.446	0.447	.00065	.00039
	Cuprous oxide	1	7.450	7.2	0.590	0.591**	.01680	.00477
<i>Botrytis cinerea</i>	Copper hydroxide	2	0.322	1.2	1.223	1.210**	.01227	.00095
	Copper zeolite	1	0.089	0	0.532	0.532	.00052	.00045
<i>Rhizopus nigricans</i>	Cobalt chloride	1	6.712	6.0	0.350	0.373	.00222	.00186
	Silver sulphate	2	13.679	15.0	0.425	0.423	.00180	.00150

* Units differ for different compounds.

** Estimated by extrapolation.

The comparison of calculated and graphic values for logarithms of LD₅₀, χ^2 , and variance of LD₅₀ are shown in Table IV. In general, the agreement between calculated and graphic values was reasonably close. The difference between the calculated and graphic logarithms of LD₅₀ was in all cases less than the standard deviation of the calculated. Ten of the graphic χ^2 values were somewhat greater than the calculated and ten somewhat less. The calculated and graphic variances agreed fairly well except for the three cases in which the LD₅₀ was estimated by extrapolation. With these exceptions, nine of the graphic variances exceeded somewhat the calculated and eight were less, while the mean difference between calculated and graphic expressed as per cent of calculated was 26.1. Hence it is concluded that under these conditions there is no significant difference between the graphic and calculated methods of obtaining LD₅₀ values. The calculated and graphic variances of LD₅₀ are in reasonably close agreement, provided the LD₅₀ is near the middle range of doses. Thus the use of the rapid graphic method is recommended for routine fungicidal tests.

Cochran (5) has shown that an analysis of variance on groups of experi-

ments differing in precision may give misleading results. A χ^2 test was made on the homogeneity of the graphic LD₅₀ variances of the copper fungicide series. The results indicated that these experiments did not differ essentially in precision; thus it is believed that the analysis of variance can be appropriately applied to the data of this study.

Analysis of variance on graphic LD₅₀ values. An analysis of variance was performed on the logarithms of the graphic LD₅₀ values of the straight line and concave type curves. The data were segregated into the compounds having somewhat similar slopes. The results on silver and mercury, and copper and cadmium are shown in Table V, while those for Bordeaux mixture, cuprous oxide, and copper zeolite, and the organic compounds appear in Table VI. The approximate values for the slopes of these compounds may be seen in Figure 3.

TABLE V
ANALYSIS OF VARIANCE ON LOGARITHMIC LD₅₀ VALUES OF SILVER SULPHATE AND MERCURIC CHLORIDE, AND COPPER SULPHATE AND CADMIUM CHLORIDE

	D.F.	Silver and mercury		Copper and cadmium	
		Variance	Significance	Variance	Significance
Compounds	1	18.1390	High	0.4770	No
Fungi	4	1.2716	Sign.	2.1057	No
Replicate Tests	5	0.0701	No	0.0459	No
Compounds × Fungi	4	0.1855	High	0.4441	High
Compounds × Tests	5	0.0536	High	0.0187	No
Fungi × Tests	20	0.0227	High	0.0045	No
Compounds × Fungi × Tests	20	0.0072		0.0171	
Within fungi					
Compounds	5	3.7762	High	0.4506	High
Replicate Tests	25	0.0340	Sign.	0.0128	No
Compounds × Tests	25	0.0164		0.0174	

TABLE VI
ANALYSIS OF VARIANCE ON LOGARITHMIC LD₅₀ VALUES OF BORDEAUX MIXTURE, CUPROUS OXIDE AND COPPER ZEOLITE, AND ORGANIC COMPOUNDS

	Copper fungicides			Organic compounds		
	D.F.	Variance	Significance	D.F.	Variance	Significance
Compounds	2	7.2052	High	4	12.9812	High
Fungi	4	0.1683	No	4	1.7767	Sign.
Replicate Tests	5	0.0070	No	5	0.0284	No
Compounds × Fungi	8	0.1475	High	16	0.4965	High
Compounds × Tests	10	0.0107	No	20	0.0491	High
Fungi × Tests	20	0.0229	High	20	0.0486	High
Compounds × Fungi × Tests	40	0.0059		80	0.0178	
Within fungi						
Compounds	10	1.5590	High	20	2.9935	High
Replicate Tests	25	0.0197	High	25	0.0446	High
Compounds × Tests	50	0.0068		100	0.0240	

These two tables substantiate the analyses on copper sulphate and Bordeaux mixture in that no general replicate test effect for all fungi on a given day can be demonstrated, while within fungi, i.e. for any particular fungus, there is a definite day-to-day variation except in the case of copper and cadmium. That is, on a particular day there is no general error such as variations in temperature or preparation of the fungicidal deposit that would tend to affect all the different fungi more or less alike. Rather, the replicate test variation is associated with the particular spores of a given species used for the test on that day. Graphic examples of replicate test variation may be seen in Figures 1 and 2.

Since it has been shown that there is in general a highly significant replicate test variation within fungi, the replicate test variances for the different fungi and compounds have been assembled and tested for homogeneity by the χ^2 method (20, p. 196). A χ^2 value of 125.030 with n of 59 was obtained showing that the replicate test variances were clearly not homogeneous. This lack of homogeneity appeared to reside in 20 per cent of the data which when omitted gave a χ^2 of 61.566 with n of 47 for the remaining 80 per cent. An examination of these unusually high replicate test variances showed that 10 out of the total of 12 were associated with *Rhizopus nigricans* or the organic compounds. Hence it is probable that replicate tests with *R. nigricans* and some of the organic compounds are not as reproducible as the other fungi or compounds.

CONTRIBUTION OF SPORE SUSPENSION ERROR

The technique of adjusting spore suspension concentrations used in this and former studies (15, 16, 21) was, following the centrifuging and decanting, to make up a temporary dilution from the stock suspension and from this dilution to make spore counts in a Fuchs-Rosenthal cell of three separate samples. From the results of these three counts the necessary dilutions are made in the stock suspension to give an estimated 50,000 spores per cc. in the final drops.

Distribution of spores in drops. In order to determine the actual distribution of the spores following this procedure, 16 pairs of drops were pipetted onto glass slides. After settling, counts were made of the actual number of spores present in a low power field (15 $\times$ ocular, 16 mm. objective) focused on the center of each pair of drops. Each test was replicated on four different days, thus using four different lots of spores and stock suspensions. Measurements of the number of drops from various 2 cc. pipettes gave a mean volume of 0.0436 cc. per drop, with a mean diameter on glass slides of 10.4 mm. The microscopic field was 1.3685 square mm. in area. Hence 50,000 spores per cc. would be expected to give approximately 35 spores per field, or 70 spores for two fields. An analysis of the results of these data appears in Table VII. It will be seen that for each fungus there is a highly

significant variation in counts made from different stock suspensions (replicate tests) as compared to counts on the same stock (within tests). In an ideal suspension it may be assumed that the spores would follow a Poisson distribution and hence the variance would equal the mean; whereas in Table VII the variance is three to six times the mean. This failure to achieve an ideal distribution of spores in drops for a given test probably contributes to the high χ^2/n internal error of an individual toxicity curve discussed previously.

TABLE VII

VARIATION IN NUMBER OF SPORES DEPOSITED ON GLASS SLIDES FROM SUSPENSION
ESTIMATED TO CONTAIN 50,000 PER CC. THEORETICALLY EXPECTED PER
TWO MICROSCOPIC FIELDS 70

Fungus	Variance		Mean number per 2 fields	Standard error replicate tests based on mean of 70 spores
	Replicate tests 3 D.F.	Counts within tests 60 D.F.		
<i>Alternaria solani</i>	4598.30	330.78	89.4	14.5
<i>Glomerella cingulata</i>	9885.30	324.93	106.2	20.1
<i>Macrosporium sarcinaeforme</i>	2192.00	256.40	40.2	15.4
<i>Sclerotinia fructicola</i>	2055.68	212.05	73.6	11.1

Relation of spore number to LD₅₀. Early studies (12) indicated a linear relation between the number of spores in suspension and the concentration of copper sulphate for a given per cent germination of *Sclerotinia (americana) fructicola*. A determination was made of this relation with respect to copper sulphate and Bordeaux mixture on the spores of *S. fructicola*, *Alternaria solani*, *Glomerella cingulata*, and *Macrosporium sarcinaeforme*. Spore concentrations of 25,000, 50,000, and 100,000 per cc. were prepared by the above method and toxicity curves obtained with a dose ratio of 1.414. The procedure was replicated at another time with a different lot of spores. Graphic LD₅₀ values were obtained by plotting on logarithmic probability paper. The logarithmic LD₅₀s were plotted against the logarithm of the spore concentration and a linear relation was found as is illustrated in Figure 5.

Contribution of spore suspension error to replicate test variation. An estimate of the error in LD₅₀ values to be expected from the errors in different lots of spore suspension was obtained by graphic projection on the spore number-LD₅₀ curves illustrated in Figure 5. The standard error of different lots of spore suspension based on three counts and a mean of 70 spores was derived from Table VII for each fungus and projected by means of the appropriate spore number-LD₅₀ curve to the LD₅₀ axis. Thus 8 standard deviations of the LD₅₀ were obtained which could not be shown by the χ^2 test for homogeneity of variances to differ significantly for fungus or compound. The mean, 0.000734, provides an estimated variance for repli-

cate tests within fungi due to the errors in spore suspension. When this variance, based on 12 degrees of freedom, is compared to that of 0.02342 and 0.06947 from Table III for copper sulphate and Bordeaux mixture respectively, it will be seen to contribute a very small amount to the total variance of replicate tests. However, this error as well as that of the individual toxicity curve could be reduced by making counts on more samples of the stock suspension for a given test.

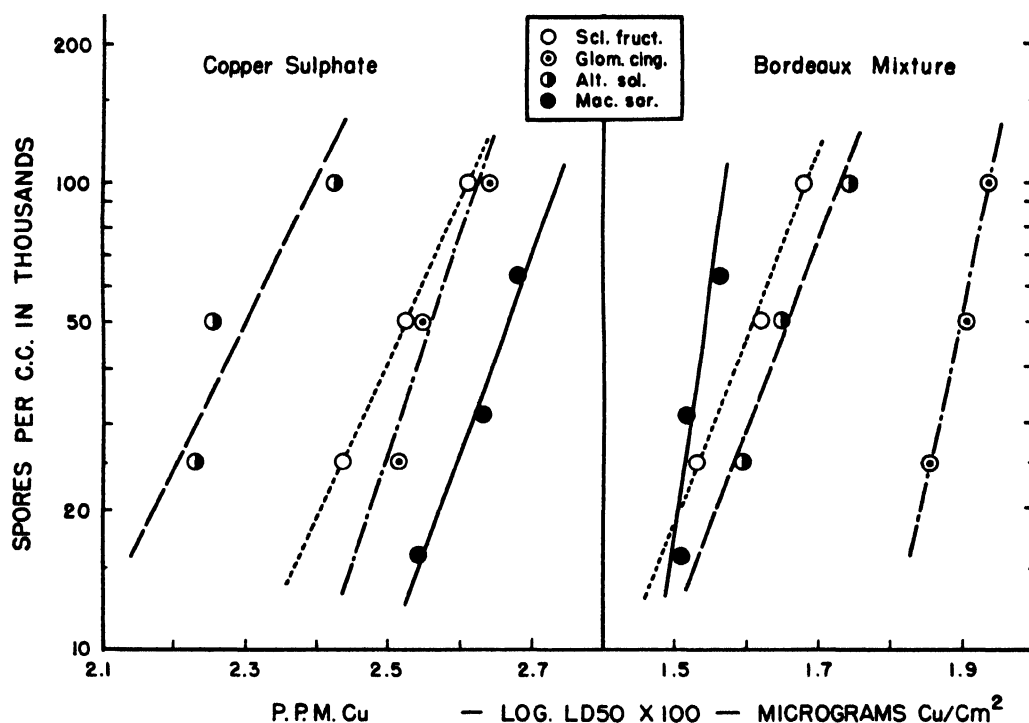


FIGURE 5. Linear relation between logarithm of LD₅₀ and of density of spore suspension for copper sulphate and Bordeaux mixture on *Sclerotinia fructicola*, *Glomerella cingulata*-*Alternaria solani*, and *Macrosporium sarcinaeforme*.

USE OF A STANDARD FUNGICIDE

The use of a standard fungicide to correct the replicate test or day-to-day variation was discussed in an earlier article (21). It has been suggested by Horsfall *et al.* (10) that every lot of spores has a particular "resistance level," and hence it is to be expected that differences in response among different lots may be adjusted by means of a standard fungicide (10, 21). In pharmacological assay the use of a standard is universal and for a valid comparison, as has been pointed out by Bliss and Marks (4, p. 186), the slopes of the standard and unknown must be substantially parallel. If the slopes are not parallel the response of a given lot of spores to the standard and unknown will vary in an irregular manner from test to test thus pro-

ducing a significant interaction of compounds and replicate tests as illustrated in Figure 6.

If the data in Tables III, V, and VI are examined, it will be seen that there is a significant replicate test effect within fungi in every case except copper and cadmium. The use of a standard would, in general, tend to correct for the replicate test effect within fungi and would be of some value in all cases examined except copper and cadmium. Here it would neither increase nor decrease the precision since there is neither a significant test

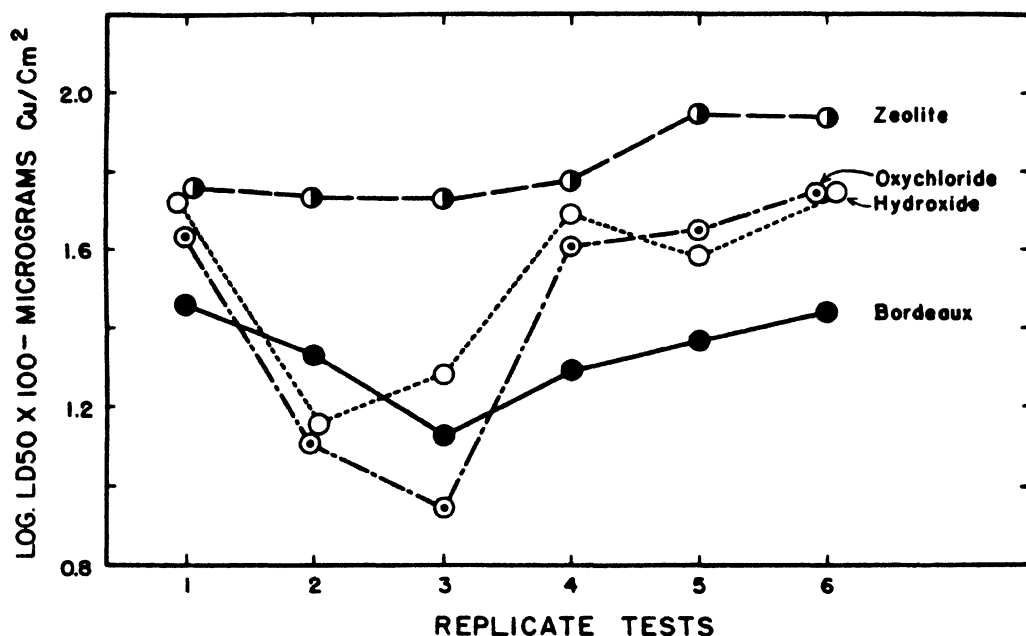


FIGURE 6. Example of compound $\times$ replicate test interaction. Spores of *Sclerotinia fructicola*. Note crossing of lines of Bordeaux and copper zeolite with copper hydroxide and oxychloride. The two former having similar toxicity curve slopes tend to respond in a parallel manner as do likewise the latter pair.

effect nor a significant compound $\times$ test interaction. The benefits to be derived from the use of a standard in correcting the test effect of any particular compound are determined by the significance of the compound $\times$ test interaction. In the case of similar compounds such as the two stocks of copper sulphate, or of Bordeaux mixture and the two copper fungicides there is little or no significant interaction of compounds and replicate tests, while with unlike compounds such as silver and mercury and the organic series there is a highly significant interaction of compounds and replicate tests, even though the slopes of the compounds within the two analyses differ but little.

A standard fungicide may be employed as a check on the reproducibility of the technique and for the purpose of orientation in preliminary tests of new compounds. Likewise a standard may be used effectively to adjust the replicate test variation provided the compounds are essentially similar

in chemical composition and slope. Wherever the compound $\times$ replicate test interaction is significant, as is the case for a series of heterogeneous compounds unlike in composition and slope, the use of a standard cannot be expected to compensate for this interaction but only for the test effect within fungi. Thus for such a heterogeneous series the usefulness of a standard is limited.

COMPARISON OF FUNGI MEAN SENSITIVITY

A comparison of the mean sensitivity at the LD₅₀ value of the fungi to the various groups of compounds, analyzed in Tables V and VI, is given in Table VIII. The mean LD₅₀ values for *Macrosporium sarcinaeforme* were adjusted to a basis of 50,000 spores per cc. by reference to their spore suspension-LD₅₀ curve discussed above, in order to make them comparable with the other fungi. By means of the *t* test, using the fungus $\times$ compound interaction as error, only *Rhizopus nigricans* can be shown to be significantly more sensitive than the other fungi.

TABLE VIII
MEAN LD₅₀ VALUES AND SPORE VOLUME

Fungus	Heavy metals Log. p.p.m. $\times 100$	Copper fungi- cides Log. micrograms Cu./cm. ² $\times 100$	Organic com- pounds Log. p.p.m. $\times 10$	Approximate spore volume Cubic μ
<i>Macrosporium sarcinaeforme</i>	1.224	2.089	3.094	8170.8
<i>Alternaria solani</i>	1.265	1.922	2.839	619.0
<i>Sclerotinia fructicola</i>	1.148	1.873	2.843	717.9
<i>Botrytis cinerea</i>	—	1.976	—	526.2
<i>Glomerella cingulata</i>	1.250	1.807	2.869	257.0
<i>Rhizopus nigricans</i>	0.751	—	2.377	940.9

Relation of spore volume. Data have been presented by Horsfall *et al.* (10) indicating that spore sensitivity is inversely proportional to spore volume. Measurements were made on 50 spores chosen at random, from each of the fungi studied. The mean dimensions in μ for length and width together with their standard errors were as follows: *Macrosporium sarcinaeforme* 28.16 ± 0.66 , 23.54 ± 0.51 ; *Sclerotinia fructicola* 15.00 ± 0.35 , 9.56 ± 0.07 ; *Botrytis cinerea* 11.72 ± 0.27 , 9.26 ± 0.19 ; *Rhizopus nigricans* 14.42 ± 0.80 , 11.16 ± 0.60 ; *Glomerella cingulata* 15.42 ± 0.21 , 4.87 ± 0.09 ; and *Alternaria solani* length 18.38 ± 0.88 , greatest width 8.22 ± 0.07 , least width 5.08 ± 0.01 . Assuming the shape of the first four to be prolate spheroids, that of *G. cingulata* to be a cylinder with hemispherical ends, and *A. solani* a frustrum with hemispherical ends, the approximate volumes were calculated in cubic μ and are given in Table VIII. The range in volume from the largest spore, *M. sarcinaeforme*, to the smallest, *G. cingulata*, is 32-fold, while *R. nigricans*, the one fungus significantly more sensitive, has the second largest spores. Thus under the conditions of these studies there

is no evidence that the average sensitivity of these six fungi to a number of compounds is inversely proportional to the spore volume.

RATING OF COMPOUNDS BY DIFFERENT FUNGI

Do different fungi rate the same compounds in like order? The compound $\times$ fungus interaction is a measure of the relative rating of the same com-

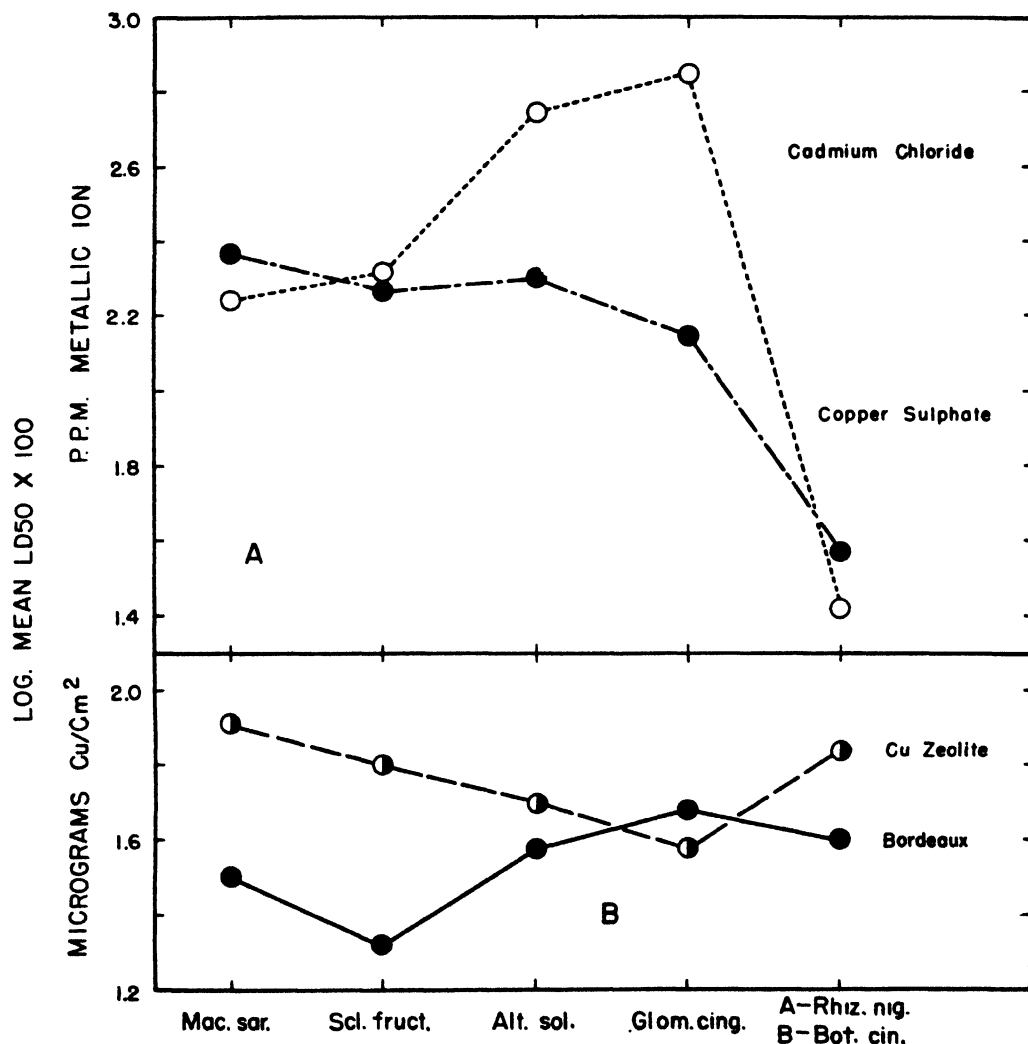


FIGURE 7. Examples of compound $\times$ fungus interaction. A. Heavy metals. B. Copper fungicides. *Macrosporium sarcinaeforme*, *Sclerotinia fructicola*, *Alternaria solani*, and *Glomerella cingulata* in both series. *Rhizopus nigricans* in A only and *Botrytis cinerea* in B only. Same scale as Figure 6.

pounds by different fungi. From Tables V and VI it may be seen that this interaction is highly significant. Thus different fungi do not rate the same compounds in the same order. Graphic examples of compound $\times$ fungus interaction are shown in Figure 7. In Figure 7 A, for instance, copper sulphate and cadmium chloride differ little in toxicity toward *M. sarcinae-*

forme, *S. fructicola*, and *R. nigricans*, but copper is much more toxic to *A. solani* and *G. cingulata* than is cadmium. Likewise in Figure 7 B the difference between Bordeaux and copper zeolite is pronounced for *M. sarcinaeforme*, *S. fructicola*, and *B. cinerea*, there is little difference for *A. solani*, and the order is reversed by *G. cingulata*.

CORRELATION OF FUNGI

Although the different fungi do not rate the compounds alike, it is perhaps possible that certain species are correlated. Correlation coefficients (6) were determined for the mean LD₅₀ values of compounds tested against all four of the fungi *S. fructicola*, *G. cingulata*, *A. solani*, and *M. sarcinaeforme*. In all cases the coefficients were highly significant except for *A. solani* and *M. sarcinaeforme* which was barely significant (r 0.588, n 10) and for *S. fructicola* and *A. solani* which was not significant (r 0.482, n 10). The highest correlation was in the case of *S. fructicola* and *M. sarcinaeforme* (r 0.929, n 10). It is to be noted that the most significant correlations do not follow generic relationships or similarities in size of spores. A further study of the data indicated that unusual responses were being produced by copper oxychloride. When the results with this compound were eliminated from the calculations, a highly significant correlation was found between all the fungi.

SUITABILITY OF DIFFERENT FUNGI AS TEST ORGANISMS

In the laboratory testing of fungicides it is necessary to select one or more test species of fungi. If one is interested only in a particular fungus or disease, that fungus will be selected provided it can be used for such tests. However, usually there is more interest in the general effectiveness of the fungicide. The selection of a fungus should be based on three main criteria: (a) reproducibility of results, (b) ease of counting, and (c) ready production of spores in quantity. Due to the common use of most of the fungi studied here, it is of interest to ascertain to what extent they meet these criteria.

Reproducibility of Results

Factors contributing to low replicate test variation are steep slope of toxicity curve (17) and small error in preparing the spore suspension. It is assumed that other environmental factors likely to affect reproducibility (15, p. 18) will be controlled. In general, as it has been shown above, the LD₅₀ values of *Rhizopus nigricans* are less reproducible than those of the other fungi.

Steepness of slope. No significant difference in steepness of slope could be shown between the fungi.

Spore suspension. The data in the last column of Table VII indicate that *G. cingulata* may be slightly more variable from day to day and *S.*

fruticola slightly less so. However, the final effect of the errors in spore suspension on the LD₅₀ is dependent also on the steepness of the spore number-LD₅₀ curve. No consistent difference could be shown for the steepness of this curve between the four different fungi, nor in the standard deviations of the derived LD₅₀ values at concentrations of 50,000 spores per cc. It will also be noted in Table VII that *G. cingulata* tends to give a higher concentration of spore suspension than expected, and *M. sarcinaeforme* much lower.

Ease of Counting

High control germination. For ease and efficiency of count, high control germination is essential. The customary procedure has been to count the control drops, then following the necessary correction, record results on all treatments on the basis of potentially viable spores. For example, if the control is 97 per cent germination, 103 spores are counted in the treatments and 3 deducted from the non-germinated results. Random samples of the germination for control lots of 200 spores were obtained from 24 months of data and the mean and standard deviation of a single test determined as follows: *Macrosporium sarcinaeforme* 99.10 ± 0.64 , *Sclerotinia fruticola* 98.94 ± 0.68 , *Botrytis cinerea* 98.75 ± 0.90 , *Alternaria solani* 95.95 ± 4.92 , *Rhizopus nigricans* 93.35 ± 3.56 , and *Glomerella cingulata* 92.93 ± 6.78 .

Visibility of spores and germ tubes. A comparison of the fungi under this criterion is very subjective. However, most workers will agree that some spores are too small for ease in counting, and for many *G. cingulata* will be so classified. Large, uniform-sized, dark-colored, single-celled spores with well defined germ tubes, probably are to be preferred. A magnification of 150 is desirable for all the fungi examined except *M. sarcinaeforme*, for which 100 is satisfactory.

Time of maximum germination. It is desirable that the spores will have reached their maximum germination in a short time, preferably within 24 hours. Another study in this series, now in progress, indicates that none of these fungi entirely meets this requirement. Certain fungi tend to produce secondary spores in the suspension drops as the time is prolonged. Such is the case for *Glomerella cingulata*; a similar effect has been observed with *Neurospora sitophila* (Mont.) Shear & Dodge.

Production of Spores

Spore yield. Random samples from data taken over a period of months show that the approximate mean yield in millions of spores per test tube slant and its standard error is as follows: *Alternaria solani* 18.7 ± 2.2 , *Glomerella cingulata* 18.4 ± 7.6 , *Rhizopus nigricans* 14.8 ± 2.5 , *Sclerotinia fruticola* 10.1 ± 2.2 , *Botrytis cinerea* 0.74 ± 0.41 , and *Macrosporium sar-*

cinnaeforme 0.41 ± 0.08 . In testing compounds by the test tube dilution method a large number of spores are required; for example, 12 compounds at six different concentrations would necessitate about 10 million spores. Hence *B. cinerea* and *M. sarcinaeforme* are not satisfactory for such tests. If sufficient tubes are used, these two fungi may be employed in spray deposit tests (16) where only about one-twentieth as many spores are needed.

Simplicity of production. Laboratory results indicate that for all the species studied except *M. sarcinaeforme*, the maximum number of spores are produced in about one week. However, for *M. sarcinaeforme* a period of about three weeks is necessary (9, 10). All six species will produce spores readily on potato dextrose agar. Recent work by Horsfall *et al.* (10) states that oatmeal agar is to be preferred for *M. sarcinaeforme*. A comparison was made of spore production by *S. fructicola*, *B. cinerea*, *G. cingulata*, and *A. solani* on 2 per cent agars of potato dextrose (see footnote 9, p. 50), oatmeal and Elliott (19) and Czapek and Barnes (18). *S. fructicola* and *B. cinerea* produced spores readily only on potato dextrose, which was also best for *A. solani*. However, *A. solani* produced some spores on all agars. *G. cingulata* produced most spores on Czapek's agar but did well on potato dextrose. If several different species are carried in culture, it is desirable that they all be cultured alike.

Temperature. High spore production and germination will take place at temperatures from 20° to 25° C. for all six fungi under consideration, except for the spore production of *B. cinerea* and *M. sarcinaeforme*. The temperature recommended for the latter is 20° C. (10).

Conclusion

These various comparisons with respect to the suitability of the six different fungi, as represented by the isolates studied, lead to the conclusion that in the long run *Alternaria solani* and *Sclerotinia fructicola* are likely to be the most satisfactory, *Macrosporium sarcinaeforme* and *Glomerella cingulata* fairly satisfactory, and *Botrytis cinerea* and *Rhizopus nigricans* generally unsatisfactory.

EXPERIMENTAL DESIGN

An experimental design will be employed most efficiently when those factors causing the greatest variation are sampled most widely. Since the primary interest is rating compounds, the factors of importance are those producing high interactions with compounds. From Tables V and VI it may be seen that the fungus $\times$ compound interaction is of greatest magnitude while the replicate test $\times$ compound interaction is significantly less. Hence the better general rating of compounds will be obtained by testing several fungi once rather than one fungus several times.

Fungi. Since fungi are the major source of variation, at least two different ones should be used as test organisms, while four would be preferable. These organisms having the same general level of sensitivity but with a high fungus $\times$ compound interaction, or low correlation, can be used to best advantage. Such a complementary pair is *Sclerotinia fructicola* and *Alternaria solani*.

Replicate tests. The greatest source of uncontrolled variation is the use of different lots of spores, i.e. replicate tests. Although a standard may be used to correct replicate test variations for compounds of like composition and slope, in general it is desirable to make all comparisons at the same time. If the number is not unduly large, such a comparison is possible. However, if a high degree of precision is desired for the comparison of a large number of compounds, the plan embodied in a Youden Square (22) may be employed. If several different fungi are used, because of the high fungus $\times$ compound interaction, not more than two tests are necessary. But if only one fungus is used, precision can be gained by replicating the tests three or four times.

Doses. Two finite points will suffice to define a simple straight line, but more are necessary for compound slopes. In general, four or five different doses giving finite responses will be satisfactory. If several dose ratios are agreed upon and standardized, the construction of reference tables becomes greatly simplified. Dose ratios of 2.000 and $\sqrt{2.000}$, i.e. 1.414, are suitable for practically all cases and are accordingly recommended.

Number of spores to count. In view of the large replicate test error, a sample of 100 spores per dose, distributed over at least two microscopic fields, is considered an efficient number. Probably even less could be counted without appreciable loss of information. An added advantage of 100 is the simplicity in computations and use of reference tables. Replicate counts can be omitted, since it was shown in the first paper of this series (15) that their variance is practically that of the binomial distribution.

Other factors. Other important factors such as resistance to weathering and the effect of time and temperature on germination have not been included in this study, but it is hoped that an investigation of these may be presented in a subsequent paper.

EVALUATION OF RESULTS

LD Levels

It is generally recognized that the LD₅₀ is the most precise point for comparing compounds of essentially similar slopes. In the bioassay of drugs an unknown is compared to a standard of like composition and slope, and for such cases the LD₅₀ is the most efficient level of comparison. However, in the laboratory testing of fungicides it is frequently necessary to compare heterogeneous compounds. These compounds may differ not only

in composition but also in steepness of slope and type of slope. An example of such heterogeneity is graphically presented in Figure 4. Comparisons made at the LD₅₀ in such cases are inadequate. Here it will be necessary to evaluate at a higher LD level, such as the LD₉₅, even though this point cannot be determined as precisely as the LD₅₀. Nevertheless, these comparisons made at the LD₉₅ will have greater practical interest. An analysis of variance on the LD₉₅ values for the copper sulphate and Bordeaux data gave a pooled variance for the triple interaction, compounds $\times$ fungi $\times$ tests, of 0.00394. If this is compared to the equivalent value obtained from the LD₅₀ analysis of 0.0022, it will be seen that in this case the precision of comparisons at the LD₉₅ have been decreased about one-half.

Thus for the general case of tests involving heterogeneous compounds of unknown slope or composition, or of slopes known to differ widely at the higher levels, it will be desirable to compare at the LD₉₅. For those cases where the slopes are essentially alike at the higher levels, the LD₅₀ will furnish the most effective point for comparison.

Significant Differences

It has been shown that the rapid graphic methods of obtaining LD values will give as satisfactory results as the long method of calculation. This graphic method is discussed in detail in an earlier paper (21). In view of the significant interaction of heterogeneous compounds with replicate tests, the determination of the error of a particular point or curve, i.e. internal error, is probably unnecessary for routine comparisons. The choice of a suitable error term to evaluate the significance of difference of compounds will depend on what question is to be answered. If it is desired to know which compound is best under all conditions of the test, i.e. for all fungi, both LD levels and all tests, it will be necessary to use the greatest interaction involving compounds. In most cases this will be the compound $\times$ fungus interaction. On the other hand, if it is desired to determine how the compounds are rated for the fungi in general or for some fungus in particular, at one LD level, the compound $\times$ replicate test error within fungi should be employed. This error is chosen within fungi because the results indicate that there is no general experiment error for a given day for all the fungi and hence each fungus may be considered a separate test. If the analysis of variance indicates a significant difference between compounds, comparisons may be made between the means of individual pairs by the *t* test. However, only the degrees of freedom appertaining to this pair should be employed (7, p. 65), though the pooled variance may be used. Since the error term is an interaction, *n* for *t* will be one less than the total LD values per compound.

The data presented in this paper as well as other unpublished results indicate that these various interactions are more or less constant under the

conditions prevailing in this laboratory. The pooled sums of squares for the compound $\times$ fungus interaction in Tables V and VI give a mean logarithmic variance of 0.364. Likewise the compound $\times$ replicate test interaction within fungi is 0.0180. These results were obtained from compounds with slopes within the approximate range of 0.1 to 0.3 λ . Thus when comparing heterogeneous compounds under these circumstances, in the former case with one test but a wider basis of inference due to the use of four different fungi a 20-fold difference between two compounds may be demonstrated with odds of 20:1, while in the latter case, four replicated tests with one specific fungus would allow a two-fold distinction to be made.

In the special case of compounds similar in composition and slope such as the two stocks, i.e. compounds, of copper sulphate and of Bordeaux mixture, no significant compound $\times$ replicate test interaction can be shown and hence the triple interaction or residual internal error may be used for error. This value is 0.0022. Hence under these optimum conditions, with one fungus, two tests would demonstrate a four-fold difference, four tests a 30 per cent difference, and 12 tests a 10 per cent difference.

SUMMARY

1. In a further analysis of the factors causing variation in spore germination tests of fungicides a study was made on 718 individual toxicity curves. The fungi employed were *Sclerotinia fructicola*, *Glomerella cingulata*, *Alternaria solani*, *Macrosporium sarcinaeforme*, *Botrytis* sp. (*cinerea* type), and *Rhizopus nigricans*. Twenty compounds were tested representing the heavy metals, commercial "insoluble" copper fungicides, and synthetic organic compounds. All tests were replicated from four to six different times.

2. Four different types of slopes were observed on logarithmic probability paper: (a) Simple straight line, (b) double slope with left-hand "break" in lower values giving a curve concave upwards, (c) double slope with right-hand "break" in upper values, or curve convex upwards, and (d) triple slope or sigmoid curve. Seventeen per cent of the curves were of the two latter types. The type and steepness of slope of the toxicity curve is determined more by the compound than the fungus. The slope of a given toxicity curve is reasonably consistent in replicate tests.

3. Heterogeneous compounds differing widely in slope as well as compounds of convex and sigmoid type curves should be evaluated at high LD levels such as the LD₉₅. Highly significant correlations were obtained between steepness of slope and toxicity at the LD₅₀ point for straight and concave type compounds of the heavy metal and copper series. There was no correlation among the organic compounds.

4. A comparison of a rapid graphic method with the detailed calculated

method of obtaining LD₅₀ values shows that the results do not differ significantly. Accordingly the use of the former is advised for general comparisons.

5. Tests replicated on the same day using the same lot of spores, in general do not vary more than is to be expected from their internal error, whereas tests replicated at different times with different lots of spores vary considerably more than is to be expected. This indicates that the replicate test variation in the main is due to the use of different lots of spores, rather than to errors of technique in applying the fungicidal dose.

6. There is a linear relation between the logarithms of LD₅₀ and of the number of spores exposed to the fungicide. Errors in adjusting the concentration of a spore suspension for a given test will account for only a small portion of the variance of replicated tests.

7. Different fungicides may be rated approximately in terms of a standard. However, the most effective use of a standard to adjust day-to-day variations is limited to compounds of essentially similar slope and composition. A single standard cannot be used for the precise comparison of heterogeneous compounds differing widely in slope and composition.

8. In their average sensitivity to a number of compounds, five of the fungi were essentially alike; only *Rhizopus nigricans* was significantly more sensitive. Nor was the sensitivity inversely proportional to spore volume. In many cases, however, the fungi were unlike in the sensitivity to specific compounds and would thus rate them differently. Fungi tending to rate compounds differently, such as *Alternaria solani* and *Sclerotinia fruticola*, can be used most efficiently in laboratory testing.

9. The selection of different fungi as laboratory test organisms should be based on three main criteria: reproducibility of results, ease of counting, and ready production of spores in quantity. The six fungi studied are compared as to these and various subcriteria.

10. Experimental designs to give the maximum in efficiency of testing and evaluating results are outlined. In general the following are advised: 5 doses; dose ratios of 2.000 or 1.414; 100 spores per count per dose; no replicate counts; graphic methods of determining LD values; evaluation at LD₅₀ for straight line or concave type compounds having similar slopes, at LD₉₅ for dissimilar slopes and curves of convex or sigmoid type; several different fungi tested twice, rather than one fungus tested a number of times; and for error term, the compound $\times$ replicate test interaction.

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CARBON DIOXIDE PREVENTS THE RAPID INCREASE IN THE REDUCING SUGAR CONTENT OF POTATO TUBERS STORED AT LOW TEMPERATURES

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From the previously reported results regarding the effect of carbon dioxide upon the sugar metabolism of potato (*Solanum tuberosum* L.) tubers, this gas would hardly be expected to *prevent* the accumulation of sugars, at least with a CO₂ concentration as low as 5 per cent.

The tests here reported upon, however, show that this result can be obtained with CO₂. In order to understand the apparently conflicting facts as to the action of CO₂ upon the sugar metabolism of potato tubers, two points regarding the conditions of such tests should be kept in mind: (a) Whether the sugar fraction is reducing sugar or is sucrose, since the development of reducing sugar may be retarded and that of sucrose increased, even under the same set of conditions; an estimate based on only total sugar would not show this difference. (b) Whether the temperature of the test is high, such as at room temperature, or low, such as at 5° C., since the response of the tubers as shown by changes in *reducing sugar* are quite different at the two temperatures.

Thornton (3) showed that the reducing sugar content was greatly increased by storage of the tubers at 21° C. in an atmosphere containing 60 per cent CO₂, and that the sucrose was increased with either 30 or 60 per cent CO₂. Barker (1), whose data unfortunately are reported only on the basis of total sugar, found that all concentrations of CO₂ used with tubers stored at 5° C. and 7.5° C. increased the total sugar content. At 1° C. *total sugar* was decreased with 20 per cent CO₂, but this was accompanied by discoloration and with the development of a peculiar odor of the tubers. Furthermore, when the concentration of CO₂ at 1° C. was decreased to 10 per cent, the total sugar was greatly increased in comparison with the controls in air. Barker did not use concentrations of CO₂ below 10 per cent in his experiments at 1° C.

In the present experiments, of which this is a preliminary report, the potato tubers were stored at low temperatures (5° C. and 8.2° C.), and in the chemical analyses separate determinations were made for reducing sugar and for sucrose. The results show that at 5° C. a concentration of 5 per cent CO₂ retarded the development of *reducing sugar* to such an extent that at the end of one month, or of even two months, the amounts of reducing sugar of the juice of CO₂-treated tubers were only one-half or one-third or one-fifth of that of control tubers in air. And yet under these same

conditions, the *sucrose* content was increased by the CO₂ treatment, so that if merely the values for total sugar were taken into consideration, it would be concluded that the effect of CO₂ was to increase the sugar.

However, our interest in the problem has been in connection with the production of potato chips with a light color, and the previous reports (4, 2) show that only the reducing sugar content is important for chip color, sucrose not having any effect upon the color, at least at the cooking temperatures ordinarily employed for this purpose.

Thus, by using CO₂ in the amount of 5 per cent in the atmosphere surrounding the tubers, the reducing sugar content has been maintained in tubers stored at 5° for two months at a value sufficiently low for the production of chips of good color, while tubers stored in air for the same period at the same temperature have increased in reducing sugar content to the point at which they have become worthless for potato chips on account of the dark color of the product. However, recommendations for the use of CO₂ in the storage of tubers for potato chip production cannot be made at this time, since, even if the method should prove to be feasible and economical, the effects upon tuber characteristics other than sugar content and color of chips have not been determined.

METHODS

The tubers used were those of the variety Irish Cobbler. A sample consisted of 16 tubers tied in a cheesecloth bag. The containers for maintaining the proper gas mixtures in the atmospheres surrounding the tubers were cylindrical steel drums, capacity approximately 54 gallons. Each drum was closed by a sheet iron cover with a rubber gasket and bolts, and at the edges where the gasket, drum flange, and cover met, a layer of modelling clay completed the seal. The cover was equipped with glass inlet and outlet tubes, through which CO₂ and O₂ were admitted to the drum in amounts to give the desired proportions (by volume) of these two gases. In each case, whatever the concentration of CO₂ (by volume), an effort was made to maintain the O₂ concentration at about 20 per cent (by volume). In some cases, the O₂ concentration dropped below this value for short periods, but in no instance was an O₂ value below 17.5 per cent observed throughout the tests. At intervals of two to three days, or more often, if necessary, especially in the early stages of a test, the proportions of CO₂ and O₂ within the containers were determined by gas analysis and a correction for the composition was made, if needed, by introducing the required gas from cylinders of the compressed gases, or from bottles of the gases obtained from these cylinders.

The methods of grinding the potato tuber tissue, squeezing the juice, and making the sugar determinations were described in a previous paper (2, p. 294).

RESULTS

FIRST MONTH OF THE TEST

The arrangement of the test and the results obtained are shown in Table I. The upper half of the table shows the results at the end of the first month after the start. At that time the lots remaining after the removal of the sample bags of tubers for analysis were divided equally into two lots as shown by the arrows in the center of the table, and the experiment was continued for another month upon the basis shown in the headings for the lower half of the table.

TABLE I

EFFECT OF CO₂ ON SUGAR CONTENT OF POTATO TUBERS STORED AT LOW TEMPERATURES
Sugar content of juice of tubers at start of experiment:

Sucrose = 3.3 " " " " "
Total = 3.5 " " " " "

Tubers sorted into six lots and placed under the conditions of storage-temperature and carbon dioxide concentration shown in Columns 2 to 7, lines 1 and 2 below:

Temp. of storage, °C. →		5°		5°		5°		8.2°		8.2°		8.2°	
Conc. of CO ₂ →		5%*		12%*		None		5%*		12%*		None	
Sugar content after 1 month	Red. sug. Sucrose Total	2.9		4.3		9.1		2.1		1.7		3.3	
		22.6		35.6		12.9		10.1		16.4		4.0	
		25.5		30.9		22.0		12.2		18.1		7.3	
The remaining samples were then subdivided into lots as shown by the arrows: →													
Temp. of storage, °C. →		5°	5°	5°	5°	5°	5°	8.2°	8.2°	5°	5°	8.2°	8.2°
Conc. of CO ₂ →		5%	None	5%	None	5%	None	5%	None	5%	None	5%	None
Sugar content after 1 additional month	Red. sug. Sucrose Total	2.5	7.5	5.2	8.4	12.6	13.1	2.9	4.9	3.6	8.4	3.4	3.8
		38.4	24.3	42.5	38.4	20.2	10.5	9.7	5.0	27.2	17.6	10.9	4.0
		40.9	31.8	47.7	46.8	32.8	23.6	12.6	9.9	30.8	26.0	14.3	7.8

* Per cent by volume; the O₂ concentration in each case was approximately 20 per cent at the start and not lower than 17.5 per cent by volume at any time.

In the first month of the test (top half of Table I) tubers were treated with 5 per cent and 12 per cent CO₂ (with O₂ at 20 per cent) at two different temperatures, 5° and 8.2°C. For comparison there were control tubers in air at each of these two temperatures. In considering the effect of CO₂ upon the sugar content of the tubers, it is desirable to discuss the reducing sugar and sucrose data separately.

Reducing sugar. The results for the first month of the test are shown for the 5° storage temperature in line 3 (main body of the table), columns 2, 3, and 4, Table I. It is seen that CO₂ retarded the development of reducing

sugar, the control lot increasing from the value of 0.2 at the start to the value of 9.1 at the end of one month, while the corresponding values for the lots receiving a treatment with 5 per cent and 12 per cent CO_2 were 2.9 and 4.3, these values being only about one-third or one-half of the control. The results for the lots stored at 8.2° are shown in line 3, columns 5, 6, and 7 in Table I. At this temperature the reducing sugar increase was not extensive as is shown by the control lot which increased from 0.2 to only 3.3 (column 7); the values for the 5 and 12 per cent CO_2 lots (columns 5 and 6) were 2.1 and 1.7, lower than the controls, it is true, but further tests would be needed to show whether there is a real difference due to CO_2 treatment.

Sucrose. The results for sucrose during the first month of the test are shown in line 4, Table I. Increases by CO_2 treatment were found for both 5 and 12 per cent CO_2 and at both temperatures (5° and 8.2°). The higher amount of CO_2 (12 per cent) caused large increases in sucrose, more than a three-fold difference in sucrose increase at 5° , i.e., a sucrose change of 35.6 minus 3.3 for 12 per cent CO_2 , as compared with 12.9 minus 3.3 for the control (see line 4, columns 2, 3, 4). At 8.2° (see line 4, columns 5, 6, 7) the ratio of the sucrose increase in a comparison between the values for 12 per cent CO_2 and the air control is even greater, e.g. $(16.4 - 3.3) \div (4.0 - 3.3) = 19$.

Total sugar. The total sugar values are shown in line 5. It is not profitable to discuss these values since they represent merely the sum of the two sets of values which have already been discussed separately. An examination of the values in line 5 will show how ineffective total sugar values alone would have been in disclosing what had been the effect of CO_2 upon the sugar changes in the tubers.

SECOND MONTH OF THE TEST

The results of the second month of the test are shown in the lower half of Table I. At the end of the first month after the removal of sample bags of tubers for analysis, there were two to four bags of tubers in each lot available for a further test. These bags of tubers from the previous test were divided equally in each case into two lots as shown by the arrows in the center of Table I, and were then placed under the conditions of temperature and CO_2 listed in lines 6 and 7 in Table I (first two lines under the center arrows). It will be noted that the tests with 12 per cent CO_2 were discontinued at the end of the first month; this was done because it appeared possible that at 5°C . the lower concentration of CO_2 , i.e. 5 per cent, was more effective in retarding the development of reducing sugar than was 12 per cent CO_2 , and the main object in these tests for the present at least, has been to find the conditions for maintaining a low value for the reducing sugar. As is shown in the lower half of Table I, the tests for the

second month were arranged to give comparisons only between 5 per cent CO_2 and air. The tubers for which the results are shown in the lower half of Table I were tubers that had already been, during the first month, under the conditions described at the top of the corresponding column.

Reducing sugar. What are believed to be conclusive results with regard to the capacity of 5 per cent CO_2 to retard the rate of development of reducing sugar at the temperatures used are shown in line 8 in Table I (third line below the center arrows). In each comparison between 5 per cent CO_2 and air the reducing sugar value is lower for 5 per cent CO_2 than for the control. The extent of the difference between the 5 per cent CO_2 and control values varies with the temperature of storage, tending to be greater after 5° storage than after 8.2° storage, and tending to be greater the lower the starting value (i.e., the value at the end of the first month's test, see line 3 in Table I). When the starting value was low, e.g., 2.9 and 1.7 (line 3, columns 2 and 6), and when the temperature of storage during the second month was low, i.e., 5°C ., the corresponding differences between CO_2 and control were large, i.e., 2.5 vs. 7.5 and 3.6 vs. 8.4 (line 8). When the starting value was high, e.g., 9.1, line 3, column 4, or the temperature was high, e.g., the data in column 7 representing tubers continuously at 8.2° during both months, the differences are small and presumably are not significant. It appears that the effect of CO_2 is not to decrease the reducing sugar but to retard its increase at low temperatures; but in column 4 no increase in reducing sugar occurred during the second month because it was already high at the beginning of the period, and in column 7 no increase occurred because the temperature was too high to induce it. In neither case was there any increase to be retarded by CO_2 .

Special attention is directed to the value 2.5 in line 8, column 2. This represents the reducing sugar value of the lot which was exposed to 5°C . with 5 per cent CO_2 for the entire period (two months), and shows the effectiveness of this relatively low amount of CO_2 in retarding the increase in reducing sugar which normally occurs in air at this temperature (5°C .). Compare this value (2.5) with the value 13.1 (in the same line, column 4) which represents the control lot in air during the same two-month period. The CO_2 treatment has kept the reducing sugar value down to a value about one-fifth of that of the air control. This lot had a reducing sugar value of 2.9 at the end of the first month and only 2.5 at the end of the second month, which shows that 5 per cent CO_2 enabled this lot to remain for a month at a temperature favorable for the rapid increase of reducing sugar without any such increase occurring. The corresponding values in column 3 also show a strongly retarding effect of CO_2 , since the CO_2 -treated lot increased during the second month from a 4.3 value to one of only 5.2, while the control during the same period increased from 4.3 to 8.4.

Sucrose. In all cases the sucrose values for the lots treated with 5 per

cent CO_2 are greater than those for the corresponding controls without CO_2 , except possibly in the pair that started from an originally high value (35.6 in column 3, lines 4 and 9). These sucrose differences due to CO_2 treatment occurred even with the lots at 8.2° which did not show a reducing sugar retardation.

Total sugar. These values taken alone do not elucidate but rather obscure the changes that have occurred in the two constituents of which the total sugar is the sum.

SUMMARY

The rapid increase in reducing sugar which occurs in potato tubers stored at 5°C. was prevented by storing the tubers in an atmosphere containing 5 per cent carbon dioxide. At the end of two months at 5°C. the reducing sugar content of the CO_2 -treated lot was approximately one-fifth of that of the control lot in air.

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RELATION OF CERTAIN AIR TEMPERATURES AND HUMIDITIES TO VIABILITY OF SEEDS

LELA V. BARTON

It has been well established by many investigators that the moisture content of seeds is of the utmost importance as a factor in the determination of their longevity. This applies not only to the absolute moisture content but also to fluctuation in moisture content, especially to fluctuations around the "critical" moisture content which varies according to the variety of seed. That temperature is of vital importance in connection with the keeping quality of seeds has also been demonstrated repeatedly. Neither moisture nor temperature can be considered alone, since the effect of one depends upon the other.

In view of these facts, it becomes highly desirable to ascertain the actual amounts of water which will be absorbed by seeds in different localities, i.e. under different conditions of humidity and temperature. Such knowledge would make it possible to determine whether air-dry seeds, in any particular region, could be stored with safety.

The present paper presents data on moisture contents of six varieties of seeds, stored at four different temperatures and three different relative humidities. Correlations with the germination capacity, under all of these conditions, have been made. Fluctuations in moisture content of seeds stored in open containers in the laboratory at Yonkers, New York, have also been determined. Data are given to show the comparative rate of deterioration of seeds of different germination capacity under unfavorable conditions of temperature and humidity.

TEMPERATURE AND HUMIDITY EFFECTS

METHODS

Seeds used for these tests were lettuce (*Lactuca sativa* L.), onion (*Allium cepa* L.), tomato (*Lycopersicon esculentum* Mill.), flax (*Linum usitatissimum* L.), peanut (*Arachis hypogaea* L.), and long-leaved pine (*Pinus palustris* Mill.). Large desiccators containing saturated solutions of magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$), calcium nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), or sodium chloride (NaCl) with an excess of the salts were placed in constant temperature chambers of 5°, 10°, 20°, and 30° C. According to Spencer (13), the relative humidities of the air over saturated solutions of magnesium chloride are approximately 35, 35, 34, and 32 per cent at 5°, 10°, 20°, and 30° C. respectively. Saturated solutions of sodium chloride provide atmospheres of 78, 77, 76, and 76 per cent at 5°, 10°, 20°, and 30° C. The relative

humidities prevailing over saturated solutions of calcium nitrate are 56 and 51 per cent at 20° and 30° C. respectively. The relative humidities over saturated calcium nitrate solutions at 5° and 10° C. have not been determined but it is probable that they lie between 55 and 60 per cent. The three relative humidities used, then, were approximately 35, 55, and 76 per cent. The seeds were placed in cheesecloth bags over the solutions in the desiccators. Moisture determinations were made on each seed lot after 8, 17, 29, 43, 105, 150, 232, and 372 days. Approximately two-gram samples of lettuce, onion, tomato, three-gram samples of flax, and five-gram samples of pine and peanut were removed from the desiccators and placed in weighing bottles and the weights determined. Seeds were removed from 5° and 10° C. desiccators in a cold room and placed immediately in a desiccator over calcium oxide which was then transferred to room temperature. All weighings were made after samples had come to room temperature. Samples were then dried in a vacuum oven with an air-jacket temperature of 78° C. for 48 hours after which the dry weights were ascertained. The moisture percentages were calculated on the basis of the dry weights of the seeds. Single lots of seeds were used for these determinations. Numerous previous tests had demonstrated the close agreement of duplicate lots under this method of treatment.

All tests for the germination capacities of the seeds were made in ovens at controlled temperatures previously determined as favorable. Moist filter paper was used as the medium for the germination of lettuce, onion, tomato, and flax at controlled temperatures of 20°, 25°, 25°, and 20° to 30° C. respectively. Twenty to thirty degrees C. represents a daily alternation of temperature where the cultures are left for 16 hours at 20° and for 8 hours at 30° each day. Duplicates of 100 seeds each were used for these tests. Pine and peanut seeds were mixed with moist granulated peat moss for germination. Duplicates of 25 seeds each of peanut were placed at 20° C. A single lot of 100 seeds of pine was placed at 5° C. for one month after which it was transferred to 20° C. for germination. Some germination of pine occurred at 20° C. without low-temperature pretreatment but a much higher and more uniform germination was obtained after pretreatment for one month at 5° C.

This experiment was begun in August, 1939. Data reported below show that the moisture contents of air-dry seeds in this locality are high in August.

RESULTS AND DISCUSSION

Moisture contents. Results of moisture determinations made 8, 29, 105, and 372 days after storage under special conditions are shown in Table I.

Tests from all conditions made after 8 and 17 days showed fluctuations which indicated that the seeds had not reached an equilibrium with the

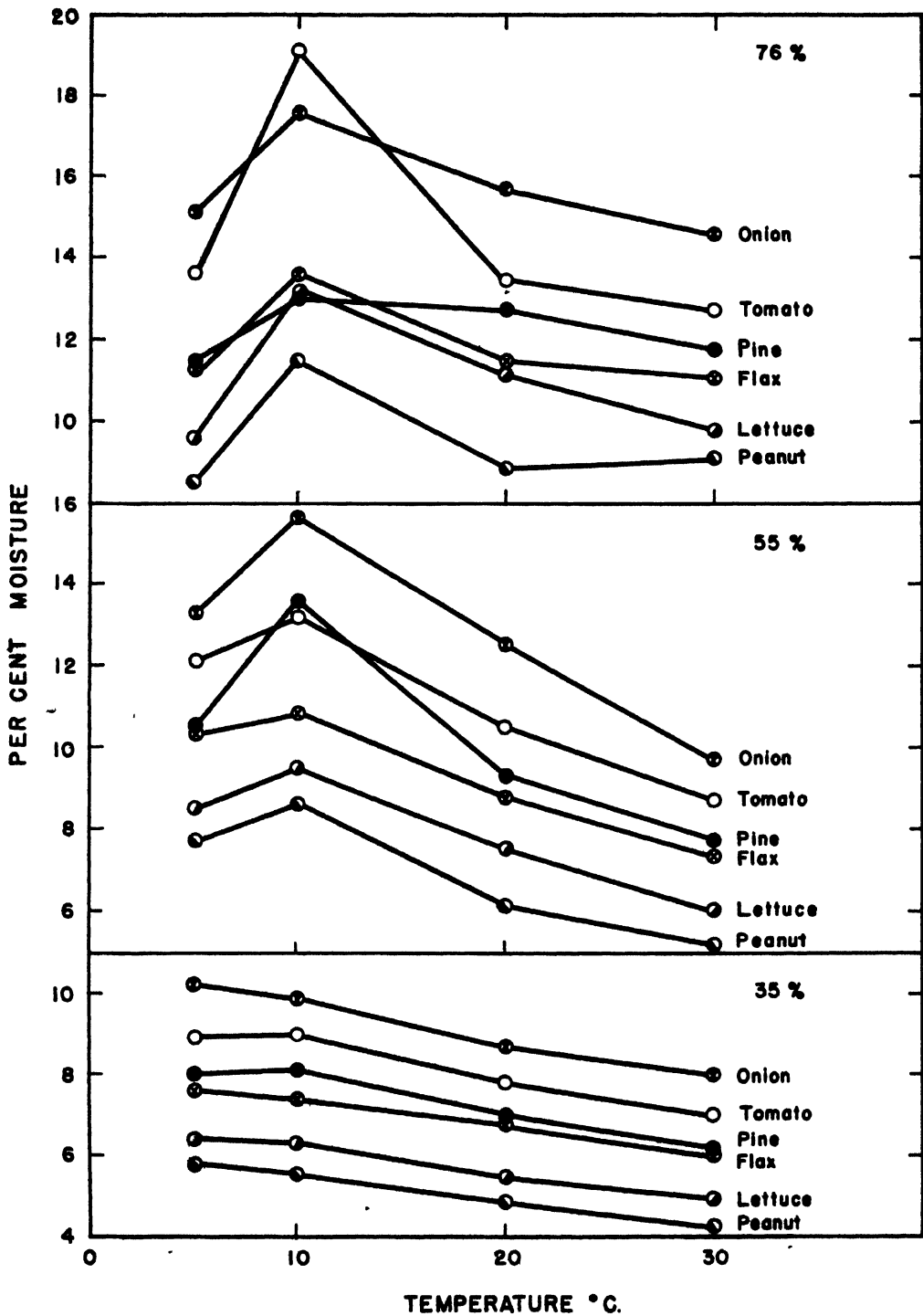


FIGURE 1. Moisture content of seeds after 43 days of storage at relative humidities of 76, 55, and 35 per cent.

moisture in the storage chamber. Tests made as long as 29 days after storage showed that an equilibrium was being established by that time and changes in moisture content of individual samples on subsequent dates were doubtless due in great part to the error of sampling.

It was noted that the seeds showed differential water absorption according to species and that the amount of moisture taken up under all conditions by the various seeds always appeared in the same relative position. This is shown in Figure 1 where the moisture determinations made 43 days after storage are used. In the order of increasing water-absorption capacity, the seeds were peanut, lettuce, flax, pine, tomato, and onion. This order persisted regardless of storage temperature or atmospheric humidity.

The chemical composition of the seeds may account, at least in part, for this variation in the amount of moisture absorbed by different seeds under identical conditions. Peanut, flax, lettuce, and pine seeds, which contain large amounts of oil, have low absorption capacities. On the other hand, tomato and onion, both of which contain larger amounts of protein and smaller amounts of oil, occupy intermediate and high positions as regards ability to absorb moisture. Ewart (10) stated that the resistant power of a seed to desiccation depended partly on the nature and thickness of the coats and partly on the form in which the reserve food was stored. Other conditions being similar, albuminous seeds were least resistant to desiccation, oily seeds next, and starchy seeds most resistant. The present tests confirm these statements for albuminous and oily seeds. The types of seed coats, also, doubtless play a rôle.

It should be pointed out that, at the beginning of the tests, seeds of the same lot possessed the same vitality in all storage conditions, but, as the storage period lengthened, loss of vitality progressed much more rapidly under conditions of high humidity and temperature. This means, then, that moisture determinations were made with increasing proportions of non-viable seeds. That the state of viability was without effect on the actual amount of water absorbed was demonstrated by the failure of the moisture content to change as the germination capacity decreased. This may be seen from a comparison of Tables I and II. Other workers have shown that seed viability does not affect the total water absorption. Heinrich (11) weighed seeds of *Lolium perenne* after 0 to 384 hours in a moist room at 20° C. and found that live seeds contained more water than dead ones up to 24 hours, after which they were approximately the same. Atkins (1) also found that seeds, living or dead, take up the same quantity of water in their initial stages and that there is no difference until germination begins.

With a relative humidity of only 35 per cent, seeds took up approximately the same amount of water at 5° and 10° C., but in every case they

TABLE II
GERMINATION PERCENTAGES OBTAINED FROM SEEDS STORED AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Seed + % germi- nation at time of storage		% Rel. hum.	Per cent germination after storage for days																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
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Lettuce 63	35 55 76	59 50 50	79 80 67	71 78 67	73 75 68	67 50 36	51 44 57	78 69 64	66 65 1	70 57 7	53 22 0	56 45 58	69 62 46	63 51 1	59 43 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46 41 52	68 51 4	33 16 0	46 41 52	41 6 0	33 16 0	41 6 0	32 3 0	46

remained drier at the higher temperatures of 20° and 30° C. (Fig. 1). At 55 and 76 per cent relative humidities, however, the peak of moisture absorption was at 10° C. and the lowest absorption was at 30° C. This was shown throughout the tests made after storage for 29 days or longer (Table I). There was no indication that equilibrium was reached more quickly at 30° C. than at any of the other temperatures tried. In all seeds, at 55 per cent relative humidity, there was an initial lag in absorption at 5° C. The moisture contents of seeds at this temperature were lower than those at 10°, 20°, or 30°, eight days after the tests were begun. After 17 days, moisture in seeds at 5° C. equalled or exceeded that in seeds at 30° C. and after 29 days equalled or exceeded that at 20° C., from which time it remained slightly in excess of that at 20° C. for all seeds (Fig. 2).

Coleman and Fellows (8) determined moisture contents of cereal grains and flax seeds exposed to 15, 30, 45, 60, 75, 90, and 100 per cent relative humidity at 25° C., but no other temperature was used. The large amount of moisture absorbed by elm and flower seeds in a high-humidity room at 5° C. has been pointed out in previous papers (5, 6). Boswell and others (7) stored seeds of bean, sweet corn, beet, cabbage, carrot, onion, spinach, tomato, and Spanish peanut at 50° and 80° F. at relative humidities of 78 to 81 per cent, 66 per cent, and 44 to 51 per cent, and reported that at a given relative humidity the seeds developed slightly higher moisture contents at 50° F. than at 80° F. They stated that this could not be explained by the physical characteristics of the air, such as differences in vapor pressure, weight of water in the air, or vapor deficit. The results reported here show significantly higher moisture contents of seeds at a given relative humidity at 10° C. than at 30° C. Less moisture was absorbed at 20° C. than at 10° C. but more than at 30° C. Approximately the same amount was taken up at 5° and 10° C. at 35 per cent relative humidity but as the relative humidity was increased to 55 and to 76 per cent, seeds absorbed more water at 10° than at 5° C. The explanation for these phenomena must be sought not only in the physical conditions of the atmosphere surrounding the seeds but in the physical conditions of the seeds themselves. The actual determination of the vapor pressures of both seeds and atmosphere at the different temperatures used and the relations of these curves to each other may provide a basis for explanation of the behavior.

Viability. Germination tests in ovens at controlled temperatures were made at the beginning of the storage period and after storage for 8, 43, 150, 232, and 372 days. The results are shown in Table II and Figure 3. When Figures 1 and 3 are compared it becomes obvious that the actual amount of water absorbed by the seed is not directly related to its loss of vitality under any given set of conditions. The three seed types exhibiting greatest retention of vitality under all conditions were peanut, flax, and tomato. On

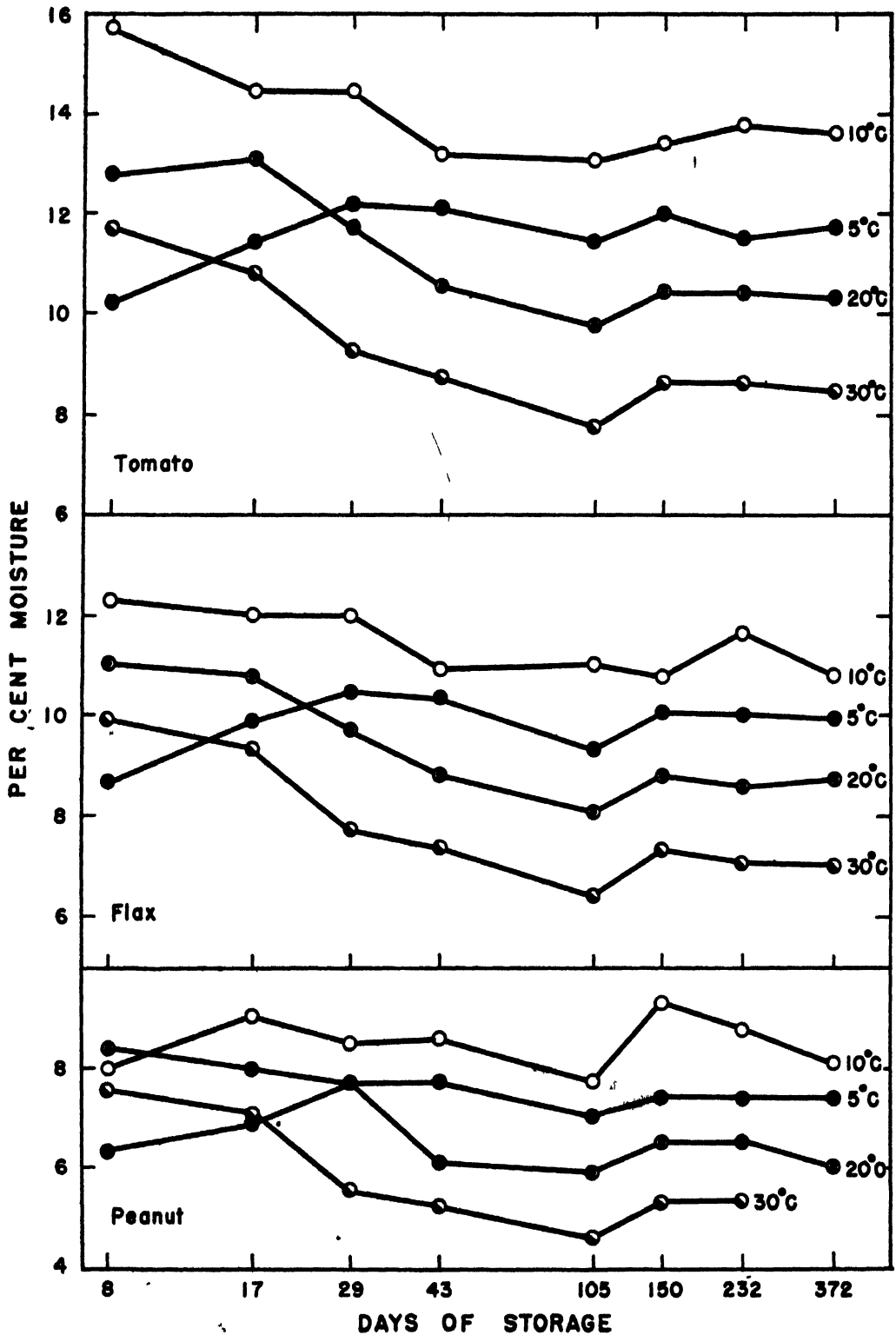


FIGURE 2. Moisture content of seeds stored at 55 per cent relative humidity.

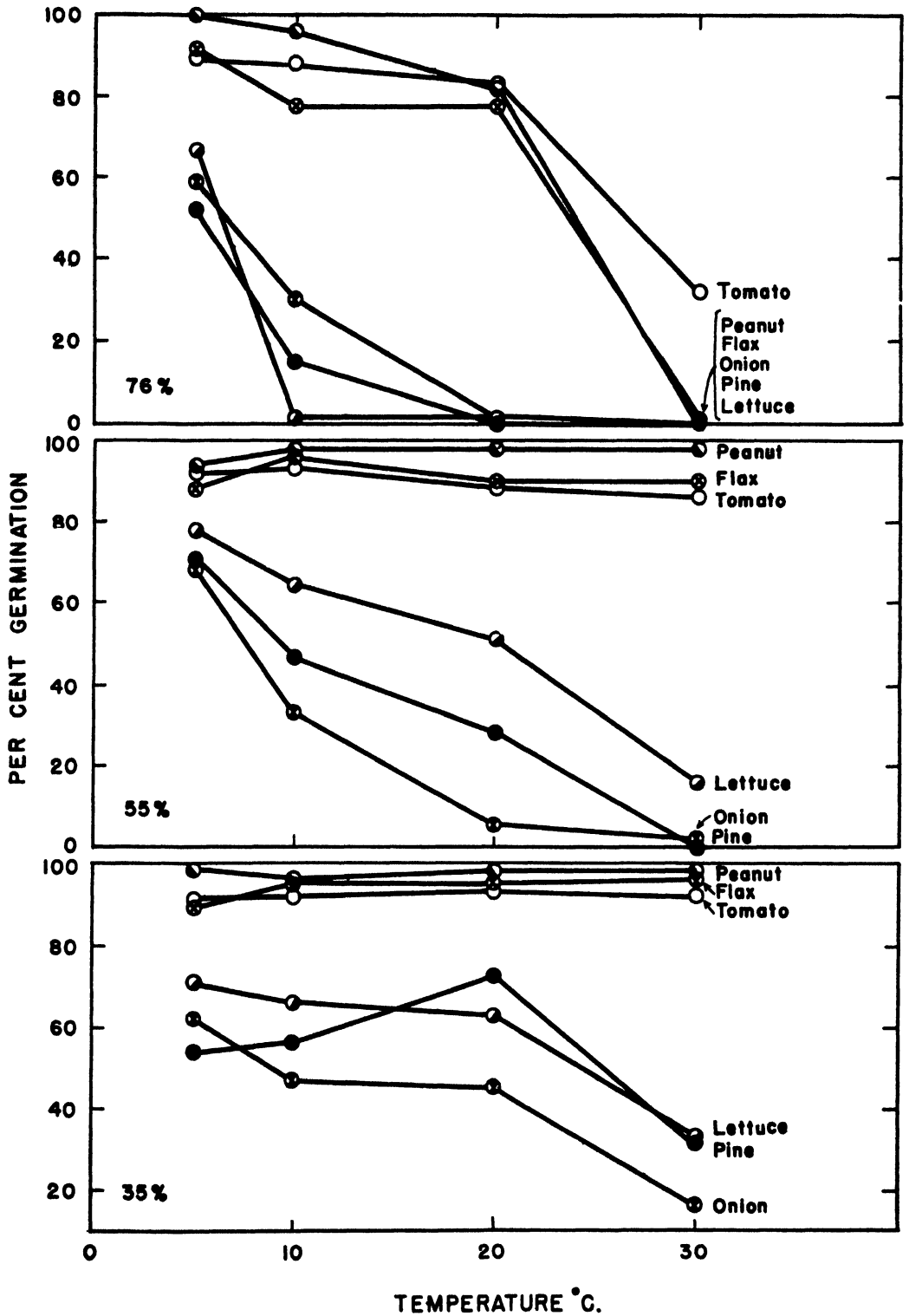


FIGURE 3. Germination after storage for 150 days at various temperatures and relative humidities.

the basis of the actual amount of moisture absorbed these seeds occupy low, intermediate, and high positions (Fig. 1). Similarly lettuce, pine, and onion seeds which lose vitality most readily under all conditions have low, intermediate, and high moisture contents. Boswell and others (7) also reported that among different kinds of seed, deterioration was not always correlated with the relative moisture-absorbing capacity of the seeds. Furthermore, under the conditions of this experiment, viability does not seem to depend on chemical composition.

A positive correlation is found, however, between the initial vitality of the seeds and the rate of loss of germination capacity. Peanut, flax, and tomato all showed initial germination capacities of over 90 per cent while lettuce and onion showed only 63 and 66 per cent.

Pine seeds represent a special case as regards initial viability. It will be seen from Table II that 96 per cent germination was obtained. The same seeds if germinated without low temperature pretreatment gave only 57 per cent germination in moist granulated peat moss at 20° C. It has been reported in a previous publication (3) that fresh seeds of *Pinus palustris* produced the same seedling stand with or without pretreatment at low temperature, but as their vitality decreased, pretreatment became necessary for maximum seedling production. Since the vitality of this lot was reduced to 57 per cent as indicated by germination test without pretreatment, it was to be expected that the seeds would lose their germination capacity at a rapid rate under unfavorable conditions. Thus it was found that behavior of these seeds was similar to those of lettuce and onion.

These facts indicate that deterioration, once initiated, proceeds rapidly to death of all of the seeds under unfavorable storage conditions but is not marked under favorable conditions. At 5° C., even in high humidity, all seeds kept well regardless of initial vitality, but as the storage temperature was increased, the need for high quality seeds was evident if vitality was to be maintained.

No effort will be made here to review all of the literature dealing with humidity and temperature in relation to loss of vitality of seeds. The advantage to the seed trade of a knowledge of the amount of moisture absorbed by seeds under varying conditions of humidity and temperature and the relation to keeping quality is obvious. Another practical application of this knowledge is in seed extraction. Eliason and Heit (9) emphasized the importance of the humidity of the kiln in the extraction of pine seeds from their cones. If the humidity was too high some of the seeds were killed. They established the limits of humidity for safety in extracting seeds at temperatures of 117° to 142° F. Many additional data are needed to provide a good working basis for the determination of standards for storage and extraction of seeds.

MOISTURE RELATIONSHIPS OF SEEDS STORED OPEN
IN THE LABORATORY

A previous publication (6) has emphasized the significance of fluctuation in moisture content as a contributing factor to deterioration of seeds in storage. In order to determine more exactly what fluctuations actually exist under ordinary atmospheric conditions during the course of a year at Yonkers, New York, moisture determinations were made in the manner described above for seeds of carrot (*Daucus carota* L. var. *sativa* DC.), eggplant (*Solanum melongena* L.), lettuce, tomato, and long-leaved pine

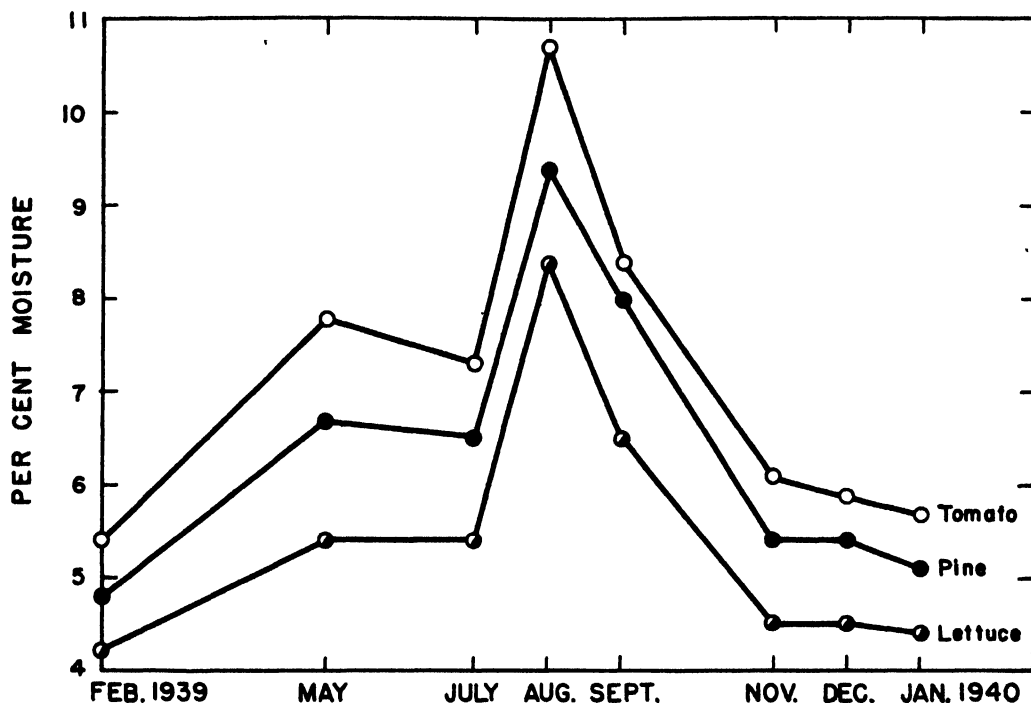


FIGURE 4. Moisture contents at various times of the year of seeds stored open in the laboratory. Moisture expressed as percentage of dry weight of seeds. Duplicates of approximately 2 grams each used for each test.

stored open in the laboratory in February, May, July, August, September, November, and December, 1939, and January, 1940. The results of these tests showed that the moisture content of seeds in August was practically double that in January or February. This applied to every type of seed tested although there was considerable variation in the actual amounts of water taken up by the different varieties. These differences are shown graphically in Figure 4, where a comparison of lettuce, pine, and tomato seeds is made. In spite of the differential water-absorbing capacities of these seeds, all of them contain the maximum moisture in August and are relatively dry during the months of November, December, January, and

February. Carrot and eggplant moisture contents at the various testing periods were practically the same as for tomato. Pine seeds took up less moisture from the air while lettuce seeds remained driest.

This test simply serves to emphasize the necessity of drying seeds to a known moisture content before storage since the amount of moisture present in "air-dry" seeds depends not only on the locality but on the time of year the tests happen to be made. "Air-dry" seeds placed in sealed containers in August in Yonkers, New York, would contain twice as much moisture as the same seed lot sealed during the winter months. In the evaluation of sealed as opposed to open storage, these facts should be considered.

The differential absorption of water by the different species furnishes additional evidence that the "critical" moisture content varies with different seeds. It is believed that fluctuation in moisture content is most deleterious to seed viability when it occurs around the "critical" point. It has been found (6) that dandelion and aster seed with moisture contents between 6 and 8 per cent deteriorated much more rapidly when fluctuation in moisture was introduced. Fluctuations at lower moisture contents were without effect.

COMPARATIVE RATE OF DETERIORATION OF SEEDS OF DIFFERENT GERMINATIVE CAPACITIES

This test was devised to demonstrate further the fact already pointed out in a previous paper (4), that deterioration, once initiated, proceeds as an autolytic process. Therefore it is very important that old seeds removed from a favorable storage condition to an unfavorable one should have lost none of their vitality at the time of transfer. As a further demonstration of these effects, seeds of eggplant, onion, and tomato of the same lots as described in previous papers (2, 4) were removed from their original storage conditions on April 18, 1940, and placed under temporary storage conditions as follows: open container at 5° C., desiccators at approximately 76 per cent relative humidity at 25° and 35° C. Seeds from the original storage conditions were selected to give a range in viability as indicated by germination tests made at the time of removal. In the cases of eggplant and tomato seeds, those which had been stored open or sealed air-dry or dried before sealing at both laboratory and below-freezing temperatures were used. Onion seeds of the dried lot sealed and kept in the laboratory as well as those stored below freezing open or sealed air-dry or with reduced moisture were also used. It should be kept in mind that all of these seeds were over eight years old at the beginning of these special tests.

It will be seen from Table III that each kind of seed was represented by lots showing variation in vitality at the beginning of the special storage tests. For example, eggplant seeds sealed air-dry and stored in the laboratory had deteriorated so that only 37 per cent were viable at the beginning

TABLE III

GERMINATION OF 8-YEAR-OLD SEEDS TESTED DIRECTLY AFTER REMOVAL FROM THE ORIGINAL STORAGE CONDITION AND AFTER FURTHER STORAGE OPEN AT 5° C., OR IN DESICCATORS WITH APPROXIMATELY 76 PER CENT RELATIVE HUMIDITY AT 25° AND 35° C. GERMINATION PERCENTAGES OBTAINED ON MOIST FILTER PAPER IN OVENS AT CONTROLLED TEMPERATURES. DUPLICATES OF 100 SEEDS EACH FOR EACH TEST

Seed	Original storage	Per cent germination after weeks of further storage																							
		5° C.								25° C.								35° C.							
		1	2	3	4	5	6	8	12	1	2	3	4	5	6	8	12	1	2	3	4	5	6	8	12
Egg-plant	Room	59	67	64	59	48	54	51	55	39	66	62	59	55	44	49	33	17	60	44	30	26	13	1	—
	Air-dry open	37	35	34	29	28	23	14	19	9	41	26	22	23	18	6	17	6	32	13	5	2	1	0	—
	Air-dry sealed	75	80	89	80	79	77	75	75	65	84	81	76	74	72	70	70	55	79	79	67	64	48	30	4
	Dried sealed	76	86	81	82	83	87	81	79	65	82	77	72	72	75	63	65	43	89	80	78	67	65	54	47
Onion	Room	87	84	84	81	84	81	75	75	80	88	87	80	78	81	72	76	70	83	83	83	79	75	73	67
	—5° C.	83	87	87	83	85	81	81	78	70	86	80	84	81	78	72	67	64	82	87	79	75	75	72	60
	Air-dry open	62	69	67	70	42	54	53	50	38	2	0	0	—	—	—	—	—	0	0	0	—	—	—	—
	Air-dry sealed	85	86	86	86	77	75	59	55	—	14	4	2	0	—	—	—	—	1	0	0	—	—	—	—
Tomato	Room	85	86	89	82	68	76	71	75	59	29	11	5	0	—	—	—	—	1	1	0	—	—	—	—
	—5° C.	94	94	94	90	93	90	83	85	84	88	83	80	59	48	24	7	—	49	13	2	0	—	—	—
	Air-dry open	48	64	61	59	52	47	41	40	46	47	35	30	12	19	5	4	0	18	9	2	2	—	—	—
	Air-dry sealed	80	84	75	90	81	84	71	76	68	81	67	68	63	58	43	45	32	64	61	43	25	16	10	1
Egg-plant	Room	86	88	90	83	82	79	80	71	55	73	66	48	33	17	9	6	1	73	52	30	15	7	1	0
	—5° C.	91	94	86	92	85	85	88	88	81	91	83	82	75	72	63	64	34	86	79	71	65	52	46	24
	Air-dry open	91	92	91	89	86	86	90	84	86	91	89	85	75	73	65	54	34	85	80	70	63	46	30	16
	Air-dry sealed	91	92	91	89	86	86	90	84	86	91	89	85	75	73	65	54	34	85	80	70	63	46	30	16

of this experiment while seeds of the same lot stored sealed air-dry in a room below freezing gave 87 per cent germination. A further study of the data in Table III, which show germination results obtained after 1, 2, 3, 4, 5, 6, 8, and 12 weeks of special storage, indicates clearly the necessity for seeds of high quality if the viability is to be retained under unfavorable storage conditions as indicated here by 25° C. and 35° C. and high humidity. The comparative rates of vitality loss at 25° C. for eggplant seeds stored at laboratory temperature demonstrates the favorable effect of drying the seeds to approximately 7 per cent moisture content before storage. Not only do the seeds give a higher percentage germination immediately after removal from storage than either those stored open or sealed air-dry, but their ability to withstand unfavorable further storage is marked as compared with air-dry seeds stored open or sealed. Seeds with a germination capacity of 75 per cent were able to withstand unfavorable storage for at least eight weeks without a significant decrease in vitality. Those less viable and showing only 59 per cent germination deteriorated more rapidly, four weeks representing the maximum safe storage. Seeds with only 37 per cent viable suffered serious deterioration by the end of two weeks at 25° C. Seeds from open storage in a room below freezing behaved in a manner similar to those dried and sealed in the laboratory. This was to be expected since the vitality was similar in the two lots (76 and 75 per cent). Lots with slightly higher vitality (87 and 83 per cent) showed a less rapid decline in germination capacity as indicated by the response after 12 weeks at 25° C. (Table III and Fig. 5).

Both onion and tomato seeds deteriorated more rapidly at either 25° or 35° C. than did those of eggplant. A comparison of the behavior of the three types of seeds at 25° C. after cold room storage under various conditions is shown in Figure 5. The differences in the responses of seeds of only slight differences in vitality after further storage at 25° C. and 76 per cent relative humidity is evident at once. When seeds of all three varieties were tested directly after removal from favorable storage in the cold room, all conditions appeared equally good for the retention of vitality. Whether storage had been open or sealed, onion, tomato, and pepper still produced a high percentage of seedlings after storage for eight years. That the storage conditions were not equally good, however, was shown by the behavior of seeds of these same lots placed at 25° and 76 per cent relative humidity.

In spite of the fact that the germination capacity of onion seed stored air-dry, open, or sealed in a cold room for eight years was still 85 per cent, these seeds were without economic value (germination reduced to 14 and 29 per cent) within a week after removal from the favorable storage condition to 25° C. That drying to a moisture content of approximately 8 per cent before sealing was beneficial, was shown by the higher germination capacity, 94 per cent as opposed to 85 per cent, upon removal from favora-

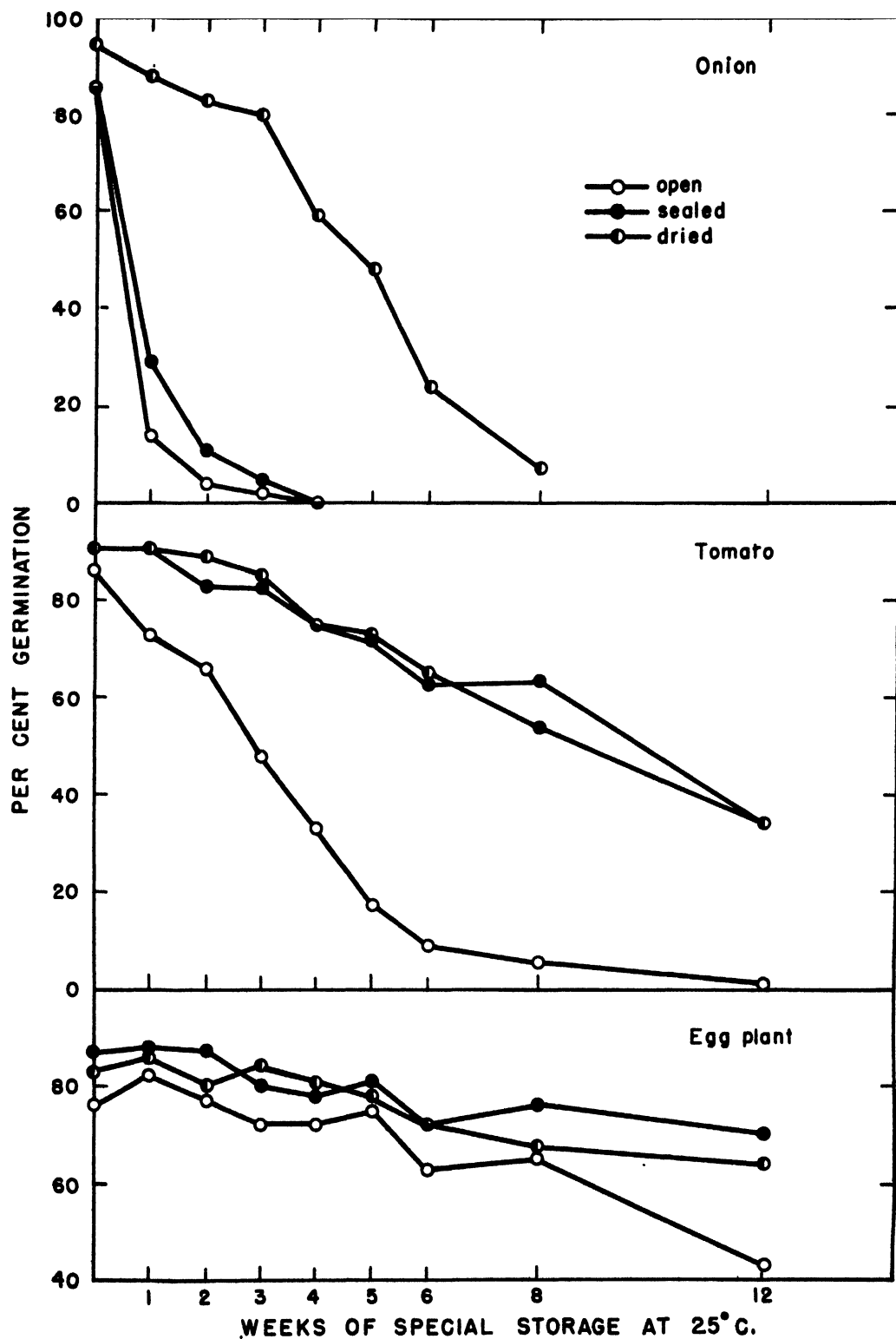


FIGURE 5. Deterioration of 8-year-old seeds after removal from cold room storage to 76 per cent relative humidity at 25° C.

ble storage condition and by the increased ability to withstand unfavorable storage conditions. In the last case, onion seeds could be kept at 25° C. for at least three weeks after removal from the cold room and still give a germination of 80 per cent.

The same effect, though less pronounced, was noted for tomato (Fig. 5). Sealing, and not drying, however, proved most beneficial for these seeds under the conditions of the present tests. This also applied to eggplant but to a lesser degree (Fig. 5).

Comparable results were obtained after storage at 35° C. with the exception that deterioration of all seeds was more rapid than at 25° C.

Deterioration of all seeds at 5° C. which is a favorable storage temperature was much less rapid in spite of the high humidity of the 5° C. room (Table III).

It is surprising that seeds giving germination up to 85 and 86 per cent as in the cases of onion and tomato should deteriorate so much more rapidly under unfavorable conditions than seeds with 90 to 94 per cent germination capacity. The indication is that deterioration of a seed lot, once initiated, proceeds rapidly to the death of all of the seeds. Different seeds vary in their sensitivity to adverse conditions. Onion and tomato are very sensitive while eggplant seeds with as low as 75 per cent viability can be kept for six weeks at 25° C. and still be 70 per cent viable.

The better the storage condition for keeping the seeds viable, the better the chance of survival when they are removed to an unfavorable condition. Apparently the seeds experience no injurious "shock" upon transfer from a storage room below freezing to 25° C. or 35° C., since in all cases seeds so treated kept better than those transferred from laboratory storage. This simply confirms the many reports which state that low temperature is best for storage. The important thing in resistance to later unfavorable storage conditions is the viability of the seed upon its removal from storage regardless of the temperature of storage. Seeds dried and stored sealed in the laboratory may keep better than those stored open in a room at 5° C. where high humidity prevails, as has been demonstrated for several flower seeds (6). In these cases, then, resistance to further storage would be in favor of the original laboratory storage.

Other conditions being equal, low temperature has always been found to be superior to higher temperatures for maintenance of viability.

The results here reported serve to confirm the fact already pointed out (4, 6), that the keeping quality of any particular lot of seeds does not depend upon initial vitality but upon storage conditions. Seeds of low germination capacity may be stored successfully for fairly long periods if the storage conditions are favorable. On the other hand, seeds in which deterioration has been initiated, even if the germination capacity is still high,

are incapable of remaining viable for long periods under adverse storage conditions.

In the light of these results, it is likely that the germination standards for onion and tomato, 70 and 75 per cent respectively, as reported by McIntyre (12) are too low, unless the seeds are planted immediately after testing since those seeds might be subject to rapid deterioration under unfavorable storage conditions. The standard of 60 per cent given by McIntyre for eggplant would probably be adequate. Similarly, the arbitrary minimum standards of viability for onion and tomato given by Boswell and others (7) as 70 and 80 per cent respectively might be too low. The time required for a significant loss of vitality or to reach a minimum standard under a given set of conditions would depend upon the initial germination capacity of the seed lot.

Heinrich (11) used seeds of the same germination capacity but of different ages and found that old seeds were less resistant to unfavorable storage conditions than fresh seeds. Tests to determine this point are now in progress in this laboratory. The present report includes seeds of different germination capacities but of the same age.

SUMMARY

The amount of water absorbed by seeds of lettuce, onion, tomato, flax, peanut, and long-leaved pine at 5°, 10°, 20°, and 30° C. at relative humidities of 35, 55, and 76 per cent was determined. It was noted that the seeds showed differential water absorption according to species. In the order of increasing water-absorption capacity, the seeds were peanut, lettuce, flax, pine, tomato, and onion. This order persisted regardless of storage temperature or atmospheric humidity. With a relative humidity of 35 per cent, seeds took up approximately the same amount of water at 5° and 10° C. but, in every case, less water was absorbed at the higher temperatures of 20° and 30° C. At 55 and 76 per cent relative humidities, however, the peak of moisture absorption was at 10° C. and the lowest absorption at 30° C.

Comparisons of curves showed no direct relation of actual amount of water absorbed to germinability. However, it was found that seeds of high initial vitality were much more resistant to unfavorable storage humidities and temperatures than were those with low initial vitality. This fact was also demonstrated in a test using 8-year-old seeds of eggplant, onion, and tomato selected to give a range in viability. It was shown that deterioration of a seed lot, once initiated, proceeds rapidly to the death of all the seeds under unfavorable storage conditions.

Moisture determinations made on seeds of carrot, eggplant, lettuce, tomato, and long-leaved pine, which were stored open in the laboratory, showed the moisture contents in August approximately double that in

January or February. It is believed that these fluctuations contribute to deterioration of seeds in open storage.

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USE OF SULPHUR AS A REAGENT FOR THE ESTIMATION OF THE SULPHYDRYL GROUPS OF EGG ALBUMIN

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Although much work has been done in recent years on the SH groups of proteins, especially egg albumin, the possibility of using sulphur as a reagent for this purpose has been neglected. While it has been known since the work of Heffter (5) that egg albumin reacts with sulphur to form H_2S , this fact is seldom mentioned in discussions of the sulphydryl groups of egg albumin or if mentioned receives only passing attention. For example, Mirsky and Anson (7, p. 309) say, "Heffter attempted to estimate protein SH groups by measuring the amount of hydrogen sulfide formed." In view of our results, it would be more accurate to say that Heffter was the *first* to estimate protein SH groups and by measuring the amount of H_2S produced from a mixture of egg albumin and sulphur found that 10 g. of egg albumin yielded 9.5 mg. of H_2S . This calculated as cysteine per cent gives a value of 0.68 which is not far from the average value of 0.60 found in the present work in which sulphur was used as a reagent for SH.

The purpose of the present paper is to show how the highly specific reaction of SH with sulphur to form H_2S may be used for the study of SH in proteins and to offer confirmation of the SH values for egg albumin obtained by workers who used less specific reagents for this purpose.

METHODS

Preparation of egg albumin. Crystalline egg albumin was prepared from eggs not over one day old by the procedure described by Heidelberger (6, page 83). In all cases the egg albumin was recrystallized three or more times. Comparison of the sulphydryl value at the third and fifth or sixth recrystallization showed no significant difference. After recrystallization the egg albumin was stored at 5° C. in the ammonium sulphate mother liquor with a few drops of toluene. For use in the experiments it was centrifuged down, taken up in water, and dialyzed in collodion bags with running tap water containing a little toluene until on dialysis for an hour or more against distilled water the dialyzate gave a negative test for the ammonium ion with Nessler's reagent and for the sulphate ion with barium chloride. This dialysis usually took 40 hours. Occasional analysis of the dialyzed egg albumin for ammonia by aeration with Na_2CO_3 into standard H_2SO_4 insured absence of ammonia from the egg albumin solutions. After dialysis a preliminary total nitrogen determination was made by the micro method of West and Brandon (12). From this value the necessary dilution to give

the desired egg albumin concentration was calculated. Two samples of 5 or 10 cc. were then taken from the filtered and diluted solution for determination of total nitrogen by the Kjeldahl-Gunning-Arnold method (2, page 21). The amount of egg albumin was then calculated by assuming the nitrogen content of egg albumin to be 15.6 per cent.

Reaction of egg albumin with sulphur. In general the procedure previously used in the determination of glutathione was used (4). The most important change was to use more sulphur with egg albumin and for this reason a colloidal solution of sulphur was used in place of the alcoholic solution previously employed. This change was necessary, since without it the amount of H_2S produced was not proportional to the amount of egg albumin when amounts over 50 mg. were used. The colloidal sulphur was prepared by rapidly pipetting 10 cc. of a hot, saturated solution of sulphur in absolute alcohol into 50 cc. of water in a 250-cc. beaker, heating to boiling on a hot plate, and then adding 10 cc. more of the hot, saturated solution of sulphur in absolute alcohol to the boiling solution. The alcohol was then removed by boiling down to about 20 cc. and the solution was made up to 25 cc. with water. Fresh reagent was prepared for each experiment.

In the case of the experiments with the native protein, 10-cc. portions containing a known amount of egg albumin were pipetted into Van Slyke-Cullen (11) aeration tubes, 10 cc. of $M/15$ phosphate buffer at pH 7.05, about 50 mg. of cetyl alcohol to prevent foaming, and 1 cc. of the freshly prepared colloidal sulphur reagent were added and the tube connected to a previously prepared receiving tube containing 25 cc. of 2 per cent zinc acetate. The tubes were then placed in a constant temperature bath at $30^\circ C.$ and aerated rapidly with nitrogen or with nitrogen containing a little HCN for four hours. After this period the amounts of ZnS in the receiving tubes were determined either iodometrically or colorimetrically.

When nitrogen containing a little HCN was used for aeration in the above procedure, it was obtained by bubbling the nitrogen through two flasks containing 90 per cent phosphoric acid (sp. gr. 1.744 at $28^\circ C.$) and then through three tubes 1 inch in diameter and 3 feet long about one-eighth full of crude calcium cyanide (Cyanogas). The nitrogen was then distributed to each determination.

In experiments in which the egg albumin was denatured, this was done by the use of alkali in the presence of KCN. The egg albumin solution, 5 cc., was placed in a Van Slyke-Cullen (11) aeration tube, 5 cc. of 1 per cent KCN and 1 cc. of $N/1$ NaOH added, and the mixture allowed to stand for one hour at room temperature (about $28^\circ C.$). The solution was then neutralized with 10 cc. of $M/15$ phosphate buffer, pH 7.05 and 2 cc. of 0.962 N HCl. This adjusted the pH to about 6.9. Cetyl alcohol and sulphur were then added and the determination continued as described above for native egg albumin.

Determination of H_2S iodometrically. In the iodometric method the H_2S produced was determined by adding 10 cc. of N/1000 or 5 cc. of N/200 KIO_3 to each receiving tube, depending on the amount of H_2S expected. This was followed by 2 cc. of 1 per cent freshly prepared KI, 10 cc. of 1:5 HCl, and finally 1 cc. of 1 per cent starch prepared according to Morrow (8, page 225). After waiting for complete solution of the zinc cyanide, if present, the excess iodine was titrated with N/1000 $Na_2S_2O_3$.

Determination of H_2S colorimetrically. In the colorimetric method, methylene blue was developed *in the receiving tube* by adding 5 cc. of 0.1 per cent solution of *p*-amino-dimethylaniline hydrochloride in 20 per cent HCl and 5 cc. of M/50 $FeCl_3 \cdot 6H_2O$ in 1:9 HCl. Standards were prepared by bubbling H_2S into 100 cc. of N/20 NaOH for ten minutes adding 10 cc. of N/1 NaOH and diluting to 1.5 liters. This solution was then roughly standardized and an aliquot diluted so that 5 cc. would contain very nearly the amount of H_2S expected from the egg albumin. The final solution was then pipetted in 5 cc. quantities into five receiving tubes containing 25 cc. of 2 per cent zinc acetate. Three of these were titrated according to the iodometric procedure outlined above and methylene blue was developed in the other two which were used as colorimetric standards. After standing closed overnight, the standards and determinations were made up to 50 cc. in volumetric flasks and carefully compared in a colorimeter. Two or three readings were taken on one side, the cups exchanged, and two or three readings taken on the other side of the colorimeter. The averages of these were used in calculation of the amount of H_2S produced.

Since it is customary to express the amount of sulphydryl in proteins in terms of cysteine per cent, this value has been calculated from the equation $2RSH + S \rightarrow H_2S + RSSR$, or 1 cc. of N/1000 H_2S is equivalent to 0.121 mg. of cysteine.

A typical experiment. Two preparations of egg albumin were used. One was recrystallized three times and the other was from the same lot of eggs but recrystallized five times. After dialysis for two days against running tap water containing a little toluene, both lots were negative for NH_4 when dialyzed against distilled water. Both were made up to 57 cc. Micro-Kjeldahls on 1 cc. of 1/11 dilution of this gave approximate egg albumin values of 164 and 161 mg. per 10 cc. of the original solution. After filtration samples were taken for macro-Kjeldahls, which later gave values of 168 and 164 mg. egg albumin per 10 cc. respectively. Portions of the solution were then used for reaction with sulphur by the procedure previously outlined. In this particular experiment the aeration was with nitrogen containing a little HCN. Determination of the H_2S was by the colorimetric procedure. In one case 10 cc. of the egg albumin solution was used and was not denatured; in the other, 5 cc. of the egg albumin solution was used which was denatured with 5 cc. of 1 per cent KCN and 1 cc. N/1 NaOH for one hour

at 26° C. The aeration was for four hours at 30° C. Standards were solutions of H_2S in dilute NaOH equivalent to 7.5 cc. of $\text{N}/1000 \text{ Na}_2\text{S}_2\text{O}_8$ and prepared as outlined previously. The results are given in Table I.

TABLE I

RESULTS OF A TYPICAL EXPERIMENT IN WHICH THE SULPHYDRYL CONTENT OF NATIVE AND DENATURED EGG ALBUMIN WAS DETERMINED BY REACTION WITH SULPHUR

Stage of recrystallization	Native egg albumin		Egg albumin denatured with NaOH and KCN	
	Mg. egg albumin	SH as cysteine per cent	Mg. egg albumin	SH as cysteine per cent
3rd	168	0.58	84	1.21
"	168	0.57	84	1.17
5th	164	0.59	82	1.15
"	164	0.60	82	1.18

RESULTS

The results of most of the experiments using various combinations of the procedures outlined in this paper are summarized in Table II. The values given are all averages of duplicates. Since the various combinations of the procedures used gave slightly different values, some study was made of the errors involved, so that correction could be made for these. One angle of approach was to determine the recovery of SH from glutathione by the various procedures and base the corrections on this. It was also found that contrary to previous statements, HCN did have some effect on the final determination of the H_2S . In the iodometric procedure the determination of known amounts of H_2S in the presence of HCN as used in the above procedures gave values of 9 per cent too high. With the colorimetric procedure the values with known amounts of H_2S were 5 per cent too low. Average values corrected on the basis of recovery from glutathione and on the effect on HCN are given in Table II. Values in which the aeration was for eight hours are high, especially where HCN was used, since HCN has a slight tendency to convert S-S to SH under the conditions of these experiments.

Taking the average of all the corrected values, one arrives at the value of 0.60 cysteine per cent for the SH content of native egg albumin and 1.29 cysteine per cent for the SH content of egg albumin denatured with alkali in the presence of KCN .

DISCUSSION

The above results are strong evidence that native egg albumin contains sulphydryl groups and supports the conclusion of Anson (1) that the sulphydryl groups of native egg albumin are free and accessible but relatively inactive toward such reagents as ferricyanide, porphyrindin, or nitroprusside, but active toward iodine and iodoacetamide. It might be stated here,

TABLE II
SULPHYDRYL VALUES FOR EGG ALBUMIN OBTAINED BY VARIOUS PROCEDURES

Native egg albumin									
Aerated N ₂ plus HCN			Aerated N ₂ without HCN			Egg albumin denatured with NaOH and KCN. Aerated N ₂ plus HCN			
Iodometric			Iodometric			Iodometric		Colorimetric	
Mg. egg albumin	SH as cysteine per cent	Mg. egg albumin	SH as cysteine per cent	Mg. egg albumin	SH as cysteine per cent	Mg. egg albumin	SH as cysteine per cent	Mg. egg albumin	SH as cysteine per cent
400	0.74	115	0.59	376	0.54	115	0.58	165	1.47
200	0.73	150	0.72*	188	0.53	150	0.58*	83	1.57
100	0.70	150	0.64	115	0.56	150	0.54	41	1.42
50	0.67	185	0.56	95	0.49			21	1.46
376	0.70	93	0.59	95	0.58*			152	1.30
188	0.70	46	0.58	100	0.50			76	1.38
124	0.61	251	0.58	100	0.53*				
124	0.63	126	0.64	150	0.58*			63	1.25
152	0.60	168	0.58					84	1.19
76	0.67	164	0.60					82	1.17
115	0.64	262	0.54					262	1.13
		131	0.59					131	1.11
Average	0.67		0.59		0.52		0.56		1.43
Corrected for recovery	0.64		0.62		0.59		0.64		1.36
Corrected for effect HCN	0.61		0.62		0.52		0.56		1.31
									1.24

* Aerated 8 hours instead of 4 hours. Omitted from average.

that after reaction with sulphur the egg albumin did not appear to be denatured. It was still coagulable by heat in acid solution and did not reduce ferricyanide.

Since the cysteine plus cystine content of egg albumin is about 1.3 per cent (9, p. 217), the sulphur procedure which gives 1.29 cysteine per cent after denaturation with alkali and KCN, shows that all of the potential SH groups of egg albumin belong to cysteine or cystine. This is in agreement with Mirsky and Anson (7) who found the SH content of reduced denatured protein to be 1.14 per cent. Greenstein (3) has found that egg albumin denatured with guanidine reduces porphyrindin to the extent of 1.24 cysteine per cent. The value 0.60 cysteine per cent for native egg albumin indicates that half of the cysteine in native egg albumin is in the reduced form, while half may be in the oxidized form (cystine). This agrees with the findings of Mirsky and Anson (7) and of Todrick and Walker (10) on denatured egg albumin.

If we assume a molecular weight of 40,000 for egg albumin, we arrive at the conclusion that native egg albumin has two SH groups and one S-S group in the molecule. It appears that two SH groups are formed from the S-S group by the reducing action of KCN, so that egg albumin after treatment with NaOH and KCN has four SH groups.

SUMMARY

Using sulphur as a reagent for the determination of SH groups in egg albumin, the SH content of native egg albumin was found to be 0.60 in terms of cysteine per cent. After denaturation with NaOH and KCN the SH content was found to be 1.29 cysteine per cent. This later value accounts for all of the cysteine plus cystine present in egg albumin. Assuming a molecular weight for egg albumin of 40,000, the above values indicate the presence of two SH groups and one S-S group in the egg albumin molecule.

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FLOWERING IN DIGITALIS PURPUREA INITIATED BY LOW TEMPERATURE AND LIGHT

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Foxglove (*Digitalis purpurea* L.) plants have been grown in the greenhouses of the Institute for several years at night temperatures as low as 55° to 60° F. without producing a single flower. The plants grow well and increase laterally by producing new clusters of leaves around new growing points so that after 18 months' growth a single plant occupies all of the available space in a 10- or 12-inch pot. *Digitalis* almost invariably flowers in June, however, if well grown plants are planted in the open soil in August or September and allowed to remain outside all winter. The long period of cold weather initiates certain changes in the plants which prepares them for flowering when warm and long days return the following spring. The present study is concerned with the proper cold treatment and lighting necessary for bringing the plants into flower at any time during the fall, winter, or spring months.

METHODS OF STUDY

The seed for this strain of *digitalis* was obtained originally from Professor Edward Kremers of the University of Wisconsin and was grown from plants used in a previous study (5) on *digitalis* glucosides as affected by ultra-violet radiation. Analysis for glucosidal content has shown it to be little different from the ordinary garden variety of *digitalis*, and probably it reacts similarly to low temperature and light treatments. Seeds were sowed in November or December and plants were repotted individually as they developed, finally reaching rosettes of leaves one foot or more in diameter, and taking an eight-inch pot by the following May or June.

A set of these plants was placed on a cold treatment schedule on May 14 and continued until September 30. The treatment during the first year consisted of moving the plants to a cold room held at 50° F. each evening, where they were left during the night and were then returned either to the greenhouse or to open sunlight (weather permitting) each morning. At the end of September the plants were stationed permanently in the greenhouse held at 55° to 60° F. each night and were given intermittent lighting (cycle 10 minutes on and 20 minutes off from 6:00 p.m. to 6:00 a.m.) using 500-watt Mazda lamps. About 50 per cent of the plants came into flower in November following this treatment. The control plant held continuously in the greenhouse (Fig. 1 A) did not flower. A plant from those receiving the cold treatment is shown in flower (Fig. 1 B). It appeared from this response

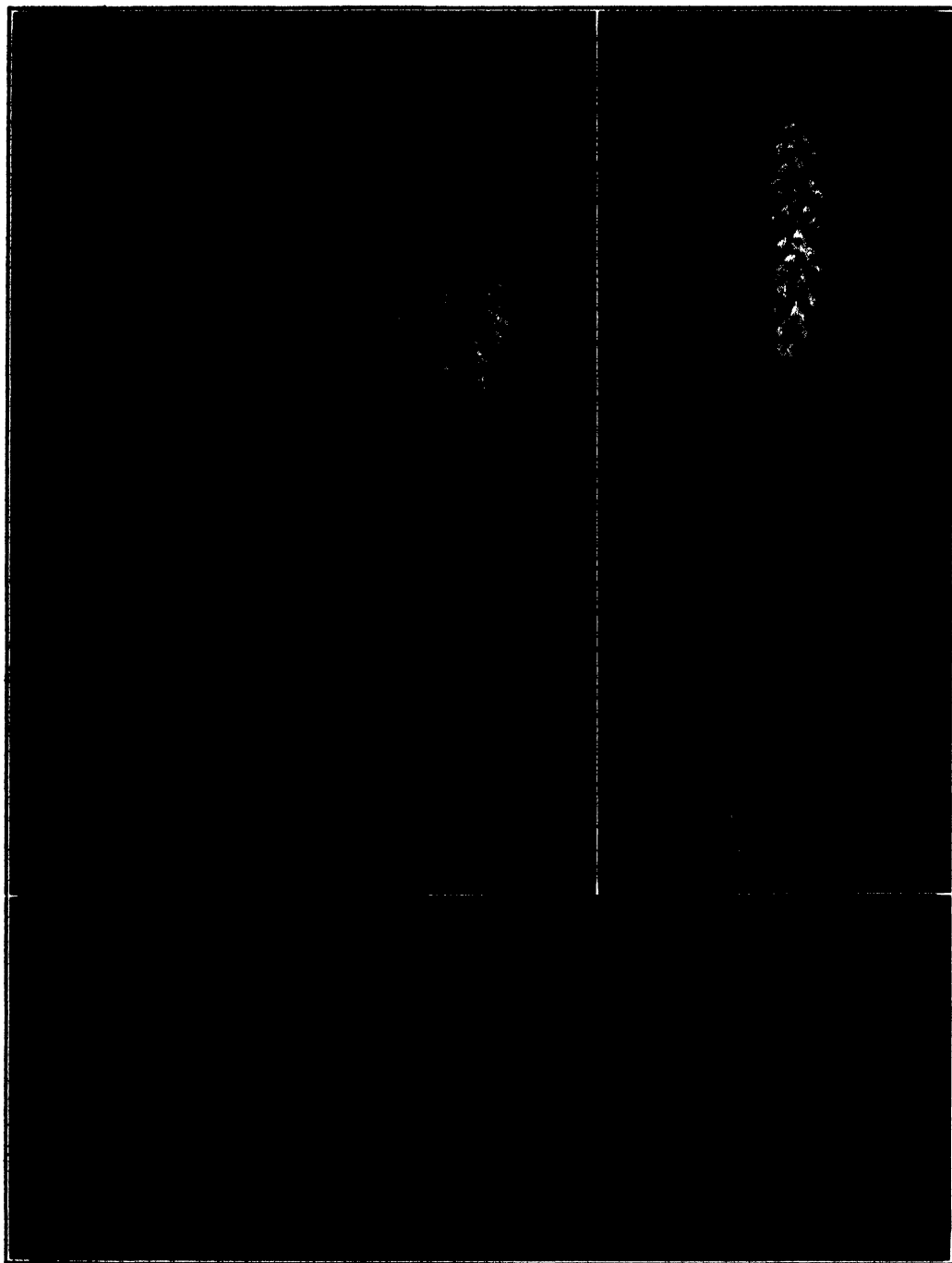


FIGURE 1. *Digitalis* after low temperature treatments. A and B, November 18, 1938. A. Control held continuously in greenhouse. B. Dark Room at 50° F. each night, May 14 to September 30, then long days in warm greenhouse. C. January 4, 1940. Same dark room as B except week-ends only outside from May 10 until September 12. D. Held continuously in 41° F. dark room from May 10 to September 12. E. Same as D, except returned to outside each week-end. F. Cold dark room each night and outside each day. (D, E, F. Condition of plants at end of cold treatment.)

that a period of low night temperatures of approximately four and one-half months was required to bring digitalis plants into flower. A note on this first year of the work has already been published (1).

During the following year tests were made to determine more convenient methods for giving these plants the required low temperature treatment. Sets of plants were kept in the cold dark room continuously at 50° F. Other sets of plants were kept in the cold dark room continuously except that they were exposed out-of-doors from Saturday morning until Monday morning when they were again returned to the cold room, thus spending only the week-end in sunlight. A third set was kept in the cold room at night and out-of-doors each day. The treatments were carried on in each case from May 10 until September 12. Representative plants from each set are shown in Figure 1. Those plants of the first set (Fig. 1 D) kept continuously in the cold dark room were severely injured by the treatment and only a few survived. The second set which spent each week-end outside came through in excellent condition (Fig. 1 E). The plants were dark green in color and in a vigorously growing condition. Many of these plants flowered when given subsequent long-day lighting treatment in the greenhouse. A few failed to flower but continued vegetative growth. The third set of plants which were in the cold only at night survived the treatment but did not grow well. They formed closely set, flat rosettes of leaves, dwarfed in height as shown in Figure 1 F. They were slower to produce flower stalks when brought into the long-day lighting treatment but many of them flowered.

The results of this second year test showed the advantage both to the plants and to the operator of keeping the plants in the cold dark room continuously except for the week-end exposure of two days and nights out-of-doors. The plants grew slowly during the long cold period at 50° F. and were in fair condition at the end of it ready for the flowering response when brought under long day conditions. At the same time the operator was relieved of the labor of moving the plants from the cold room to the open sunlight and back again each day. The flowering response following the return to long day conditions was not as uniform as desired. A few of the plants failed to flower. One of the flowering plants was photographed on January 4, 1940 and is shown in Figure 1 C.

In the third year of the tests the plants were started on the cold treatments on June 7 and continued until October 12. Two cold dark room temperatures, 41° and 50° F., were used in the study. Only one set of plants was used in each cold room treatment and the plants were kept continuously in the cold rooms all of each week except that they were brought out into sunlight Saturday morning and left until Monday morning, thus giving the plants the same treatment which was found best the preceding year, except that a lower temperature was used in one case. Practically all of the plants

from the 50° F. cold room died from injury brought about by the sudden shift to low temperature, and were discarded following the second week-end exposure outside, while those from the 41° F. cold room showed very little injury. It is not known at present whether this failure to survive was due to the higher outside temperatures in June when the treatments were started as compared to the lower temperatures prevailing at the start in May, the year before, or to the fact that a light of low intensity was inadvertently left burning in this cold room for two weeks at the start of the cold treatment. Further work must be done to determine the cause of such lethal shocks when starting the cold treatments. The plants transferred to the 41° F. cold room withstood the shock without any great injury. The lower temperature apparently protected the plant against such injury. However, a slat shelter was provided after the first two week-end exposures out-of-doors, which shielded the plants appreciably from the high intensity June sunlight.

The plants were removed from the cold treatment and placed permanently in the greenhouse on October 12 and were exposed to various lighting arrangements each night. These plants were in excellent condition at the end of the treatment. Some plants received intermittent light from 500-watt lamps (cycle 10 minutes on and 20 minutes off from 6:00 p.m. until 6:00 a.m.). Another group was exposed continuously from 6:00 p.m. to 6:00 a.m. to the light from an 85-watt fluorescent lamp (Cooper Hewitt type). A third group received intermittent light from a 400-watt high intensity mercury lamp (type H-1, lighting cycle 1 hour, 33 minutes on alternated with 2 hours, 20 minutes off from 6:00 p.m. until 6:00 a.m.). All plants exposed to these lights came into flower in November and December. This cold treatment seemed to be the most satisfactory since more than 95 per cent of all individual plants so exposed came into flower following the return to long days. In most cases only four or five weeks of lighting was required to bring the plants into flower. Plants treated at 50° F. the year before required nearly three months of lighting to bring them into flower. The lower temperature of 41° F. was therefore much more favorable. Two or three of the two-year-old plants which were carried over from the tests of the preceding year were given the cold treatment, but did not flower when exposed to the long days. These plants were in very poor vegetative condition when removed from the cold treatment so that this might account for their failure, rather than the fact that they were one year older than those which came into flower. A single plant which had received the cold treatment was placed in a greenhouse with no supplementary light at night. A flower stalk developed but many of the terminal florets were abortive. Late in December this plant produced several laterals which flowered normally. It is therefore possible to produce some flowers on *digitalis* under short day conditions, but long days greatly favor rapid flowering. On the

other hand, no plants in our experience have ever reached the flowering stage without first having a period of low temperature treatment.

EXPOSURES TO OUTDOOR TEMPERATURES REQUIRED FOR FLOWERING

All of the tests reported so far have been with plants exposed for a certain period in a cold room at a constant low temperature. It has been mentioned previously that plants set out-of-doors during the summer or early fall and left out all winter come into flower normally the following June. In tests made during the first year of the present study it was found that three out of five plants removed from a heated greenhouse in January and placed in an unheated greenhouse or cold frame would flower in June. During the past summer a number of plants were set in the open soil in June and grown until October. They were then placed in large pots and removed to a cold frame where they would be exposed to the normally cool temperatures prevailing in this region during the fall and winter months. Two or three plants were brought into the warm greenhouse (55° to 60° F. at night) each month from October to January and given supplementary light. The first plants came into flower in early March from those which were brought in on December 18. Only one out of three plants brought in during November had produced a flower bud by April, while those brought in during January were also showing several buds by April. Flowering response was at a slower rate for all of these plants as compared with those treated in the cold room and exposed to light for a short period each weekend. This may have followed from the poor vegetative condition since plants which appeared to be in good vegetative condition at the end of the cold treatment were first to flower under the lights. It was apparent, however, from these studies that plants left out-of-doors in the fall had reached their potential flowering stage by the middle of December. Since outdoor temperatures at night often drop to 50° F. during the last half of September in this region, this would allow the plants a natural cold treatment of approximately three months necessary to bring them to the flowering stage. It would then require an additional two and one-half months of lighting to bring the flowers out. In contrast with this natural cold treatment it has already been pointed out that flowers are brought out by approximately one month of lighting in October and November following the carefully regulated cold storage treatment from June to October as already described. Since the red-purple flower spikes are large and attractive (often reaching a height of five feet) these plants might meet some demand for flowers at Christmas time.

DISCUSSION OF COLD TREATMENTS

Thompson (9) has summarized the results of considerable study of the relation of temperature and day length to the flowering of certain vegetable

crops, especially biennials. Such plants as cabbage, celery, and beets produce flowers and seed stalks the first year from seed if grown for a period of 50 to 60 days at a comparatively low temperature of about 50° to 60° F. The essential difference between winter wheat and spring wheat as regards the response to flowering on long days is that winter wheat requires a period of low temperature at some time during its growth before long days are effective in bringing it into flower. Gassner (4) found that winter wheat seeds, germinated slowly at temperatures just above freezing, came into flower very quickly. Lyssenko and others (reviewed by Whyte, 10) have shown that this cold treatment can be given during a slow germination of the seeds and has named the process by a Russian term, which we translate as "vernalization." The conclusion might be drawn at once from this work that there might be vernalization of any seed which produces a plant requiring at some stage in its development a cold temperature period preliminary to flowering. If we were prone to generalize on such responses we might expect that soaked seeds of beets, cabbage, celery, or digitalis might be vernalized by cold temperature so that they would produce flowers at once when returned to long days at high temperature. Lojkin (6), working at this Institute for more than two years on this problem, failed to find positive evidence of such vernalization in these seeds of biennial plants, although she confirmed the findings of previous workers as to winter wheat and published only on this part of the work. Slogteren (8) and others (2) have shown that hyacinths and daffodils require a period of low temperature storage after planting before they can be brought into the proper condition for flowering. This period is normally from two to three months for hyacinth at a temperature of 50° F. in a dark storage room. During such preliminary cold treatment the young shoot slowly emerges above the soil level and at the end of the treatment the plants can be brought into flower rapidly by exposure to long days and higher temperatures.

Digitalis clearly belongs to that group of plants which must be well grown before it can be converted into the flowering stage by a long exposure to cool temperatures. This type of response should not be confused with vernalization or other effects which take place in seeds. Owen, Carsner, and Stout (7) have proposed the term "photothermal induction" for a combination response to both thermal and photoperiodic induction in sugar beets. Since the comparable flowering response brought about by day length has been called "photoperiodism" by Garner and Allard (3), we propose that this flowering response in plants brought about by low temperature be called *thermoperiodism*, a response considered independent of day length however important this may be in the subsequent rate of development of the flower stalk.

SUMMARY

1. Plants of *Digitalis purpurea* which had been given a cold treatment of approximately four months (June to October) at 41° F. came into flower rapidly when returned to a long day and higher temperature in a greenhouse.

2. The best method found for preserving a slow growth and an excellent appearance of the plants during the long storage treatment was to remove the plants to a slatted shade in sunlight for two days and nights each week.

3. Plants left out-of-doors in cold frames until December 18, then returned to a long day and higher temperature in the greenhouse, flowered after two and one-half months, while approximately one month of lighting was required after the more carefully regulated cold room treatment at 41° F.

4. It is proposed that this flowering response in plants brought about by low temperature be called *thermoperiodism*.

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PRELIMINARY EXPERIMENTS ON THE CONTROL OF THE HOLLY LEAF MINER

ALBERT HARTZELL AND GEORGE F. MCKENNA

The holly leaf miner (*Phytomyza ilicicola* Loew) is the most serious insect pest of holly (*Ilex opaca* Ait.) in eastern North America. The chief injury is caused by the larvae mining the leaves, but puncturing of the leaves by the female with the ovipositor for the purpose of egg laying, and possibly for feeding, causes leaf distortion (Fig. 1). The miner confines its attack chiefly to *Ilex opaca*, and is not a serious pest of *I. cornuta* Lindl. or *I. aquifolium* L.

Nicotine and soap sprays have been recommended for the control of the holly leaf miner by Felt and Rankin (5) and by Britton (1). Excellent results are reported by Downes (3) in the control of European holly leaf miner (*Phytomyza ilicis* Curt.), a related species, in British Columbia, with a 2 per cent nicotine-lime dust.

A spray consisting of 5 lb. lead arsenate and 1 lb. S.S.S. Spreader made up to 100 gal. with water was found by Felt and Bromley (4) to give satisfactory control of *Phytomyza ilicicola* in Connecticut.

All authors stress the importance of applying the spray or dust at the time the adult flies are present on the foliage, if the desired results are to be obtained.

While satisfactory control has been reported by a number of investigators, Langford and Cory (6) failed to obtain control in Maryland with nicotine, rotenone, pyrethrum, and cyanide gas.

In the present investigation some of the more promising sprays reported by others were tested together with new combinations. There was a heavy infestation of holly leaf miner at Yonkers, New York, where these experiments were conducted. The work extended over three seasons.

MATERIALS AND METHODS

Arrangement of plots. Randomized plots were laid out in a holly (*Ilex opaca*) planting eight years old grown from seed and from cuttings. Each treatment was replicated at least three times. The plot arrangements for 1940 are shown in Figure 2.

Types of treatment. The types of treatment during the season of 1938 consisted in spraying or dusting the foliage of holly with the following mixtures: (a) powdered lead arsenate (5 lb.) and soybean flour (1 lb.) made up to 100 gal. with water, applied twice; (b) molasses (24 gal.) and nicotine sulphate (1 qt.) made up to 100 gal. with water, applied once; (c) powdered



FIGURE 1. Holly leaf miner. A. Adult fly ($\times 15$); B. mines produced by larvae in holly leaf ($\times 5$); C. lower side of the leaf showing punctures by ovipositor ($\times 0.5$); D. distorted leaf induced by oviposition punctures ($\times 0.5$); E. a young leaf (enlarged) photographed 4 days after-exposure to adults ($\times 5$). (Note punctures made by ovipositor.)

North plot				
		T 191	TL 997	CK 698
			F 535	FL 470
T 497	FL 114	CK 1216	TL 456	F 443
CK 1395	TL 276	F 368	FL 401	T 237
South plot				
FL 298	FN 45	CK 722	FNL 11	
CK 770	FNL 147	FL 365	F 929	
F 593	FNL 33	FN 73	CK 1138	
FL 205	F 209	F 288	FL 348	
FN 84	CK 812	FN 110	FNL 234	

CK = No treatment.

T = Textac.

TL = Textac + lead arsenate.

F = Fish oil.

FL = Fish oil + lead arsenate.

FN = Fish oil + nicotine sulphate.

FNL = Fish oil + nicotine sulphate + lead arsenate.

FIGURE 2. Holly leaf miner plot arrangement—1940.

calcium arsenate (1 part) and hydrated lime (9 parts), applied twice; (d) β , β' -dichloroethyl ether¹ (1%) and powdered calcium arsenate (99%), applied twice; (e) non-treated check.

During the season of 1939 treatments were made as follows: (a) powdered lead arsenate (2.5 lb.) and calcium caseinate (1 lb.) made up to 50 gal. with water, applied once; (b) a miscible oil (Scale-O)² (5 gal.) and powdered lead arsenate (2.5 lb.) made up to 100 gal. with water, applied once; (c) Scale-O (5 gal.) made up to 100 gal. with water, applied once, followed by Scale-O (1 gal.) and powdered lead arsenate (2.5 lb.) made up to 100 gal. with water, applied once to the same plot as (c); (d) non-treated check.

Five applications were made of the following spray mixtures during the season of 1940, as follows: (a) fish oil (2 qt.) and powdered lead arsenate (4 lb.) made up to 100 gal. with water; (b) fish oil (2 qt.), nicotine sulphate (1 qt.), and powdered lead arsenate (4 lb.) made up to 100 gal. with water;

¹ Furnished by Carbide and Carbon Chemicals Corporation, New York, N. Y.

² Product of Andrew Wilson, Inc., Springfield, N. J.

(c) fish oil (2 qt.) made up to 100 gal. with water. Four applications were made during the same season of (a) diethylene glycol diabietate (Textac)³ (1 pt.), and powdered lead arsenate (4 lb.) made up to 100 gal. with water to which a 10 per cent aqueous dioctyl ester of sodium sulphosuccinate (Aerosol OT)⁴ at a dilution of 1 to 1000 was added to the last application; (b) Textac (1 pt.) and Aerosol OT 1 to 1000 for the last application; (c) fish oil (2 qt.) and nicotine sulphate (1 qt.) made up to 100 gal. with water; (d) check.

The sprays with the exception of Textac were applied by means of a 50-gal. capacity power sprayer at a pressure of 300 lb. Textac was applied by means of a hand sprayer. The dusts were applied by means of a hand duster.

RESULTS

The sprays and dust were applied in 1938 during the week of May 19 to 25, and on June 22. In 1939 all the sprays were applied May 31, except Scale-O, which was applied May 10 and June 5. The results of the first and second year's work were not promising.

The results obtained in 1940 indicate that fish oil (2 qt.), and nicotine sulphate (1 qt.) made up to 100 gal. with water gave a control of 91 per cent (Table I). No advantage was gained by the addition of lead arsenate to this spray (Table II).

The fact that three applications of Textac alone followed by a single application of Aerosol OT and Textac gave a control of 72 per cent may be of interest since both Textac and Aerosol OT are spreading agents. The presence of lead arsenate seems to detract from rather than add to their efficiency.

In addition to the randomized field plots referred to above, eight holly trees ranging in height from 10 to 12 feet and with a 6-foot spread at the base were sprayed on June 3, 1940, with a mixture consisting of Aerosol OT at a dilution of 1 to 1000 in water, nicotine sulphate (1 qt.), and powdered lead arsenate (2.5 lb.) made up to 100 gal. with water. Counts of mines in the leaves resulted in a total of 72 mines present in the leaves of these eight trees, while the corresponding unsprayed check which consisted of three trees showed a total of 547 mines. This is a reduction of 95 per cent when compared with the check.

No injury that could be attributed to the sprays was observed on the foliage in 1940, except with Textac alone and in combination with lead arsenate.

³ Product of Hercules Powder Co., Wilmington, Del.

⁴ Product of American Cyanamid and Chemical Corp., Bridgeville (Pittsburgh), Pa. According to a private communication from the New York office of this corporation, Aerosol OT is identical with Vatsol OT.

TABLE I
EFFECT OF SPRAYS APPLIED DURING THE SEASON OF 1940 IN REDUCING
THE NUMBER OF MINES IN LEAVES

Spray mixture (Water added to make up 100 gal.)	Dates applied	Av. No. punctures per leaf	No. mines	% Reduction of mines
North plot				
Fish oil—2 qt. Lead arsenate—4 lb.	May 3 May 17 June 7 June 17 July 17	67	984	70
Fish oil—2 qt.	May 6 May 17 June 7 June 17 July 17	114	1346	59
Textac—1 pt. Lead arsenate—4 lb. (added Aerosol OT at 1:1000 on June 14)	May 8 May 20 June 7 June 14	78	1730	48
Textac—1 pt. (added Aerosol OT at 1:1000 on June 14)	May 10 May 17 June 7 June 14	97	925	72
Check		113	3309	0
South plot				
Fish oil—2 qt. Nicotine sulphate—1 qt. Lead arsenate—4 lb.	May 6 May 17 June 7 June 14 June 17	101	425	88
Fish oil—2 qt. Lead arsenate—4 lb.	May 3 May 17 June 7 June 17 July 17	92	1216	64
Fish oil—2 qt. Nicotine sulphate—1 qt.	May 10 May 17 June 14 July 17	102	312	91
Fish oil—2 qt.	May 6 May 17 June 7 June 17 July 17	77	1719	50
Check		115	3442	0

Counts of mined holly leaves (Fig. 1 B) were made in October of each year. In 1940 counts were made of all the mines in the leaves on three representative trees in each plot. Control was estimated by comparing the number of mines in the treated with its corresponding check.

TABLE II
MINES FOUND IN PLOTS TREATED WITH NICOTINE AND LEAD ARSENATE EXPRESSED
AS PER CENT OF MINES FOUND IN FISH OIL CHECK PLOT

Mixture with fish oil		Nicotine	
		Present	Omitted
Lead arsenate	Present	21	60
	Omitted	15	100

In 1940 the number of punctures in leaves on one representative tree in each plot were counted. There appears to be no correlation between the number of punctures and the number of mines. It is well known that species of *Phytomyza* make many punctures in leaves with the ovipositor for the purpose of feeding in which eggs are not deposited or if deposited the eggs never hatch. Cohen (2), for example, reports finding only one larva to approximately 25 punctures for *Phytomyza atricornis* Mg., a related species of leaf miner in chrysanthemum. He points out the fact that male flies are dependent on the female feeding punctures and will starve when caged on unpunctured leaves.

SEASONAL OCCURRENCE AND CLIMATIC FACTORS

In 1938, pupation took place under natural conditions between April 10 and April 15. Adults (Fig. 1 A) reared from detached mined holly leaves containing puparia collected April 15 emerged April 29 and April 30. The first flies were observed in the field on May 16. As they were present in considerable numbers some of the individuals probably emerged several days previous to this date. The flies continued to be present in the field as late as July 12. Leaves were considerably injured by oviposition punctures at this date but mines remained inconspicuous.

Adults emerged in cages in the greenhouse from mined leaves containing puparia on May 28, 1940, when 20 individuals were observed, on May 29, 26 adults emerged, and on May 30, 118 adults emerged, after which date the number of individuals emerging became so abundant that counts were discontinued because of the difficulty in making accurate records of individuals. All the adults had emerged by June 8. The flies were first observed in the field on June 3.

According to the local weather station (7) the mean temperature for

May, 1938, was 59.8° F., and the total precipitation was 3.36 inches. Both these figures are within a degree and an inch respectively of the 70-year average. The mean temperature for June, 1938, was also normal, but the total precipitation was 7.13 inches, which exceeded by 3.61 inches the 70-year average.

The mean temperature for May, 1939, was 64.8° F., which was 4 degrees above the 71-year average. Precipitation was light, only 0.89 inch, which was 2.39 inches below the 71-year average. The mean temperature for June, 1939, was 72.2° F., or 2.2° above the 71-year average. The total precipitation (3.8 inches) was within 0.28 inch of the 71-year average.

The season of 1940 was late. The mean temperature for May was 60.6° F., and the total precipitation was heavy (7.61 in.). The mean temperature for June was 70.4° F. while the total precipitation was 3.01 inches.

During an average season such as in 1938 the flies made their appearance about the middle of May, but in a late season such as 1940 the flies were not observed until the first week in June.

DISCUSSION

Felt and Bromley (4) have stressed the importance of the proper timing of lead arsenate spray for the control of the holly leaf miner. They state that the spray should be applied when the adults are found in abundance on the leaves. Our results tend to show that foliage protected by application of fish oil and nicotine sulphate spray during the period that the flies are present in the field tends to be relatively free from mines. The nicotine sulphate spray has the advantage of being relatively non-poisonous to higher animals and does not leave an unsightly residue. It is probable that our failure to control in 1938 and 1939 may have been due to improper timing of the first application.

SUMMARY

A series of sprays and dusts were tested as to their efficiency to control holly leaf miner (*Phytomyza ilicicola*).

A spray consisting of two quarts of fish oil, and one quart of nicotine sulphate made up to 100 gal. with water sprayed on the foliage gave a reduction in mines of 91 per cent in field plots at Yonkers, New York, as compared with the unsprayed check.

Spray applications were made the second and third weeks in May, followed by an application about the middle of June and a final application around the middle of July.

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A SURVEY OF PLANT PRODUCTS FOR INSECTICIDAL PROPERTIES

ALBERT HARTZELL AND FREDERICKA WILCOXON

The use of plant products as insecticides may be traced through the centuries to ancient times. The Chinese mandarins are said to have used camphor to ward off bubonic plague and thus unwittingly repelled the rat flea, the vector of *Pasteurella pestis* Yersin & Kitasato.

The commercial development of plant insecticides is attributed to a lady of Ragusa, Dalmatia, who noticed dead insects on a discarded bouquet of pyrethrum flowers. She began milling pyrethrum into powder and thus was born the pyrethrum industry.

Another line of development has come through the use of fish poisons by primitive peoples. Derris is used by the Malay natives for killing fish as are also cubé and other rotenone-containing plants in South America.

While many plant products have been used as insecticides in combination with perfumes and repellents (32) particularly with household insecticides, aside from a few well known plant insecticides such as pyrethrum, derris, cubé, and nicotine, the amount of definite information on their toxicity to a given insect is relatively meager.

No attempt will be made here to cite the voluminous literature on the above named, well known plant insecticides or on other rotenone-containing plants except to call attention to a few articles that give reviews of the literature on these subjects.

The literature on plant insecticides up to 1924 has been reviewed by McIndoo and Sievers (18). As the result of testing products of 54 species of plants on various species of insects they conclude that, aside from pyrethrum, temperate zone plants are not worth testing. They considered the chances of finding insecticides in tropical plants, especially fish poisons, very good. Of those tested, derris and cubé were found to be the best.

Stäger (29) found that powders and volatile constituents of nutmeg, eucalyptus, cloves, caraway, silver linden flowers, and a number of other plants are toxic to ants.

Anabesine was found by Orechoff and Menschikoff (21) in *Anabasis aphylla* L., a weed common in Turkistan. Its insecticidal efficiency was tested by Richardson, Craig, and Hansberry (25).

Spies (28) reports that croton resin is more toxic to goldfish than rotenone.

Fagundes (9), in Brazil, lists 89 toxic or insecticidal plants.

Tattersfield (31) reviews the literature of fish poisons up to 1936.

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Plummer (23) reports on the toxicity of *Haplophyton cimicidum* A. DC. to fruitflies. This plant has been used in Mexico to control cockroaches and other household pests. The toxic principle is said to be an alkaloid.

Tests made by Kayumov (15) with essential oils from geranium and lavender indicate that they are toxic to red spider and cotton aphids.

It was reported in India (20) that the addition of cashew nut oil to kerosene increased the toxicity to culicine mosquito larvae threefold.

Landreth (16) observed that Japanese beetles are attracted to castor bean plants and that thousands of dead beetles were found under the plants, presumably killed by eating the foliage. Experiments conducted by Christensen (6) also indicated that castor bean foliage would kill grasshoppers. The suggestion is made that castor beans be planted around grain fields to ward off grasshopper attacks. On the other hand, experiments conducted by Metzger (19), Smith (26), Spain (27), and by the Oklahoma Agricultural Experiment Station (4) indicated that castor bean was not toxic to grasshoppers to the extent that it can be used as a practical insecticide. Drake (7) claims that castor bean plants used as barriers will not control grasshoppers. It was reported (5) from experiments in Brazil that the powdered leaves of castor bean will repel aphids, mosquitoes, and the rust fly. Castor oil was used in combination with pyrethrum as a fly spray by Lefroy (17).

Gibbon (11) found dead Japanese beetles under the fleece vine (*Polygonum auberti*), killed by eating the foliage of this plant.

Pan (22) reports that leaves and flowers of several species of *Prunus*, including *P. persica* and *P. americana*, are toxic to insects.

Infusion of bulbs of *Cyclamen elegans* was found by Pylnov (24) to be toxic to fruit tree pests such as *Paratetranychus*.

Bushland (2) tested various volatile oils as ovicides for screwworm and reported a number that possess high toxicity.

It was reported (8) that the flowers of dwarf buckeye (*Aesculus parva alba*) attract and kill Japanese beetle.

Hwang (14), in China, isolated an unknown alkaloid designated by him as tripterygine, from *Tripterygium wilfordii* Hook., which he found to be a potent insecticide. Recently Swingle, Haller, Siegler, and Swingle (30) found that *Tripterygium wilfordii* is toxic to the silkworm, eastern tent caterpillar, southern army worm, Colorado potato beetle, diamond back moth, imported cabbage worm, codling moth, and several other species of insects.

Haller (12) found that the fruit of the Amur cork tree (*Phellodendron* spp.) yields a quick-acting insecticide toxic to mosquito larvae, houseflies, and codling moth.

MATERIALS AND METHODS

The crude botanical drugs were furnished through the courtesy of S. B.

Penick and Company, New York, N. Y. The plant products in Tables I and II, with the exception of balm of Gilead, European linden, oil of bay leaves, boneset, and wormwood, were obtained locally, chiefly from the Boyce Thompson Arboretum, Yonkers, N. Y., as were also white pine, Scotch pine, Norway spruce, hemlock, wild black cherry, sumac, sassafras, wild geranium, skunk cabbage, quack grass, and various species of ferns except male fern (Tables III and VII). Spearmint, peppermint, sage, chive, rhubarb, Russian tarragon, chamomile, and ground ivy were grown in the Institute herb garden, while gladiolus and geranium were grown in the greenhouses (Tables II, III, and VII).

Covillea tridentata, *Baileya multiradiata*, *Eriodictyon californicum*, and various species of *Ephedra* were obtained from and identified by the Boyce Thompson Southwestern Arboretum,¹ Superior, Arizona (Table VII).

The plants were dried at room temperature. When thoroughly dry they were passed through a meat grinder after which they were ground in a power-driven ball mill overnight or as long as necessary to obtain a granulated product suitable for extraction.

The essential oils, thymol, oleoresin of aspidium, and santonin were purchased from reliable drug firms (Tables IV and VI).

METHODS OF EXTRACTION

Preliminary extractions of plant products were made as follows: Four grams of an essential oil or of ground plant material were placed in 10 cc. of acetone overnight (16 hr.) at room temperature. One and one-half cc. of the resulting extract were used in one liter of water. Water extracts were prepared in a similar manner by substituting water as a solvent for acetone.

Known concentrations were prepared by evaporating the extracts to dryness on a steam bath, reweighing and diluting with the required amount of water or acetone for a given concentration.

In a few cases a Soxhlet extractor was used, but this was found to be unnecessary for preliminary extractions.

BIOLOGICAL TESTS

Preliminary tests were made on guppies (*Lebistes reticulatus* Peters) but this species was discontinued as a test animal when it was discovered that extracts from the roots of *Symplocos paniculata* would kill these fish but did not give an appreciable kill of mosquito larvae (*Culex quinquefasciatus* Say). Thereafter a standard method of testing insecticides was used. As mosquito larvae are relatively easy to kill with insecticides any substance that shows promise is likely to be detected. Tests were made essen-

¹ Through the courtesy of Frederick Gibson.

tially according to the method of Campbell, Sullivan, and Smith (3). Mosquito eggs² were obtained from Orlando, Florida. The eggs were shipped weekly to Yonkers, New York, via air mail. As soon as the eggs were received they were placed in tap water in four-liter beakers at room temperature. The eggs hatched within 24 hours. When the larvae had hatched, yeast was added at the rate of 575 mg. to 150 cc. of water which amount was in turn added to each four-liter beaker containing the mosquito larvae. The following day 500 mg. of blood albumen were dissolved in 150 cc. of water which was added to each four-liter beaker containing the mosquito larvae. Thereafter yeast and blood albumen were added on alternate days in amounts specified above. Larvae five days old were used for testing. Test tubes (25 cc. capacity) containing larvae in solutions to be tested and the checks in tap water or in acetone and water solutions were placed in an oven at $30^{\circ} \pm 1^{\circ}$ C. overnight (16 hr.). It was found by experiment that the larvae would tolerate one part acetone to 330 parts of water. In none of these tests was acetone used at a concentration greater than 1:660 and usually at much lower concentrations.

The solutions containing the larvae were poured into porcelain dishes and living and dead larvae were counted. Tests were run in duplicate, i.e. two tubes containing ten larvae each. If any larva was found to be dead in the checks, the tests were repeated. Any substance giving a 50 per cent or more kill was considered worthy of further testing. These retests were made at four or more concentrations.

Tests to determine whether a material possessed promise as a contact insecticide were made on *Aphis rumicis* L. on nasturtium according to the method of Hartzell and Wilcoxon (13), as described in a previous publication.

Tests to determine whether a substance is a stomach poison were made by spraying the material with a wetting agent (0.5 per cent Penetrol) on wheat plants and colonizing grasshopper (*Melanoplus differentialis* Thomas) nymphs on the sprayed plants after the foliage had dried. The checks consisted of tests on grasshopper nymphs that were colonized on wheat plants that had been sprayed with the wetting agent (0.5 per cent Penetrol) and allowed to dry.

RESULTS

TOXICITY OF PLANT EXTRACTS TO MOSQUITO LARVAE AND APHIDS

Products of plants relatively resistant to insect attack. It was thought that plants known to be relatively resistant to insect attack may contain an active principle that could be extracted and be used as an insecticide. Felt (10, p. 47) has published a list of the deciduous trees giving a numerical resistance rating. Trees known to be highly resistant to insect attack are

² Obtained through the courtesy of C. H. Bradley.

given a rating of 3, while trees known to be very susceptible to insects are given a rating of 0.5. Intermediate ratings are given also to species whose resistance is known to lie between these extremes.

Water and acetone extracts of dried leaves obtained from 14 species and the buds of one species of deciduous trees were tested on mosquito larvae. The results are shown in Table I. Only two leaf extracts of this series gave kills of 50 per cent or more to mosquito larvae. There appears to be no correlation between resistance rating and toxicity to mosquito

TABLE I

TREES ARRANGED ACCORDING TO COMPARATIVE INJURY BY INSECTS COMPARED WITH KILLS OBTAINED ON CULEX QUINQUEFASCIATUS WITH LEAF EXTRACTS

Common name	Scientific name	Resistance rating by E. P. Felt	% Kill of mosquito larvae by leaf extracts	Solvent*
Tulip tree	<i>Liriodendron tulipifera</i> L.	3.0	85	Water
Tree of heaven	<i>Ailanthus altissima</i> Swingle	3.0	20	Acetone
Catalpa, hardy	<i>Catalpa ovata</i> Don	3.0	10	Acetone
Ginkgo	<i>Ginkgo biloba</i> L.	3.0	0	Acetone and water
Oak, scarlet	<i>Quercus coccinea</i> Muenchh.	2.5	35	Acetone
Plane, oriental	<i>Platanus orientalis</i> L.	2.5	20	Acetone
Plane, American	<i>Platanus occidentalis</i> L.	2.5	5	Acetone
Maple, sycamore	<i>Acer pseudo-platanus</i> L.	2.5	10	Acetone
Maple, Norway	<i>Acer platanoides</i> L.	2.0	0	Acetone and water
Oak, white	<i>Quercus alba</i> L.	2.0	25	Water
Oak, black	<i>Quercus velutina</i> Lam.	2.0	10	Acetone and water
Linden flowers and leaves**	<i>Tilia europaea</i>	1.5	50	Acetone
Horse chestnut	<i>Aesculus hippocastanum</i> L.	1.5	5	Acetone
Elm, American	<i>Ulmus americana</i> L.	1.0	10	Water
Balm Gilead buds, N. F., true**	<i>Populus</i> sp.	0.5	95	Acetone

* Water and acetone extracts were made of each plant product and tested on mosquito larvae. The solvent giving the highest kill is reported in each case, except with extracts that gave identical kills.

** Obtained through the courtesy of S. B. Penick & Co., New York, N. Y., and listed here according to the names and spelling given in their Price List and Manual of Botanical Crude Drugs, December 1, 1937.

larvae of the materials tested. The tulip tree, for example, has a resistance rating of 3, according to Felt, while an acetone extract of the leaves gave a kill of 85 per cent to mosquito larvae. An extract of balm of Gilead buds gave a kill of 95 per cent; this, according to Felt's resistance rating, is 0.5 which places it among the least resistant trees to insect attack. Yet an acetone extract of the buds proved the most toxic in this series to mosquito larvae.

In addition to the trees reported above, several plants known to be relatively free from insect attack also were tested. The results obtained with water and acetone extracts of these plants appear in Table II. Again

there appears to be no correlation between resistance to insect attack and toxicity to mosquito larvae.

Crude botanical drugs and essential oils. It was thought that drug plants (35) might offer a promising source of insecticides non-poisonous to hu-

TABLE II
TOXICITY TO CULEX QUINQUEFASCIATUS OF EXTRACTS FROM
INSECT-RESISTANT PLANTS

Common name	Scientific name	Extract of	% Kill of mosquito larvae	Solvent*
Bay	<i>Pimenta acris</i> Kostel.	Oil of leaves	100	Acetone
Boneset	<i>Eupatorium perfoliatum</i> L.	Leaves and stems	0	Acetone and water
Catnip	<i>Nepeta cataria</i> L.	Leaves and stems	30	Acetone
Kudzu vine	<i>Pueraria hirsuta</i> Schneid.	Leaves	0	Acetone and water
Sage	<i>Salvia officinalis</i> L.	Leaves	80	Acetone
Wormwood	<i>Artemisia absinthium</i> L.	Leaves	10-5	Acetone and water

* See footnote *, Table I.

TABLE III
EXTRACTS OF CRUDE BOTANICAL PRODUCTS NOT POISONOUS TO HUMANS GIVING A KILL OF
FROM 50 PER CENT TO 100 PER CENT TO MOSQUITO LARVAE
(CULEX QUINQUEFASCIATUS)

Common or trade name	Scientific name	Plant part	Solvent	% Kill
Barberry	<i>Berberis</i> sp.	Root	Acetone	70
Butternut bark of root*	<i>Juglans cinerea</i>	Bark of root	Water	55
Caraway	<i>Carum carvi</i> L.	Seed	Acetone	90
Chive	<i>Allium schoenoprasum</i> L.	Whole	Water	70
Coltsfoot root*	<i>Tussilago farfara</i>	Root	Water	70
Dogwood, flowering	<i>Cornus florida</i> L.	Leaf	Acetone	60
Echinacea root, N.F.*	<i>Brauneria</i> sp.	Root	Acetone	100
Elecampane root*	<i>Inula helenium</i>	Root	Acetone	100
Fern, male	<i>Aspidium filix-mas</i> [L.] Sw.	Rhizome	Acetone	100
Fringe tree bark of root, true, N.F.*	<i>Chionanthus virginica</i>	Bark of root	Acetone	65
Gold thread*	<i>Coptis trifolia</i>	Whole	Acetone	55
Golden seal root, N.F.*	<i>Hydrastis canadensis</i>	Root	Water	70
Grindelia robusta herb, N.F.*	<i>Grindelia</i> sp.	Whole	Acetone	65
Hydrangea root, N.F.*	<i>Hydrangea arborescens</i>	Root	Acetone	90
Ironwood*	<i>Ostrya virginiana</i>	Wood	Acetone	65
Juniper berries, hand pkd., mature*	<i>Juniperus</i> sp.	Berry	Acetone	70
Mustard seed, black, U.S.P.*	<i>Brassica nigra</i> , etc.	Seed	Water	100
Prickly ash berries, N.F.*	<i>Xanthoxylum</i> sp.	Berry	Acetone	60
Pumpkin	<i>Cucurbita pepo</i> L.	Seed	Acetone	100
Rhubarb	<i>Rheum officinale</i> Baill.	Root	Water	70
Sage	<i>Salvia officinalis</i> L.	Root	Acetone	95
Skunk cabbage	<i>Symplocarpus foetidus</i> [L.] Nutt.	Root	Acetone	65
Spice bush	<i>Benzoin aestivale</i> [L.] Nees	Bud	Acetone	65

* Obtained through the courtesy of S. B. Penick & Co., New York, N. Y., and listed here according to the names and spelling given in their Price List and Manual of Botanical Crude Drugs, December 1, 1937.

mans. Through the centuries there has been a gradual selection and discarding of botanical drugs poisonous to humans. Insecticides have not had this advantage because of their more recent development. Crude botanical drugs and essential oils which gave kills of 50 per cent or more to mosquito larvae are listed in Tables II, III, and IV. Balm of Gilead buds and European linden flowers and leaves (Table I) also gave kills of this magnitude. Drug plants which gave kills of less than 50 per cent will be found also in the miscellaneous lists in Tables II and VII. Toxicity curves of some of the more promising products appear in Figure 1.

TABLE IV
TOXICITY OF ACETONE EXTRACTS OF ESSENTIAL OILS TO MOSQUITO LARVAE
(*CULEX QUINQUEFASCIATUS*)

Oil of	P.p.m.	% Kill
Bay leaves	25	55
	50	100
Cubebs	50	50
	100	100
Cypress	50	90
	100	100
Patchouli	50	85
	100	100
Rosemary	50	20
	100	70
Santal	25	65
	50	100
Sweet basil	50	95
	100	100
Sweet marjoram	50	50
	100	95
Wormseed or chenopodium	11.1	35
	25.0	90

The median lethal dose (LD₅₀) for mosquito larvae of echinacea root was found to be 16.5 p.p.m., for balm of Gilead buds 5.8 p.p.m., for sage root 18.3 p.p.m., for filicin, a product of male fern, 11.0 p.p.m., oil of cypress 31 p.p.m., oil of rosemary 78 p.p.m., and oil of sweet basil 28 p.p.m. These compare with 0.06 p.p.m. for rotenone.

Elecampane, echinacea, and filicin were tested on *Aphis rumicis*. The results appear in Table V. A steam distillate of elecampane gave kills of 90.0 and 79.3 per cent in duplicate tests, which compared with 99.3 and 96.4 per cent for filicin. The kills obtained with acetone extracts of elecampane and echinacea were below 50 per cent.

Anthelmintics. It will be noted that among the most toxic products listed in Table III are several anthelmintics. It was thought advisable to test a series of anthelmintics at known concentrations on mosquito larvae. The results appear in Table VI. Oleoresin of aspidium (male fern) and thymol at 25 p.p.m. gave a kill of 100 per cent, while oil of chenopodium at a

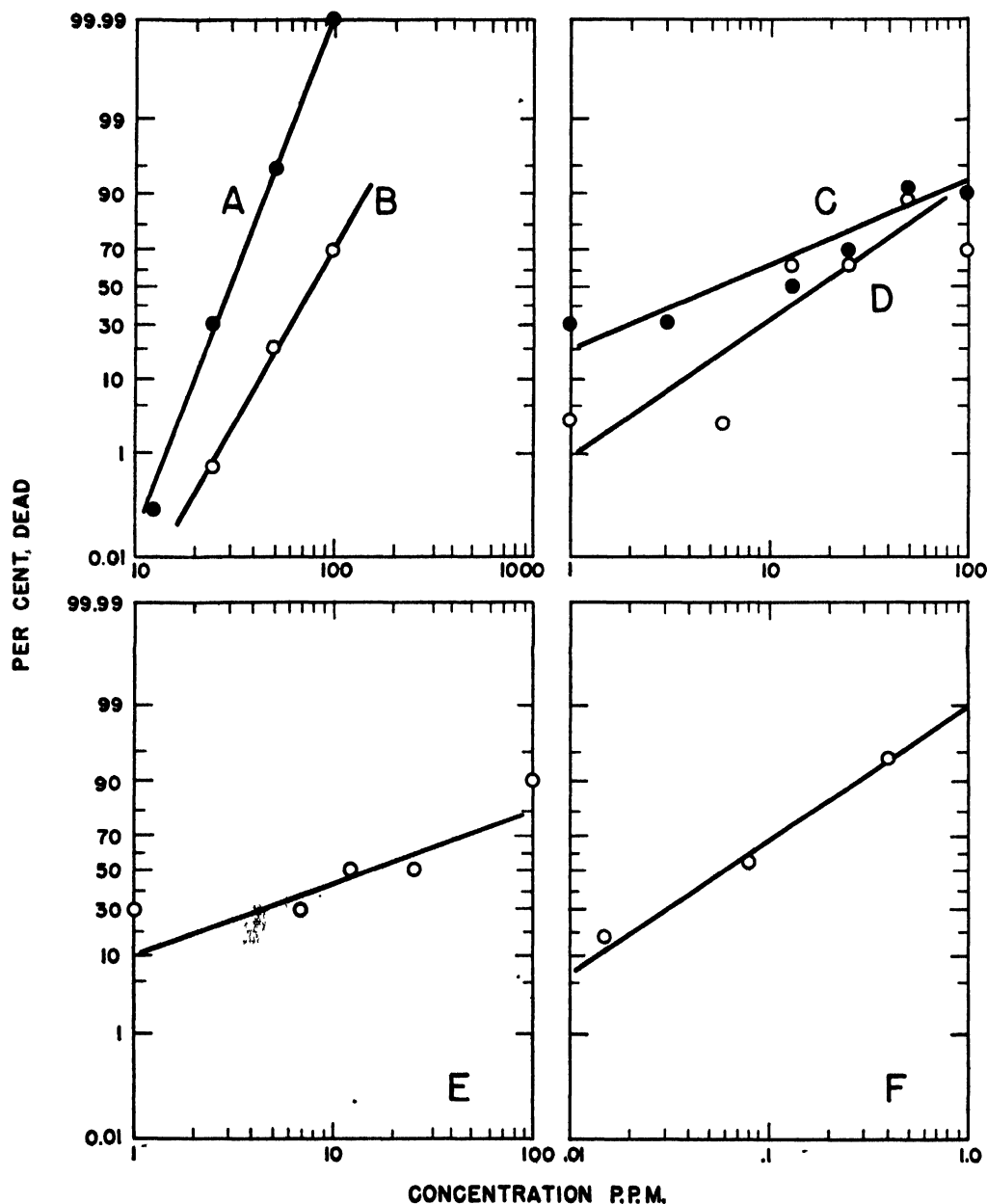


FIGURE 1. Effect of acetone extracts of plant products on mosquito larvae (*Culex quinquefasciatus*). A. Oil of cypress, LD₅₀ 31 p.p.m.; B. Oil of rosemary, LD₅₀ 78 p.p.m.; C. Balm of Gilead buds, LD₅₀ 5.8 p.p.m.; D. Sage root, LD₅₀ 18.3 p.p.m.; E. Echinacea root, LD₅₀ 16.5 p.p.m.; F. Rotenone, LD₅₀ 0.06 p.p.m.

TABLE V
TOXICITY TO APHIS RUMICIS OF EXTRACTS OF BOTANICAL DRUGS
(DUPLICATE TESTS)

Name	Conc., %	% Kill
Elecampane root	0.2	34.3 40.6
Elecampane root (steam dist.)	0.18	90.0 79.3
Echinacea root	0.2	49.4 38.6
Filicin (male fern)	0.1	99.3 96.4
Spreading agent (Penetrol)	0.5	58.6 42.0

TABLE VI
TOXICITY OF ANTHELMINTICS TO MOSQUITO LARVAE
(CULEX QUINQUEFASCIATUS)

Name	Therapeutic use	% Kill of mosquito larvae	P.p.m.
<i>Botanical drugs</i>			
Oil of chenopodium	Roundworms	95	16.7
Oleoresin of aspidium	Tapeworms	100	25.0
Pepo (pumpkin seed)	Tapeworms	85	> 600.0
Quassia	Pinworms	30	600.0
Santonin	Roundworms	10	100.0
Thymol	Hookworms	100	25.0
<i>Compound not obtained from plants</i>			
Carbon tetrachloride	Hookworms	85	200.0

concentration of 16.7 p.p.m. gave a kill of 95 per cent to mosquito larvae. Pepo (pumpkin seed) was much less toxic, requiring a concentration of over 600 p.p.m. to give a kill of 85 per cent. No appreciable kill was obtained with quassia chips at 600 p.p.m. or with santonin at 100 p.p.m. It will be noted that the botanical drugs of this series compare favorably with carbon tetrachloride which gave a kill of 85 per cent at a concentration of 200 p.p.m.

Both oil of chenopodium and santonin are poisonous to humans at very low concentrations.

The situation with anthelmintics is analogous with that of insecticides in that both are required to kill the parasitic organism without causing appreciable injury to the host.

Miscellaneous plant products. Of the miscellaneous plant products tested (Table VII), all gave kills to mosquito larvae of less than 50 per cent. In-

TABLE VII

ACETONE AND WATER EXTRACTS OF PLANT PRODUCTS GIVING KILLS OF LESS THAN
50 PER CENT TO MOSQUITO LARVAE (*CULEX QUINQUEFASCIATUS*)

Common or trade name	Scientific name	Plant part	Solvent*	% Kill
Aletris root (Unicorn true), N.F.†	<i>Aletris farinosa</i>	Root	Acetone	15
Angelica root, Amer.†	<i>Angelica</i> sp.	Root	Acetone	40
Arbor vitae†	<i>Thuja occidentalis</i>	Leaves	Water	25
Baccharis	<i>Baccharis sarothroides</i> Gray	Seed	Acetone	0
Baileya	<i>Baileya multiradiata</i>	Flowers	Acetone & water	0
Balm	<i>Melissa officinalis</i> L.	Leaves & stem	Acetone & water	0
Bamboo brier root†	<i>Smilax tamnoides</i>	Root	Acetone	30
Barberry	<i>Berberis vulgaris</i> L. var. <i>atropurpurea</i> Reg.	Root & stem	Acetone	10
Basil, sweet	<i>Ocimum basilicum</i> L.	Whole plant	Acetone & water	0
Bayberry bark of root†	<i>Myrica cerifera</i>	Bark of root	Acetone	35
Birch, sweet	<i>Betula lenta</i> L.	Wax	Acetone & water	10
Black cohosh root, N.F.†		Bark	Water	10
Black haw bark of root, N.F.†	<i>Cimicifuga racemosa</i>	Root	Water	25
Black willow bark†	<i>Viburnum prunifolium</i>	Bark of root	Acetone & water	30
Blessed thistle herb, select†	<i>Salix nigra</i>	Bark	Acetone	5
Blood root, natural†	<i>Cnicus benedictus</i>	Whole plant	Water	35
Blue flag root, N.F.†	<i>Sanguinaria canadensis</i>	Root	Acetone	35
Brake (Bracken)	<i>Iris</i> sp.	Root	Acetone & water	5
Buckbean leaves†	<i>Pteridium latiusculum</i> (Desv.) Maxon	Rhizome	Acetone	0
Bugle weed†	<i>Menyanthes trifoliata</i>	Leaves	Acetone & water	0
Burdock root, N.F.†	<i>Lycopus virginicus</i>	Whole plant	Acetone & water	0
Castor bean	<i>Arctium</i> sp.	Root	Acetone	5
Chamomile	<i>Ricinus communis</i> L.	Seed	Acetone	0
Cherry, wild black	<i>Matricaria chamomilla</i> L.	Leaves & stem	Water	5
Cladrastis	<i>Prunus serotina</i> Ehrh.	Leaves	Water	10
Comfrey root†	<i>Cladrastis (Maackia) amurensis</i> Benth.	Root & stem	Acetone	5
Creosote bush	<i>Symphytum officinale</i>	Root	Water	45
Culvers root, N.F.†	<i>Covillea tridentata</i>	Leaves	Acetone	20
Ephedra	<i>Veronica virginica</i>	Stem	Acetone	0
Fern, Christmas	<i>Ephedra</i> sp.	Root	Acetone	0
Fern, cinnamon	<i>Ephedra altissima</i>	Root	Water	15
Fern, flowering	<i>Ephedra procera</i>	Root	Acetone & water	0
Fern, hay-scented	<i>Polystichum acrostichoides</i> [Michx.] Schott.	Stalk	Water	5
Fern, sensitive	<i>Osmunda cinnamomea</i> L.	Rhizome	Acetone & water	0
Figwort herb†	<i>Osmunda regalis</i> L.	Rhizome	Acetone	0
Geranium	<i>Dennstaedtia punctilobula</i> (Michx.) Moore	Rhizome	Acetone	10
Geranium, wild	<i>Onoclea sensibilis</i> L.	Rhizome	Acetone	10
* Gladiolus	<i>Scrophularia nodosa</i>	Whole plant	Acetone	25
	<i>Pelargonium zonale</i> Willd.	Leaves & stem	Acetone	0
	<i>Geranium maculatum</i> L.	Root	Acetone	20
	<i>Gladiolus</i> sp.	Leaves	Acetone	5

* See footnote, * Table I.

† Obtained through the courtesy of S. B. Penick & Co., New York, N. Y., and listed here according to the names and spelling given in their Price List and Manual of Botanical Crude Drugs, December 1, 1937.

** Obtained through the courtesy of Dr. David Fairchild, Fairchild Tropical Garden, Coconut Grove, Florida.

TABLE VII (Continued)

Common or trade name	Scientific name	Plant part	Solvent*	% Kill
Golden rod leaves and tops†	<i>Solidago odora</i>	Leaves & tops	Acetone & water	5
Gourd, Missouri	<i>Cuburbita foetidissima</i> HBK.	Root	Acetone	0
Hemlock	<i>Tsuga canadensis</i> Carr.	Needles	Water	10
Horehound herb†	<i>Marrubium vulgare</i>	Whole plant	Water	0-30
Horse nettle berries†	<i>Solanum carolinense</i>	Berry	Acetone	40
Ivy, ground	<i>Nepeta hederacea</i> [L.] Trevisan.	Whole plant	Water	30
Lady slipper root†	<i>Cypripedium</i> sp.	Root	Water	30
Maple, Norway	<i>Acer platanoides</i> L.	Leaves	Acetone & water	0
Maple, sugar	<i>Acer saccharum</i> Marsh.	Leaves	Acetone & water	0
Marjoram, sweet	<i>Origanum vulgare</i> L.	Whole plant	Acetone	5
Mountain ash bark†	<i>Sorbus americana</i>	{ Bark	Acetone	10
Mountainashberries†		{ Berries	Acetone	20
Pagoda-tree, Japan.	<i>Sophora japonica</i> L.	{ Root	Acetone	20
Passion flower, herb†	<i>Passiflora incarnata</i>	{ Stem	Acetone	0
Pennyroyal leaves, American†	<i>Hedeoma pulegioides</i>	Whole plant	Water	5
Peppermint		Leaves	Acetone & water	0
Peppermint herb, American†	<i>Mentha piperita</i> L.	Whole plant	Acetone	10
Pine, Scotch	<i>Pinus sylvestris</i> L.	Needles	Acetone	25
Pine, white	<i>Pinus strobus</i> L.	Needles	Water	10
Pink root, genuine†	<i>Spigelia marilandica</i>	Root	Water	30
Pipsissewa herb†	<i>Chimaphila umbellata</i>	Whole plant	Acetone & water	0
Pleurisy root, true†	<i>Asclepias tuberosa</i>	Root	Acetone & water	0
Prayer beads (Crab's eye bean)	<i>Abrus precatorius</i> **	{ Root	Acetone	10
Quack grass	<i>Agropyron repens</i> [L.] Beauv.	{ Stem	Acetone	10
Sarsaparilla root, American†	<i>Aralia nudicaulis</i>	Root	Acetone & water	35
Sassafras	<i>Sassafras officinale</i> Nees & Eberm.	Root	Acetone	45
Scullcap herb, N.F.†	<i>Scutellaria lateriflora</i>	Leaves	Acetone	5
Senega root, N.F.†	<i>Polygala senega</i>	Whole plant	Acetone	25
Snake root, Canada	<i>Asarum canadense</i> L.	Root	Acetone	10
Southernwood	<i>Artemisia abrotanum</i> L.	Button	Water	10
Spearmint	<i>Mentha spicata</i> L.	Whole plant	Acetone & water	0
Spruce, Norway	<i>Picea abies</i> [L.] Karst.	Leaves & stem	Acetone & water	0
Squills, red†	<i>Rhus</i> sp.	Leaves & small branches	Acetone & water	0
Sumac		Bulb	Acetone	20
Sweetleaf		{ Leaves	Water	5
Tansy herb†		{ Berry	Water	20
Tarragon, Russian	<i>Symplocos paniculata</i> Miq.	Root	Acetone	10
	<i>Tanacetum vulgare</i>	Whole plant	Acetone	10-30
	<i>Artemisia sacrorum</i> Ledeb.	Leaves & stem	Acetone	15
Tung-oil tree	<i>Aleurites fordii</i> Hemsl.	{ Leaves	Acetone	40
		{ Stem	Acetone	5
Vervain herb, blue†	<i>Verbena hastata</i>	Root	Acetone	0
Wahoo bark of root, N.F.†	<i>Euonymus atropurpureus</i>	Whole plant	Acetone	30
Wild yam root, N.F.†	<i>Dioscorea villosa</i>	Bark of root	Acetone & water	15
Wintergreen herb†	<i>Gaultheria procumbens</i>	Root	Acetone & water	0
Wormwood leaves†	<i>Artemisia absinthium</i>	Whole plant	Water	20
Yellow parilla root†	<i>Menispermum canadense</i>	Leaves	Acetone	15
Yellow root†	<i>Xanthorrhiza apiifolia</i>	Root	Acetone	35
Yerba santa	<i>Eriodictyon californicum</i>	Root	Acetone & water	5
		{ Leaves	Acetone & water	0
		{ Stem	Acetone	35

cluded in this list were a number of drug plants, xerophytic plants, evergreens, garden vegetables, and flowers.

TOXICITY OF CASTOR BEAN FOLIAGE TO GRASSHOPPERS

It was thought that the conflicting reports of the toxicity of castor bean foliage to grasshoppers may be due to differences in toxicity exhibited by different varieties of castor bean. The following 11 varieties of castor bean were tested on nymphs and adults of *Melanoplus differentialis*: *Ricinus communis* var. *Africanus*, *Bourboniensis arboreus*, *Brazilian*, *Cambo-gensis*, *Communis*, *Duchess of Edinburgh*, *Gibsonii*, *Panormitanus*, *Red Spire*, *Sanguineus*, *Zanzibarensis*.

From 10 to 25 partly-grown grasshopper nymphs were caged on potted castor bean plants. Counts of living and dead grasshoppers were made daily. This method was discontinued because of cannibalism. Later individual nymphs were caged on leaves and the amount eaten noted daily. Of the several varieties of castor bean tested it required 8 days with *Zanzibarensis* and 37 days with *Red Spire* to obtain a 50 per cent kill. The other varieties showed intermediate periods. Some of the nymphs that survived transformed to adults on the castor bean foliage.

It was soon discovered that the amount of foliage eaten by the grasshopper nymphs and adults which died was barely sufficient to sustain life so that the factor of starvation could not be eliminated.

There can be no doubt that castor bean contains a toxin as a number of nymphs that fed on the foliage were affected with a paralysis of the legs although some so affected continued to live for days.

Water and acetone extracts of the seed and leaves of castor bean gave kills of less than 50 per cent to mosquito larvae and were not toxic to grasshoppers.

It was thought that ricin, a poisonous protein of castor bean, which is known to affect hemoglobin in warm-blooded animals, might be toxic to insects as a stomach or contact poison. Grasshoppers fed for seven days on wheat plants dusted with ricin were not appreciably affected although their bodies were covered with the white powder by coming in contact with it while feeding on the plants. Preparations of ricin, ricinin, and crushed castor bean seeds, each incorporated in bran mash baits and fed to grasshoppers, also showed no toxicity whatever.

It is believed that the utility of castor bean as an insecticide awaits the application of proper chemical methods of extraction of a specific toxin for insects.

DISCUSSION

One of the objects of this investigation was to reveal chemical groups that possess insecticidal properties. Many of the plants tested contain

terpines known to be potent insecticides. On the other hand, filicin, a toxic constituent of male fern, is a phloroglucinol propyl ketone, while the active principle of elecampane is helenin. In the case of pumpkin (seed) and a number of other plants the active principle is not known.

There appears to be no correlation between botanical classification and toxicity to insects. The plants tested ranged from Pteridophytes through the Angiosperms to Compositae. A similar lack of correlation has been noted for botanical drug plants and pharmacological effect (34).

Water and acetone extracts do not necessarily remove the toxic principle from plant products. Of the two, acetone was found to be the better solvent. Geranium leaves and flowers are toxic to Japanese beetle (1), but water and acetone extracts are not toxic even to mosquito larvae.

McIndoo and Sievers (18) relied chiefly on water as a solvent and thus possibly failed in many cases to obtain extracts toxic to insects.

The fact that a plant extract is toxic to mosquito larvae does not necessarily indicate that it possesses marked toxicity to other species of insects. As an illustration, an acetone extract of balm of Gilead buds is toxic to mosquito larvae but possesses little or no toxicity to *Aphis rumicis*; on the other hand, filicin (33) possesses a toxicity comparable with that of pyrethrum to mosquito larvae, *Aphis rumicis*, and houseflies (*Musca domestica* L.)

None of the plant products tested caused injury to plants nor showed promise as stomach poisons, although in this respect only a limited number were tested.

Plants known to contain toxins to warm-blooded animals were avoided, with few exceptions, in this study as it was hoped to find toxins specific to insects.

It should be remembered that mere toxicity to insects is not the only requirement of a satisfactory insecticide. Availability, cost, stability, and other factors are involved.

The results given in this paper are presented because it is believed that they may be of scientific interest and may help to form a basis for a broader understanding of insecticidal plants.

SUMMARY

Products of 150 species and varieties of plants were tested as possible insecticides. Of this number water and acetone extracts of 36 species gave kills of 50 to 100 per cent to mosquito larvae (*Culex quinquefasciatus*). Products of the following 18 botanical drugs gave kills of 90 to 100 per cent: balm of Gilead buds, caraway seed, echinacea root, elecampane root, hydrangea root, male fern rhizome, black mustard seed, pepo (pumpkin seed), sage root, oil of bay leaves, oil of cubebs, oil of cypress, oil of patch-

ouli, oil of rosemary, oil of santal, oil of sweet basil, oil of sweet marjoram, and oil of wormseed or chenopodium.

In addition, a steam distillate of elecampane gave an average kill of 89 per cent to *Aphis rumicis*.

Of the 18 plant products giving the highest kill to mosquito larvae all are botanical drugs, although many species not listed as drug plants were tested. It is of interest to note that among the drug plants giving high kills to mosquito larvae are several well known anthelmintics.

No correlation was found to exist between insect resistance to plants and toxicity of leaf extracts to mosquito larvae.

Of 11 varieties of castor bean tested on grasshopper (*Melanoplus differentialis*), the foliage of none was found to be sufficiently toxic to be satisfactory as a practical insecticide. Preparations of ricin, ricinin, and crushed castor bean seeds, each incorporated in bran mash baits and fed to grasshoppers, also showed no toxicity.

Extracts that will kill tropical fish will not necessarily give an appreciable kill to mosquito larvae.

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FURTHER TESTS WITH VITAMIN B₁ ON ESTABLISHED PLANTS AND ON CUTTINGS

A. E. HITCHCOCK AND P. W. ZIMMERMAN

Reports that certain plants were benefited by small additions of vitamin B₁ to the growing medium (3, 4) were the principal basis for subsequent interest in the possible use of vitamin B₁ in horticultural practice. Unfortunately, before the above-mentioned claims could be substantially confirmed, and before any practical application could be tested adequately, there appeared a number of popular articles with exaggerated accounts of practical results which might be expected from the use of vitamin B₁. The erroneous impression created by these popular articles has led to considerable confusion concerning the status of vitamin B₁, not only among laymen, but also among scientists. The status of vitamin B₁ in horticultural practice has been reviewed by one of the present authors (13).

The importance of vitamin B₁ in the growth of green plants is by no means clear at present. The results of certain experiments with isolated roots (2, 8, 3, 9, 12) are essentially in agreement, but the results with cuttings (11, 10, 7) and with established plants (3, 4, 1, 5, 13) lack agreement. Thus the claims of Bonner and Greene (3, 4) that certain plants benefited from an external supply of vitamin B₁ were not substantiated by the results of Arnon (1) and Hamner (5), who showed that all of the plants they used were capable of synthesizing adequate amounts of vitamin B₁ when the nutrient medium contained no source of this vitamin. At present it seems unlikely that under normal field and greenhouse conditions the growth of plants is limited directly by the lack of an external source of vitamin B₁.

With respect to the importance of vitamin B₁ in the rooting of cuttings, the situation is similar to that described for established plants. Our results (7) showed that root development in cuttings was not accelerated by deferred treatment with vitamin B₁. In some instances it appeared that vitamin B₁ and also several other substances functioned as activators. With optimum mixtures of a root-inducing substance and vitamin B₁ the number of initiated roots was increased but their rate of growth either remained the same or was reduced. This is in contrast to the suggestion of Went, Bonner, and Warner (11) and Warner and Went (10) that the development of initiated roots in cuttings is greatly accelerated by an external supply of vitamin B₁.

Although brief mention has been made of results obtained with vitamin

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B₁ in this laboratory (7, 13), it is the purpose of the present paper to describe the results of other experiments in which provision was made to determine the influence of other factors in addition to vitamin B₁. There are relatively few reports which give detailed data relating to treatment of green plants and cuttings (grown in light) with vitamin B₁.

MATERIALS AND METHODS

The vitamin B₁ used in all experiments was obtained from Merck & Co. Inc. under the name of thiamin hydrochloride or Betabion. Freshly-prepared solutions were used in all cases.

A white-flowered China aster (*Callistephus chinensis* Nees var. Queen of the Market) was used in the potted plant tests. Cuttings of the following plants were used in other tests: *Actinidia arguta* Miq., *Ardisia japonica* Thunb., *Celastrus articulatus* Thunb., *Citrus* sp., *Euonymus radicans* Sieb. var. *vegeta* Rehd., *Fuchsia hybrida* Voss, *Hibiscus syriacus* L., *Ilex glabra* Gray, *Juniperus chinensis* L., *Ligustrum ovalifolium* Hassk. (privet), *Rosa* var. Briarcliff, and *Taxus media* Rehd. var. *hicksii* Rehd.

Flat-grown plants. Twenty aster seedlings were planted in each of four flats containing a standard soil mixture consisting of composted sod soil amended with peat moss and a 5-8-7 commercial mineral fertilizer. The root systems were not washed free of soil as was done in the second test. Three flats were watered weekly with 350 cc. of a solution containing vitamin B₁ in concentrations of 0.01, 0.1, and 1.0 mg./l. respectively, and at other times with tap water. The control flat received tap water only. The test was started February 17 and terminated April 29.

In a second test similar aster seedlings were first washed free of all soil and then the root systems were dipped into solutions containing vitamin B₁. The concentrations of vitamin B₁ were 0.1, 1.0, and 10 mg./l. respectively. Root systems of control seedlings were dipped into tap water. After the dip treatment the seedlings were planted in soil in flats as described for the preceding test.

Potted plants. Aster seedlings were transferred from flats to 4-inch pots when the rosette of leaves was about 10 cm. in diameter. The soil was a composted sod soil mixture containing at the start ample nutrients and organic matter for the initial growth of most plants used in this laboratory. The same soil mixture was used in all pots. A mineral commercial fertilizer (5-8-7) was applied thereafter at such times and in sufficient quantities to maintain the plants in an actively growing state.

One hundred aster plants were arranged in five rows of 20 plants each on a greenhouse bench occupying a space of 3 × 14 feet. The treatments were administered according to the Latin Square arrangement shown in Table I. Each of the five concentrations of vitamin B₁ was applied to a total of 20 plants which were arranged in five sets of four each so that all

five treatments appeared in every row and in every block. This arrangement not only tends to minimize differences due to chance variation, but it also makes possible the determination of any important influence due to position in different rows or in different blocks.

TABLE I

ARRANGEMENT OF PLANTS ON BENCH SHOWING THE CONCENTRATION OF VITAMIN B₁
(P.P.M.) APPLIED TO THE SOIL WEEKLY*

Row number	Block number				
	I	II	III	IV	V
5	10.0	1.0	0.5	0.1	0.0
4	1.0	0.5	0.1	0.0	10.0
3	0.5	0.1	0.0	10.0	1.0
2	0.1	0.0	10.0	1.0	0.5
1	0.0	10.0	1.0	0.5	0.1

* Each concentration applied to 20 plants which were distributed in 5 lots of 4 each.

Deferred treatment of cuttings. Duplicate sets of cuttings were treated initially for 24 hours, as indicated in the first column of Table IV. When the cuttings were removed from the rooting medium on the sixth and twelfth days for the deferred treatment (Part B, Table IV), comparable sets were removed and replanted without treatment (Part A, Table IV). Vitamin B₁ was applied as a solution for a period of 24 hours to the basal ends of cuttings. A mixture containing one-fourth German peat moss and three-fourths sand, by volume, was used as the rooting medium.

Treatment of the rooting medium. Duplicate sets of cuttings were planted in sand (pH 6.8) on December 4 and 5. The control plot of sand was watered with tap water having a reaction of pH 6.5 to 7.1. The treated plot of sand was watered every three to five days with tap water containing 1 p.p.m. of vitamin B₁ and at other times with tap water. The sand plot to which vitamin B₁ was added had received 27 liters of the vitamin solution by December 27, 37 liters by January 6, 47 liters by January 15, and 62 liters by January 31. Different types of cuttings were selected, but an attempt was made to include comparable types in each replicate set, as illustrated for *Taxus* in Figure 3.

EXPERIMENTAL RESULTS

Flat-grown plants. The average height and the fresh weight of aster seedlings grown in the three flats receiving weekly applications of vitamin B₁ were greater than for plants in the control flat. The total number of flowers was also greater for the plants in the treated flats as compared with the number of flowers on control plants. The maximum differences which were obtained with a concentration of 0.01 mg./l. of vitamin B₁ are illustrated in Figure 1, A and B. Data for this test are recorded in Part A of

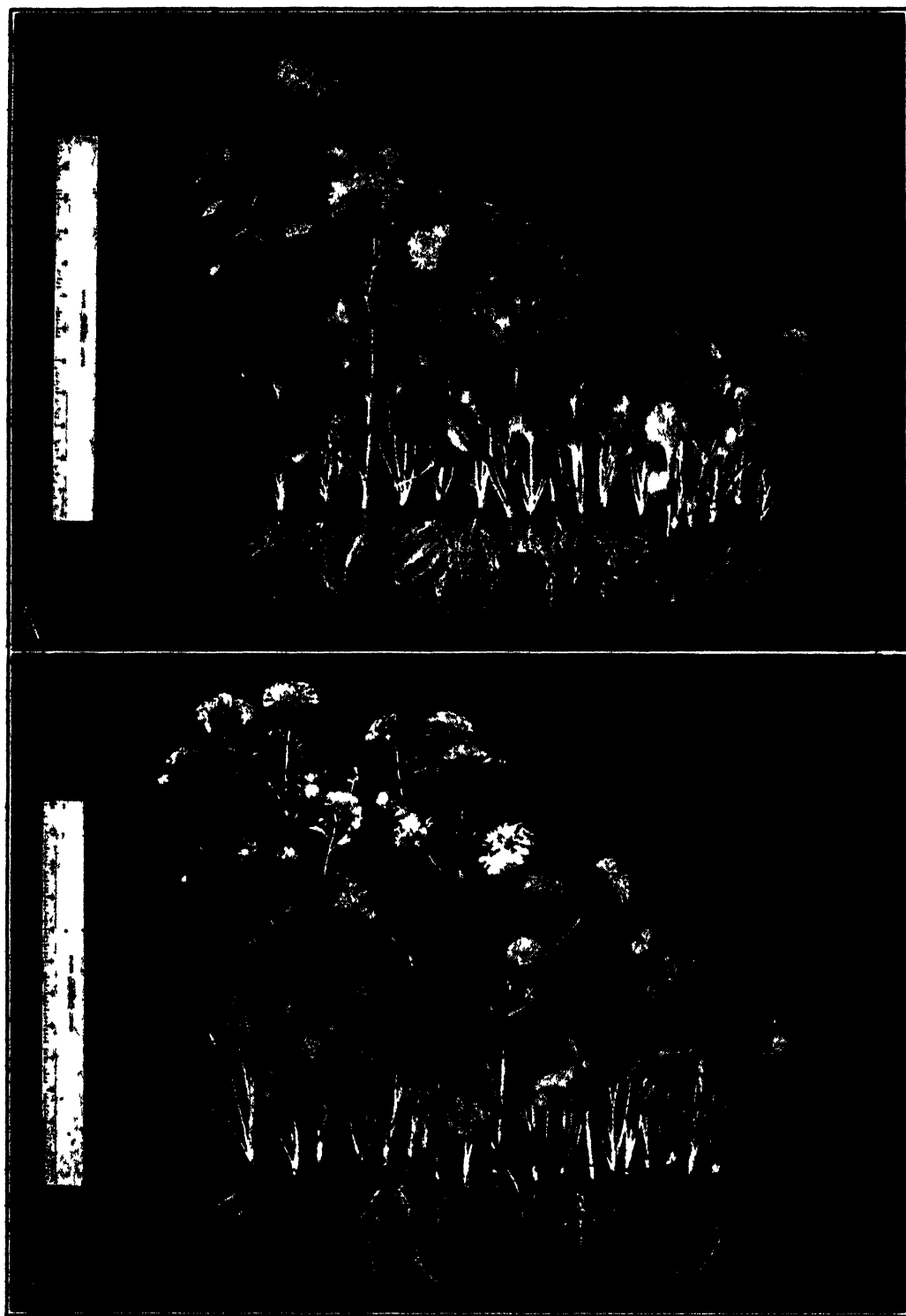


FIGURE 1. A. Flat-grown aster controls after 10 weeks. B. Similar plants grown in soil watered weekly with 0.01 p.p.m. of vitamin B₁.

Table II. The relative size or density of the root systems was approximately proportional to the relative size of the top growth (Fig. 1, A and B).

TABLE II
INFLUENCE OF VITAMIN B₁ ON THE GROWTH OF ASTER SEEDLINGS GROWN IN FLATS

Conc. vit. B ₁ , mg./l.	Total No. flowers	Relative height of plants			Total wet wt. (g.)	Av. wet.wt. (g.) per plant
		Tall	Medium	Short		
A. Soil watered weekly with 350 cc. of solution						
1.00	19	10	6	4	239	12.0
0.10	17	2	10	8	235	11.8
0.01	21	5	6	8	244	13.8
0.00	12	1	9	8	156	8.7
B. Root systems dipped into solution before planting						
10.0	9	1	5	9	147	9.8
1.0	8	0	5	15	170	8.5
0.1	10	3	6	11	208	10.4
0.0	5	0	7	13	173	8.7

Seedlings similar to those used in the preceding test were washed free of soil and the roots were then dipped into a solution of vitamin B₁ before planting in flats. Data for the second test are shown in Part B of Table II and the maximum differences which were obtained with 0.1 mg./l. of vitamin B₁ are illustrated in Figure 2, A and B. In this test the differences in growth of treated and control plants were less pronounced than in the preceding test. Although the highest concentration of vitamin B₁ (10 mg./l.) caused a mortality of 25 per cent, the 15 plants which survived were essentially the same in appearance as plants in the other flats.

The fresh weights of control plants were about the same in both tests so that washing the roots free of soil had relatively little effect on the fresh weight, but exerted a noticeable influence on the relative height of the plant and the total number of flowers produced (Table II). In both tests there was considerable variation in the height of plants in the same flat. Thus the treatment with vitamin B₁ did not cause a more uniform growth of the aster seedlings. While the results of these tests with flat-grown plants indicate a favorable influence of vitamin B₁ on the growth of aster seedlings, there was no influence of vitamin B₁ on the growth of potted aster plants during June and July.

Potted plants. Since the marked variation in the size of flat-grown plants indicated that the crowded conditions may have been partly responsible for causing differences in growth, the potted plant test was undertaken for the purpose of minimizing the factor of competition. The final height measurements made on July 10 (Table III) show no significant influence of

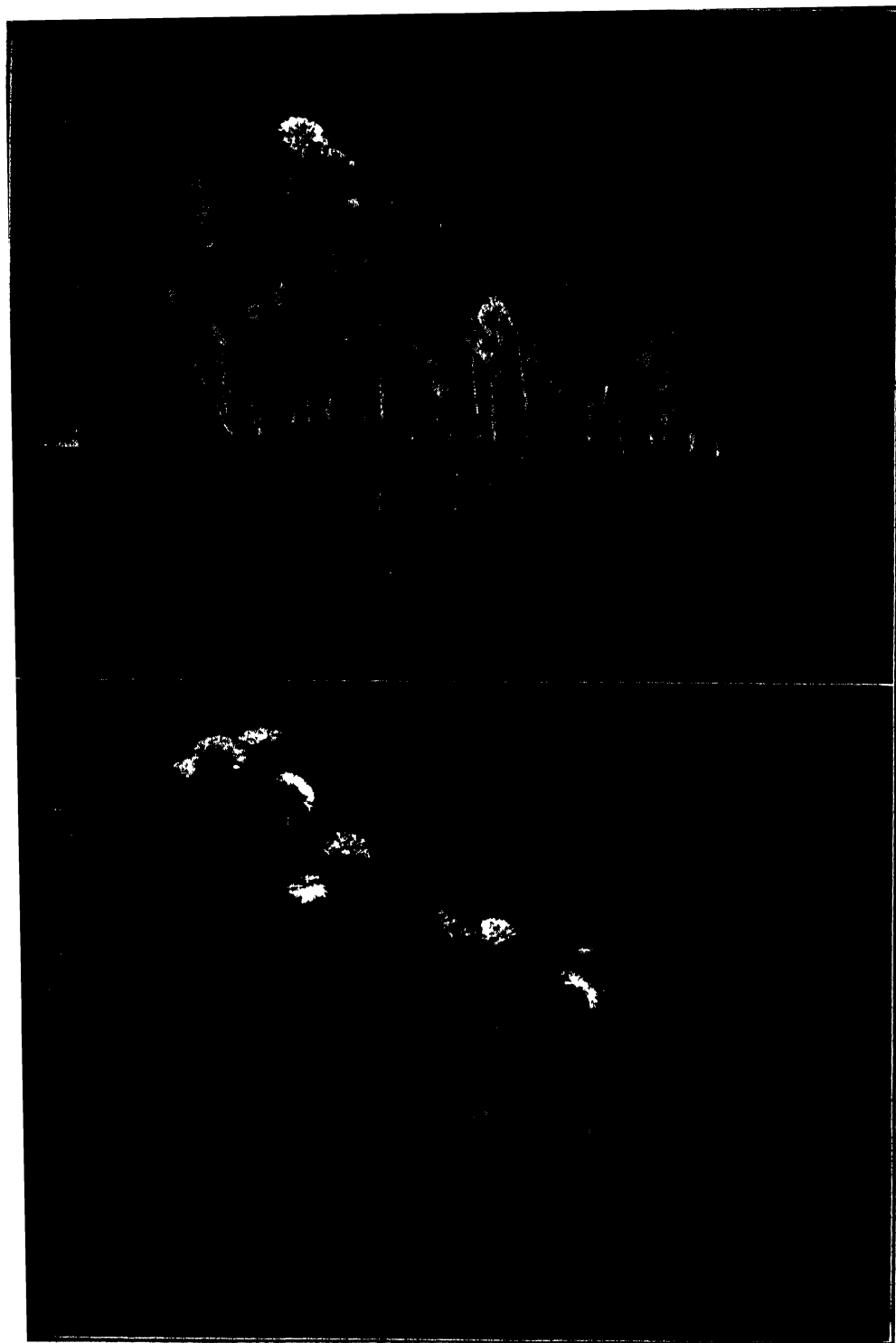


FIGURE 2. A. Control asters 10 weeks after transplanting. B. Similar plants treated (dots) with 0.1 p.p.m. of vitamin B₁ at the time of transplanting. Plants in A and B washed free of soil when transplanted.

vitamin B₁ on the growth of the China aster. The totals for the vitamin B₁ treatments ranged from 262 to 271 as compared with 287 for the water controls (Table III). Differences due to the position of plants in different rows (240, 286, 281, 275, 271) were more significant than those (258, 267, 268, 272, 288) due to position within the row. The measurements made two weeks previously (June 26) showed similar trends. In this case the totals for vitamin B₁ treatments were 158 to 167 as compared with 174 for the water controls. The corresponding row totals were 147 to 174 and the block totals were 157 to 192. With respect to the ineffectiveness of vitamin B₁, these results are similar to those reported previously for 20 varieties of plants grown in pots in the greenhouse (7).

TABLE III
INFLUENCE OF VITAMIN B₁ ON THE GROWTH OF ASTER SEEDLINGS.
HEIGHT OF PLANTS IN CENTIMETERS* ON JULY 10, 1940

Block number	Row number					Block totals	Block number	Conc. vitamin B ₁ (p.p.m.)				
	1	2	3	4	5			0.0	0.1	0.5	1.0	10.0
I	53	61	61	59	54	288	I	53	61	61	59	54
II	50	62	55	48	57	272	II	62	55	48	57	50
III	46	54	59	54	55	268	III	59	54	55	46	54
IV	48	54	51	61	53	267	IV	61	53	48	54	51
V	43	55	55	53	52	258	V	52	43	55	55	53
Row totals	240	286	281	275	271		Column totals	287	266	267	271	262

* Average of four plants.

Results with potted asters grown during June and July revealed no influence of vitamin B₁ on growth as in the case of the seedlings grown in flats during March and April.

Deferred treatment of cuttings. Data in Table IV show that deferred treatment of cuttings of *Euonymus*, privet, and *Hibiscus* with vitamin B₁ (1 p.p.m.) did not increase the average number of roots per cutting. On the contrary, the number of roots was reduced by the deferred solution treatment. Thus the group totals are in all cases lower for deferred treatment of cuttings (Part B, Table IV) than for cuttings which did not receive a deferred treatment (Part A, Table IV). Some of the adverse effect of deferred treatment is to be attributed to the 24-hour period of solution treatment, since the values in Part A of Table IV show that the removal and replanting procedures caused no pronounced reduction in the average number of roots. There was no beneficial effect resulting from an initial treatment with a mixture of indolebutyric acid and vitamin B₁ and, in addition, a deferred treatment with vitamin B₁ applied on the sixth or twelfth day.

TABLE IV
INFLUENCE OF A DEFERRED TREATMENT WITH VITAMIN B₁ ON THE AVERAGE
NUMBER OF ROOTS PER CUTTING

Initial basal treatment 24 hours	Part A				Part B			
	Without deferred treatment			Group totals	Deferred treatment with vitamin B ₁ (1 p.p.m.) 24 hrs.			Group totals
	2nd day	6th day*	12th day*		2nd day	6th day	12th day	
<i>Euonymus</i> (Oct. 3 to Oct. 22)								
Water	2	1	0	3	3	0	1	4
IB** 20 mg./l.	62	64	60	186	50	68	35	153
IB 20 mg./l. }	60	55	55	170	53	50	41	144
B ₁ 1 p.p.m. }	1	0	0	1	1	0	0	1
B ₁ 1 p.p.m.								
Column totals	125	120	115	360	107	118	77	302
<i>Privet</i>								
Water	0	0	0	0	0	0	0	0
IB 80 mg./l.	33	43	26	102	17	44	26	87
IB 80 mg./l. }	49	36	30	115	45	18	23	86
B ₁ 1 p.p.m. }	0	0	0	0	0	0	0	0
B ₁ 1 p.p.m.								
Column totals	82	79	56	217	62	62	49	173
<i>Hibiscus</i>								
Water	3	2	0	5	1	0	1	2
IB 40 mg./l.	13	17	26	56	1	14	8	23
IB 40 mg./l. }	16	15	16	47	9	7	11	27
B ₁ 1 p.p.m. }	2	0	0	2	2	1	0	3
B ₁ 1 p.p.m.								
Column totals	34	34	42	110	13	22	20	55

* Cuttings planted after initial treatment (indicated in first column) and then removed and replanted without treatment.

** Indolebutyric acid.

The average length of roots on *Euonymus*, *Hibiscus*, and privet cuttings was about the same for all lots which had been treated initially with solutions containing indolebutyric acid. For example, in the case of *Euonymus* the total average length of roots in millimeters was 115, 119, and 113 for lots not receiving a deferred treatment, and 119, 111, and 113 for lots which received a deferred treatment with vitamin B₁. The corresponding root count values shown in Table IV are 125, 120, and 115 (Part A) and 107, 118, and 77 (Part B). Thus the deferred treatment reduced the number of roots without influencing appreciably the length of the roots.

The results here described for the ineffectiveness of deferred treatment of cuttings with vitamin B₁ are essentially the same as those reported previously for *Taxus*, *Celastrus*, *Actinidia*, and *Hibiscus* (7).

Treatment of the rooting medium. Cuttings treated initially with indolebutyric acid produced approximately the same number of roots with and without application of vitamin B₁ to the rooting medium (Table V). Increasing the concentration of indolebutyric acid caused an increase in the average number of roots on cuttings planted in control sand (Part A,

TABLE V

AVERAGE NUMBER OF ROOTS ON CUTTINGS PLANTED IN SAND WITH AND WITHOUT THE APPLICATION OF VITAMIN B₁ (1 P.P.M.) TO THE ROOTING MEDIUM

Name of cutting	No. days in sand	Part A				Part B			
		Without vitamin B ₁				With vitamin B ₁			
		Conc. IB in talc, mg./g.				Conc. IB in talc, mg./g.			
		0	1	3	8	0	1	3	8
<i>Ardisia japonica</i>	39	0	—	13	42	0	—	22	27
<i>Celastrus articulatus</i>	33	0	—	4	13	0	—	10	2
<i>Citrus aurantium</i>	57	1	—	—	4	1	—	—	2
<i>Fuchsia hybrida</i>	22	16	10	26	—	14	20	30	—
<i>Ilex glabra</i>	30	2	3	12	—	0	1	10	—
<i>Juniperus chinensis</i>	58	0	—	1	2	0	—	4	3
<i>Rosa</i> (var. Briarcliff)	22	3	20	—	—	1	25	—	—
<i>Taxus media hicksii</i>	58	0	—	5	10	2	—	6	11

Table V). The corresponding values for treated sand (Part B, Table V) show that relatively fewer roots were induced on cuttings of *Ardisia*, *Celastrus*, and *Juniperus* pretreated with the higher concentration of indolebutyric acid as compared with the number of roots induced by pre-treatment with the lower concentration. The reduction in number of roots on cuttings of these three genera was associated with a noticeable decrease in average root length and with one or more of certain other symptoms characteristic of a relatively high concentration of root-inducing substance (pronounced swelling and proliferation of the stem, retardation of shoot growth, and the reduction in the amount of basal callus).

Retardation of shoot growth was most pronounced on *Celastrus* cuttings pretreated with the higher concentration of indolebutyric acid and then planted in sand watered with vitamin B₁. Pronounced overtreatment effects were absent in cuttings of *Ilex* and *Taxus*. On cuttings of *Fuchsia* pretreated with the higher concentration of indolebutyric acid, the roots emerged entirely from the stem tissue above the base in the case of the cuttings planted in sand watered with vitamin B₁. Similarly treated *Fuchsia* cuttings planted in control sand rooted at the base in addition to rooting from stem tissue above the base. The lower root count value for *Citrus*

cuttings planted in the treated sand (Part B, Table V) was due to a lower (25 per cent) percentage of rooted cuttings. Seventy-five per cent of the cuttings rooted in control sand. This difference in rooting response is attributed to variability in the type of cuttings and not to any adverse influence of vitamin B₁.



FIGURE 3. Cuttings of *Taxus media hicksii* 58 days after treatment with talc preparations of indolebutyric acid. Left to right: control talc, 3 mg./g., and 8 mg./g., respectively, of indolebutyric acid. Cuttings planted in sand (top row) and in sand watered weekly with 1 p.p.m. of vitamin B₁ (lower row).

The rooting response of *Taxus* cuttings shown in Figure 3 is typical of the results obtained with most other genera in showing no increase in root growth resulting from additions of vitamin B₁ to the rooting medium. On the contrary, in those cases where the number of roots on cuttings in the treated sand was noticeably different from the number of roots on control cuttings, the response in the former case was characteristic of a relatively higher concentration of root-inducing substance. In this respect, the results are similar to those previously reported (7) in which case vitamin B₁ and several other substances appeared in some of the tests to function as activators but not as factors for root growth.

DISCUSSION

The fact that vitamin B₁ appeared to influence the growth of flat-grown aster seedlings but not pot-grown asters, indicates that any favorable effects of small additions of vitamin B₁ to the soil must depend upon special conditions which at present are not known. Even though not all conditions were identical in the two cases, such differences as existed do not appear to offer a substantial basis for a satisfactory explanation of the inconsistent results obtained. The larger number of flowers on aster plants receiving vitamin B₁ treatments was not to be anticipated. Neither Bonner and Greene (4) who reported beneficial effects of vitamin B₁ on growth nor Hamner (5) who reported no beneficial effects, found any differences in flowering. It seems probable that the differences in the responses of flat-grown aster plants were not entirely due to vitamin B₁.

The soil mixture was the same in all tests with aster seedlings, but there was more competition for nutrients and for light in the case of flat-grown plants than in the case of potted plants. Although the flat-grown plants exhibited no pronounced symptoms of nutrient deficiency, there was marked variation in the size of the plants in each flat, regardless of the treatment. It is thus possible that vitamin B₁ may be beneficial to growth under conditions in which the nutrients are limited. In the tests with flat-grown plants no provision was made to determine this point. On the other hand, Arnon (1) and Hamner (5) reported no influence of vitamin B₁ on the growth of plants given an adequate supply of complete nutrients.

In addition to the possible limitation in nutrient supply in the case of the flat-grown plants, there is the possibility that the China aster does not respond to small additions of vitamin B₁ during June and July in the same manner as during February and March. Hamner (5) obtained no influence of vitamin B₁ regardless of whether the plants were grown under long or short day conditions.

Considering the smaller differences resulting from dipping the root systems in vitamin B₁ solutions as compared with the differences obtained by applying the solution to the soil, it is not certain that in the former case the difference in growth was due to vitamin B₁. All plants washed free of soil were retarded more than those transferred to flats without having their roots washed, regardless of the concentration of vitamin B₁ used. Thus the treatment of roots at the time of transplanting did not reduce appreciably the setback or so-called "shock" as claimed by others (10).

Variability in the response of aster to treatment with vitamin B₁ is similar to the variability in the responses of other plants to vitamin B₁ reported by different workers. However, more weight is to be attached to the results with potted asters than to the results with flat-grown asters. In the potted plant test 20 replicate lots of soil were used for each of the four treatments with vitamin B₁ and also for the controls, whereas in the

flat test a single lot of soil was used for each of the three treatments with vitamin B₁ and also for the controls. The more substantial potted plant test makes the results far more convincing than the results with flat-grown plants. Lacking any substantial confirmation of the results of Bonner and Greene (4), it must be concluded that the growth of established plants is not significantly influenced by small additions of vitamin B₁ to the growing medium.

Went, Bonner, and Warner (11) and Warner and Went (10) have suggested that cuttings treated initially with an optimum concentration of a root-inducing substance form root primordia, many of which do not grow due to lack of vitamin B₁. They stated that if vitamin B₁ is applied as a deferred treatment after the root primordia are formed, root development is accelerated, resulting in the production of a larger number of roots and a faster rate of growth. Data in Table IV reveal no such beneficial effects of vitamin B₁ under conditions in which indolebutyric acid proved highly effective for rooting. Similar results were obtained previously with *Celastrus*, *Actinidia*, *Hibiscus*, and *Taxus* (7). Furthermore, we have shown (6, 7) that cuttings of camellia varieties which are difficult to root, respond readily to proper treatment with an effective root-inducing substance (indolebutyric acid or naphthaleneacetic acid) notwithstanding that camellia leaves are reported (4, 10) to be low in vitamin B₁. The consistently lower root count values resulting from a deferred treatment indicate that the procedure of subjecting cuttings to a 24-hour solution treatment 6 to 12 days after planting in the bench is likely to retard rather than accelerate rooting.

The application of vitamin B₁ to the rooting medium was not effective in promoting the root growth of cuttings of nine genera which had been treated initially with indolebutyric acid. However, in this case the retardation in certain instances was not due to the mechanics of the method as in the case of a 24-hour deferred treatment of the basal ends of cuttings. These results and also those reported previously (6, 7) indicate that cuttings of the plants used are capable of meeting their own vitamin B₁ requirements. Differences in rooting response in these tests were characteristic of a root-inducing substance and not of a root growth factor. Certain unpublished data which will be the basis of a future report, also indicate that the rooting responses of cuttings planted in mixtures containing different kinds and amounts of peat moss are not explainable on the basis of a single limiting factor such as vitamin B₁. In addition, tests with internodal and budless cuttings are now under way to determine the influence of vitamin B₁ under conditions in which the synthesis of vitamin B₁ is eliminated or greatly reduced.

SUMMARY

China aster seedlings grown in flats (one flat per treatment) during February and March produced taller plants with a greater fresh weight when the soil was watered weekly with a solution of vitamin B₁ (0.01, 0.1, and 1.0 mg./l.) than when watered only with tap water (control flat). In contrast, there was no influence of vitamin B₁ on stem elongation of potted asters grown individually in pots (20 pots per treatment) during June and July. Stem elongation of potted asters varied significantly for plants located in different rows on the greenhouse bench but not according to treatment with vitamin B₁ in concentrations of 0.1, 0.5, 1.0, and 10 mg./l. These results appear to be typical of the conflicting reports in the literature relating to treatment of the soil with vitamin B₁. However, the more substantial experimental plan for potted asters makes the results more convincing than those obtained with flat-grown asters.

Application of vitamin B₁ every three to five days to the rooting medium (sand) or as a deferred treatment to the basal ends of cuttings did not accelerate the development of roots as reported by Warner and Went. Differences in rooting response were characteristic of a root-inducing substance (indolebutyric acid) and not of a root growth factor. In a few cases vitamin B₁ appeared to function as an activator, thus increasing the activity of indolebutyric acid as previously reported for several substances in addition to vitamin B₁.

The results of these tests indicate that vitamin B₁ should not be recommended for practical use in treating cuttings or as a soil amendment until there is more substantial evidence than at present to show that plants or cuttings can benefit from an external supply of vitamin B₁.

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EMBRYOLESS DILL SEEDS

FLORENCE FLEMION AND ELIZABETH WATERBURY

Recently a grower of dill (*Anethum graveolens* L.) seeds sent us a sample of seeds which, in spite of being apparently well filled and in good condition, gave only about 50 per cent germination. Examination of these seeds showed that the seeds which failed to germinate had no embryos. The embryo lies at one end of the seed while the remaining content consists of endosperm. The embryoless seeds had apparently normal endosperm.

Various lots of dill seeds were obtained from other sources and examination of these seeds for germinative capacity and for presence of embryos has shown that failure to germinate is due to lack of embryos. Seeds with embryos showed satisfactory germination. These results indicate that the occurrence of seeds without embryos but with endosperm is quite frequent in dill and accounts for low germination results with fresh seeds.

TECHNIQUE AND RESULTS

Duplicate lots of 100 seeds each were soaked in water at room temperature for about 20 hours, then examined in order to determine the percentage of seeds containing embryos. The embryo is quite small and lies embedded in the endosperm at one end of the seed (Fig. 1 C). In seeds lacking embryos there is a cavity in the endosperm where the embryo would normally be found (Fig. 1 B). Such seeds have apparently normal endosperm while the embryos either aborted at an early stage of growth or never developed. By slitting the seeds open longitudinally those with cavities could be readily detected. A few were empty, that is devoid of both endosperm and embryo, while in a few others the entire contents of the seed had deteriorated. In macroscopic preparations there was no evidence of the presence of embryos in the cavities. However, in preliminary studies of microscopic preparations made by Norma E. Pfeiffer, evidence of disintegrated tissue was found in the cavities.

The variation in size of the embryo is shown in Figure 1 A. Frequently, but not invariably, the smaller embryo was found in the smaller seeds. In general, the smaller seeds in a given lot contained a greater proportion of embryoless seeds than did the larger seeds from the same lot.

At the time the cutting tests were made (February and March, 1941) duplicate lots of 100 seeds each from the same lots were planted in soil and kept in a greenhouse maintained at approximately 70° F. except during warm days when the temperature frequently rose higher. The soil mixture

consisted of one-third peat moss, one-third sand, and one-third sterilized sod soil. The figures presented in Table I are the total germinations obtained by the twentieth day, although most of the seeds had germinated by the tenth day. All of the seeds were of the Long Island Mammoth variety with the exception of the set grown in North Africa which was labeled common dill.

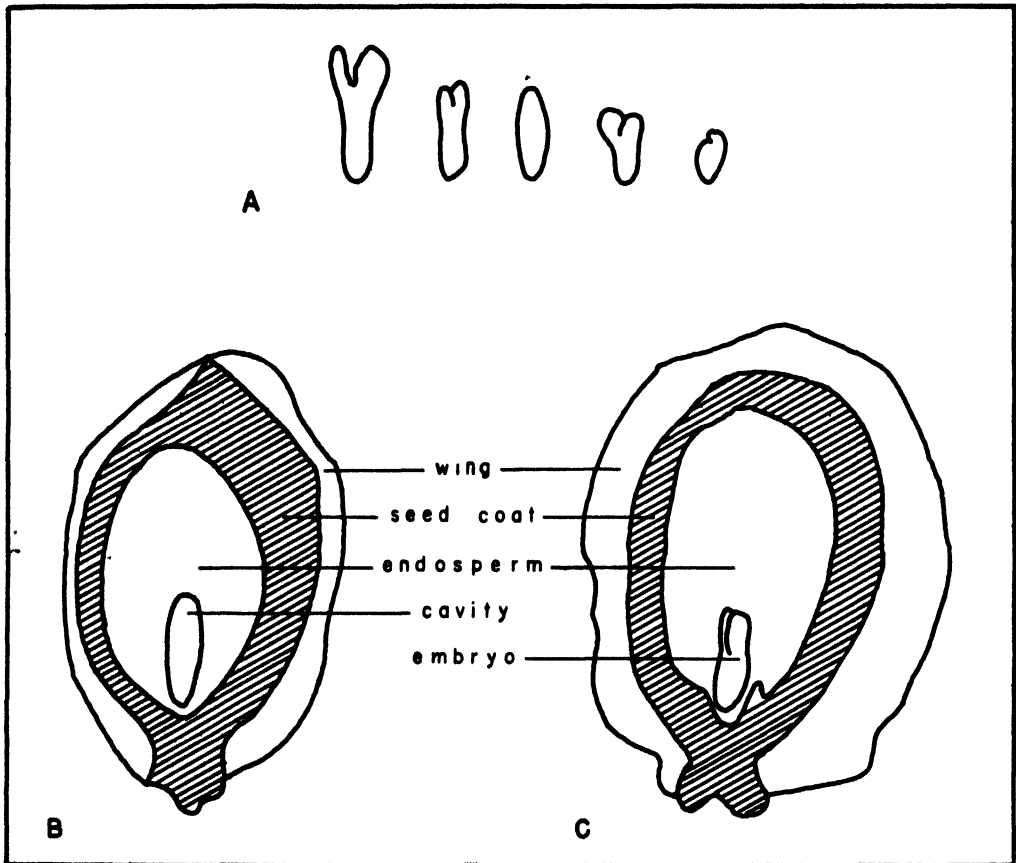


FIGURE 1. Dill seeds—isolated embryos and free-hand longitudinal sections. A. Shows variation in size of embryos. B. Section shows cavity in embryoless seed. C. Section shows embryo embedded in endosperm. Camera lucida drawings $M \times 11$.

The data obtained from 12 different lots of dill seed (Table I) illustrate the close agreement of the percentage germination and the percentage of seeds containing embryos. In several instances the germination is somewhat lower and this is probably due to some of the embryos lacking sufficient vigor to germinate. It is readily seen that most of the seeds which failed to germinate lacked embryos.

One other member of the Umbelliferae was examined for this absence of embryos. A lot of common parsley (*Petroselinum hortense* Hoffm.), 1940

crop grown in California, contained 21 per cent embryoless seeds. When planted under the same conditions as described above for dill seeds, 73 per cent germinated.

TABLE I

PER CENT OF EMBRYOLESS SEEDS AND GERMINATION OF VARIOUS LOTS OF DILL SEEDS

Origin	Crop	Cutting test, per cent*				Germination, per cent*
		Embryoless	Empty	Non-viable	Embryos	
American	1940	62	1		38	29
California	"	13			87	87
"	"	48		4	48	46
"	"	9			91	87
Connecticut	1939	39**	2		59**	54†
Holland	"	14		1	85	82
"	"	8	1		91	88
Holland or Denmark	"	12			88	88
New York	1940	9**			91**	94†
North Africa	"	11			89	83
North Dakota	"	7			93	87
?	?	60		5	35	24

* Averages of duplicate lots of 100 seeds.

** Only 1 set of 100 seeds.

† Duplicate lots of 50 seeds.

DISCUSSION

As far as the authors are aware, the phenomenon of embryoless seeds in dill has not been previously reported. However, embryoless seeds have been reported in a number of species especially in the cereals.

The frequency of occurrence as observed in wheat, rye, and bald barley was about 0.1 per cent (13), in rice from 0.01 to 0.02 per cent (12), and in seeds of castor-oil plant about 0.18 per cent (15). This occurrence is quite low in contrast to the percentage found in dill seeds which in several instances was about 60 per cent. In *Ginkgo* (5) 12 per cent were found to be without embryos, but it is not clearly stated whether such seeds contained endosperm.

Several workers (6, 14, 17) correlated the occurrence of embryoless seeds in corn with inheritance and linkage relations. In wheat and rye (7, 11) it appeared to be a varietal character. Another embryoless type has been observed which is believed to be non-hereditary in character and is probably due to some irregularity during fertilization (8, 14). In some cases (9) it appears that without the stimulus of fertilization apparently normal seeds develop which contain endosperm but lack embryos. Various causes have been discussed such as age of pollen as well as external factors, the combined action of which might be responsible (14, 15). Even if the fertilization mechanism has functioned properly, the growing seed is under the

influence of various genetic factors which may cause an arrested development at any stage of growth.

Many crops of carrot yield poorly germinating seeds. An examination (2, 3) has shown that the failure of a large percentage of the seeds to germinate was due to defective or immature embryos. Cole, as stated by Borthwick (3), found that some of the immature embryos would germinate when kept for long periods under germination conditions. Double fertilization occurs in this family (10). In carrot (4) not only is the pollen apparently short-lived but it appears that cross-pollination occurs between umbels, while in parsnip (1) cross-pollination within umbels seems to be the rule. In carrot (3), seeds produced in umbels of the first and second order are of somewhat better germinating quality than those produced in umbels of the third and fourth order. The seed crop of carrot is about equally divided between the first two and the last two orders of umbels.

In plantings of dill (16) spacing which gives a high yield per acre is much closer than where the maximum individual plant development occurs, but unfortunately no tests were made to determine the germinative capacity of seeds obtained from various parts of the plant grown at various spacings. Some growers report that oftentimes the poorest yielding fields (sparse stand) will produce seeds of high germinating quality, while fields with perfect stands giving a high yield per acreage will produce seeds of poor germinating quality. Dill has been grown on various types of soil and under varying climatic conditions such as temperature and moisture, but the growers still continue to have difficulty.

Dill seed has been produced chiefly in Europe but due to cutting off the supply from abroad there will be a large increase in production in the United States this year. The only seeds grown in Europe which were tested were of the 1939 crop from which a high germination was obtained. From correspondence with various seedsmen, the consensus of opinion is that European-grown dill also fluctuates from year to year but not to such a great extent as does dill grown in the United States. It has been estimated by some growers that about 90 per cent of their dill seed crops are of below-normal germination.

An examination of twelve different lots of dill seeds revealed that most of the seeds which failed to germinate were without embryos although the endosperm was present. The occurrence of these embryoless seeds is quite frequent and accounts for the great variation in the germinative capacity found in the various lots.

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SIMULTANEOUS FORMATION OF A β -GENTIOBIOSIDE AND A β -GLUCOSIDE IN GLADIOLUS CORMS TREATED WITH CHEMICALS

LAWRENCE P. MILLER

Experiments on the artificial production of glycosides in *Gladiolus* corms from absorbed chemicals which serve as aglycons have shown that the sugar component of the glycosides formed depends on the nature of the introduced aglycon. Thus, with ethylene chlorohydrin β -2-chloroethyl-*d*-glucoside is formed (1) but, with *o*-chlorophenol or trichloroethyl alcohol, β -gentiobiosides are produced (3, 4). Gentiobiose [6-(β -*d*-glucosido)-*d*-glucose] is not known to occur normally in gladiolus corms although a detailed study of the carbohydrates present does not appear in the literature. Preliminary tests on the action of emulsin on the expressed juice of control corms have shown only a small increase in reducing power after emulsin hydrolysis indicating that, if any gentiobiose is present, the quantity is low. It seems quite probable, therefore, that the gentiobiose is produced as a result of the chemical treatment. The introduced chemical may induce the formation of gentiobiose which is then combined with the aglycon to form the β -gentiobioside, or the β -gentiobioside could arise without the presence of any free gentiobiose at any stage through condensation of the chemical with *d*-glucose to the β -glucoside and subsequent condensation of the β -glucoside in the 6-position with another mol of *d*-glucose. If the β -gentiobioside is formed as the result of the stimulation of the production of gentiobiose by the chemical treatment, the presence in the tissue of another chemical, which of itself does not stimulate the production of gentiobiose, might result in this chemical also being condensed with gentiobiose. It, therefore, seemed of interest to subject gladiolus corms to the simultaneous action of *o*-chlorophenol and ethylene chlorohydrin to see whether the specificity of these chemicals for condensation with either glucose or gentiobiose in gladiolus corms would persist in such a dual treatment.

Acetylation of purified extracts of corms treated as indicated above yielded a product which was readily separated into two fractions, the one being β -*o*-chlorophenyl-gentiobioside heptaacetate (m. p. 207.5° to 208.5° C., corr.), and the other β -2-chloroethyl-*d*-glucoside tetraacetate (m. p. 118° to 119°). No evidence was obtained for the presence in the acetylated mixtures of either β -*o*-chlorophenyl-*d*-glucoside tetraacetate (2) (m. p. 150.5° to 151°) or β -2-chloroethyl-gentiobioside heptaacetate (m. p. 167.0° to 167.5°, unpublished results). It is thus seen that the type of glycoside

resulting from the absorption of one chemical was not influenced by simultaneous treatment with another chemical which combined with a different sugar.

EXPERIMENTAL

Gladiolus corms of the Alice Tiplady variety (2300 g.) were placed in a 7-liter desiccator connected to several series of Van Slyke-Cullen tubes (5) and to a vacuum pump so that a stream of air which had first passed through two tubes containing either *o*-chlorophenol or ethylene chlorohydrin could be drawn through the desiccator. The switch from one chemical to the other was made every few hours with the *o*-chlorophenol treatments being continued for somewhat longer periods than the chlorohydrin treatments because of the lower vapor pressure of *o*-chlorophenol. Usually the chemical treatment was carried out for only about eight hours each day; air which had not been in contact with either chemical was drawn through for the other 16 hours. With this method of treatment it would be expected that glycoside formation from the absorbed *o*-chlorophenol and ethylene chlorohydrin would take place simultaneously in the treated corms. During a 247-hour period the corms were subjected to the vapor of *o*-chlorophenol for 52 hours and to ethylene chlorohydrin for 39 hours. Twelve days after the end of this treatment period the corms were sampled for the characterization of the glycosides formed.

The corms were ground through a food chopper and the juice expressed by squeezing through cheesecloth. The residue was covered with water and a little toluene and again squeezed through cheesecloth. This was repeated a second time and an additional aqueous extract obtained. The expressed juice and the aqueous extracts, handled separately, were centrifuged, heated to 80° C., and again centrifuged. A portion of the expressed juice was subjected to the action of emulsin for 16 hours at 35° C. in a M/20 acetate buffer at pH 4.75. Distillation of the sample after emulsin hydrolysis resulted in the recovery of 0.54 millimol of *o*-chlorophenol and 0.38 millimol of ethylene chlorohydrin per 100 cc. of expressed juice. The sample without added emulsin yielded no *o*-chlorophenol and only 0.08 millimol of ethylene chlorohydrin per 100 cc.

The expressed juice and the aqueous extracts were combined and precipitated with an excess of lead acetate. The filtrate from the lead precipitate was freed from excess lead by H₂S. The lead-free filtrate was then concentrated under reduced pressure to a thin syrup and extracted with two portions of 200 cc. of acetone and two portions of 200 cc. of 90 per cent acetone. The combined acetone extracts were concentrated under vacuum and the aqueous residue remaining after the removal of the acetone extracted a number of times with ethyl ether. The aqueous solution was again concentrated to a thin syrup and extracted with 50 cc. of acetone followed by two 50-cc. portions of 90 per cent acetone. The first acetone

extract and the last two acetone extracts were evaporated to dryness and acetylated in separate portions. The crude product on solution in acetone and crystallization after the addition of absolute alcohol yielded two fractions in each case, the one (total weight 1.29 g.) melting at 203° to 206° C. (before recrystallization), and the other (0.87 g.) at about 115° .

The residue remaining after the original acetone extractions was freed of acetone and extracted in a continuous extractor with ethyl acetate. Acetylation of the material extracted by ethyl acetate yielded a further 1.22 g. of the higher melting fraction and 1.05 g. of the lower melting product.

Since β -2-chloroethyl-*d*-glucoside tetraacetate is several times as soluble in absolute alcohol as β -*o*-chlorophenyl-gentiobioside heptaacetate, the two substances separated out well even in the first crystallization of the crude product. Recrystallization from absolute alcohol gave pure β -*o*-chlorophenyl-gentiobioside heptaacetate, m. p. 207.5° to 208.5° C., $[\alpha]_D^{25} = -49.3^{\circ}$ (concn., 2.96 g. in 100 cc., CHCl_3) and β -2-chloroethyl-*d*-glucoside tetraacetate, m. p. 118° to 119° , $[\alpha]_D^{25} = -13.4^{\circ}$ (concn., 3.6 g., CHCl_3). These constants compare with a melting point of 207.5° to 208.5° and a specific rotation of -49.7° found for synthetic β -*o*-chlorophenyl-gentiobioside heptaacetate (3) and a melting point of 118.5° to 119.0° and a specific rotation of -13.4° for synthetic β -2-chloroethyl-*d*-glucoside tetraacetate (1). Mixed melting point determinations of the isolated products with the corresponding synthetic compounds gave no depression.

There was no indication that either β -2-chloroethyl-gentiobioside heptaacetate or β -*o*-chlorophenyl-*d*-glucoside tetraacetate was present in the acetylated mixture. It seems safe to conclude that neither of these glycosides was formed in any appreciable quantity if at all.

SUMMARY

Condensation of ethylene chlorohydrin with *d*-glucose to form the β -glucoside and formation of the β -gentiobioside from absorbed *o*-chlorophenol took place in gladiolus corms even when the chemical treatments were carried out under conditions involving the simultaneous production of the two glycosides. No evidence was obtained for the formation of either β -2-chloroethyl-gentiobioside or β -*o*-chlorophenyl-*d*-glucoside.

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FATE OF CHLORAL HYDRATE ABSORBED BY GROWING PLANTS OF *LAGENARIA LEUCANTHA*

LAWRENCE P. MILLER

The absorption of chemicals which can serve as aglycons by higher plants leads to the formation of β -glycosides (2). Characterization of such glycosides in a number of species has shown that the sugar component may vary with the species or in the same species with different aglycons (3). In the species studied thus far the sugar involved has been either *d*-glucose or gentiobiose. The formation of β -gentiobiosides was an unexpected result since gentiobiose is not known to be widely distributed in plants and its occurrence as the sugar component of natural glycosides is rare. Studies of additional species will yield information as to the frequency of such β -gentiobioside formation and will disclose whether some species will utilize sugars other than *d*-glucose or gentiobiose in glycoside formation from introduced chemicals.

Results given in the present report show that plants of *Lagenaria leucantha* Rusby form β -2,2,2-trichloroethyl-*d*-glucoside from absorbed chloral hydrate. Chloral hydrate is an aldehyde and has to undergo reduction to the alcohol before glycoside formation occurs. This reduction apparently takes place readily in the plant. The identity of the glycoside formed in *L. leucantha* was established through the synthesis of β -2,2,2-trichloroethyl-*d*-glucoside tetraacetate, which was prepared for the first time, and which was found to be identical with the acetyl glycoside obtained from the treated plants.

EXPERIMENTAL

Treatment of Growing Plants with Chloral Hydrate

Seeds of *Lagenaria leucantha* were placed on moist filter paper and after the emergence of the hypocotyl were transferred to sand cultures in 8-inch shallow earthenware pots, 2 seeds to each pot. Nutrient was supplied by the drip culture method of Shive and Robbins (5). The solution was made up according to Formula I of these authors and in addition a minor element solution was added so that the final solution contained 3 p.p.m. of iron as ferric tartrate, 0.50 p.p.m. of manganese as $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.25 p.p.m. of boron as H_3BO_3 , and 0.125 p.p.m. of zinc as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. For the first five days after transfer of the seeds to the sand cultures the nutrient solution was diluted to one-fifth its usual strength. The flow was so regulated that about one liter of solution was added to each culture in 24 hours. As the plants grew larger this was increased to meet the increasing demands of

the plants for moisture. After 37 days the plants had reached a height of about 8 feet and were in vigorous condition. For treatment with chloral hydrate the drip culture method was discontinued, the pots supplied with saucers and 1.0 millimol of chloral hydrate added to each of 12 cultures 6 times weekly for 8 applications. The equivalent of 25 cc. of each of the salt solutions used previously for sand cultures (3) was also added to supply nutrients during this period.

Isolation of β -2,2,2-Trichloroethyl-d-glucoside as the Tetraacetate

The plants were sampled four days after the last addition of chloral hydrate. The tops and roots were ground separately through a food grinder and the juices expressed through cheesecloth. The expressed juices were centrifuged, heated to 80° C. and again centrifuged. Analysis for chlorine before and after heating with N KOH indicated the presence of non-ionic chlorine equivalent to 0.47 and 0.36 millimol of a trichloro-compound per 100 cc. of the expressed juice of the tops and roots respectively. On distillation non-ionic chlorine equivalent to 0.04 and 0.09 millimol per 100 cc. was recovered; after emulsin hydrolysis an additional 0.12 and 0.06 millimol was obtained from the top and root juices respectively.

A purified preparation suitable for acetylation was obtained from the top juice by the same procedure previously used with tomato plants treated with chloral hydrate (4). The portion acetylated contained non-ionic chlorine equivalent to 4.4 millimols of a trichloro-compound and yielded 1.80 g. of crude product melting at 141° to 142° C. Treatment with Norite and recrystallization from absolute alcohol gave 1.16 g. melting at 144.5° to 145.5° (corr.) with a specific rotation $[\alpha]_D^{25} = -29.3^\circ$ (concn., 4.1 g. in 100 cc., CHCl_3). A mixed melting point determination with synthetic β -2,2,2-trichloroethyl-d-glucoside tetraacetate gave no depression.

Analysis: Calcd. for β -2,2,2-trichloroethyl-d-glucoside tetraacetate, $\text{C}_{16}\text{H}_{21}\text{O}_{10}\text{Cl}_3$: Cl, 22.17. Found: Cl, 22.12, 22.09.

Synthesis of β -2,2,2-Trichloroethyl-d-glucoside Tetraacetate

A solution of 29.8 g. (0.20 mol) of trichloroethyl alcohol (1) in 90 cc. dry benzene was cooled in an ice bath and 16.5 g. (0.04 mol) acetobromoglucose and 14 g. Ag_2CO_3 added. The mixture was shaken frequently during the first few hours after which it was allowed to stand at room temperature overnight. The mixture was then filtered, washed well with benzene, and the filtrate evaporated *in vacuo*. Water was added to aid in the removal of unreacted trichloroethyl alcohol. Finally the product was crystallized from absolute alcohol. Yield of crude product 1.66 g., m. p. 137.5° to 138.5°. After three recrystallizations from absolute alcohol, 0.98 g. melting at 144.5° to 145.5° was obtained. Specific rotation was found to be $[\alpha]_D^{25} = -29.0^\circ$ (concn., 3.095 g., CHCl_3).

Analysis: Calcd. for $C_{16}H_{21}O_{10}Cl_3$: Cl, 22.17. Found: Cl, 22.01, 22.15.

SUMMARY

Plants of *Lagenaria leucantha* have been shown to form β -2,2,2-trichloroethyl-*d*-glucoside when grown in a medium containing chloral hydrate.

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X-RAY STUDIES REGARDING THE FORMATION AND ORIENTATION OF CRYSTALLINE CELLULOSE IN THE CELL WALL OF VALONIA¹

WAYNE A. SISSON

During the past ten years, the mature cell wall of the large, single-celled, marine alga, *Valonia ventricosa* Agardh, has been the subject of X-ray diffraction studies by several investigators. Sponsler (11, 12), in 1930, first focused the attention of X-ray workers upon *Valonia* when he pointed out the unique possibility which it offered for determining the orientation of the two minor axes of the cellulose crystal lattice. Since the first X-ray work on cellulose, the two major axes of the unit cell were known to be oriented in most fibers either parallel or at some spiral angle to the fiber axis. Owing to the cylindrical form of cellulose fibers, however, it was impossible to obtain further definite evidence regarding the orientation of the cellulose lattice with reference to the surface of the cell wall. Sponsler (12) pointed out that the difficulty in splitting longitudinally such a small cell as the cotton fiber and pressing it flat for X-ray analysis could be easily overcome in the large wall of *Valonia*, which often grows to a diameter of over three centimeters. By cutting flat pieces of the *Valonia* cell wall, several of which were superimposed horizontally, Sponsler was able to show that the 101 (6.1 Å) and the 101⁻ (5.4 Å) crystallographic planes intersect each other in the cellulose unit cell at an angle of approximately 90°. Of special biological interest (11) was the fact that the 101 planes were found to lie roughly parallel to the surface of the cell wall.

In 1932 Astbury, Marwick, and Bernal (1) made X-ray patterns of single pieces of the *Valonia* membrane. In addition to the orientation of the 101 plane showed by Sponsler's superimposed sections, they found two principal sets of crystallites crossing each other at an angle of approximately 80°. When compared with Preston's microscopic data (6), it was found that the X-ray crystallites are parallel to the direction of the fine crossed striae of the wall which are visible in the microscope. Preston and Astbury (7) later published in greater detail their X-ray and microscopic studies and showed that the cell wall consisted of layers in which the cellulose chains in any one layer are inclined to those in the adjoining layers at an angle of approximately 80°. Van Iterson (5) has discussed some of the reasons why alternate layers in *Valonia* should have opposite orientations.

In 1936, Farr (3) reported the existence of cellulose particles in *Valonia* which were similar to particles previously found in the young cotton fiber

¹ A contribution from the former Cellulose Department of the Chemical Foundation, Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

and other developing plant cells by Farr and Eckerson (4). Microscopic mounts from the liquid portions of the cytoplasm showed separate cellulose particles, while in the layers of dense cytoplasm many of the particles were arranged end to end in the form of fibrils. In the outer layers of the membrane the fibrils cross at an angle of approximately 80° .

Since the *Valonia* cell consists of a large volume of liquid cytoplasm surrounded by a thin layer of dense or viscous cytoplasm which in turn is limited by the compact cellulose membrane, the present investigation will be discussed under three headings: (a) liquid cytoplasm, (b) viscous cytoplasm, and (c) mature cellulose membrane. The studies on liquid cytoplasm are concerned primarily with the identification of crystalline cellulose, those on the viscous cytoplasm deal with the production of orientation, while the orientation of the mature cell wall is discussed in the third section.

METHODS AND RESULTS

Cells of *Valonia ventricosa* were used in the present investigation. The sample was collected by Captain John W. Mills at Dry Tortugas in June 1937. The live cells were placed in fruit jars containing sea water, brought by boat to Key West and hence by airplane to New York. In the laboratory at Yonkers, the material was kept green and apparently alive for several weeks. The cytoplasmic studies were made immediately on the green cells, while the orientation studies were made later on cells preserved in either a 95 per cent ethyl alcohol solution or in a solution of 1.4 per cent formalin and 70 per cent alcohol.

The X-ray diagrams were made with unfiltered copper radiation as described in an earlier paper (9).

LIQUID CYTOPLASM

The liquid cytoplasm was obtained by piercing the wall of the green *Valonia* cell with a razor blade and collecting the fluid contents in a beaker, care being taken not to dislocate material from the inner cell wall which would pass out with the liquid. The last few drops of the liquid were discarded. The liquid (250 cc.) was centrifuged and decanted to obtain a residue which gave a diffraction pattern showing Laue spots. This indicated the presence of salts. The residue was then washed several times with water, after which it gave the X-ray pattern shown in Figure 1 A. This pattern contains a broad amorphous band and two sharp diffraction rings, but no evidence of the characteristic cellulose diffraction rings. In order to show the presence of crystalline cellulose it was necessary to subject the residue to a further purification. This was accomplished by heating the residue in a 2 per cent sodium hydroxide solution, followed by bleaching with a 2 per cent hypochlorite solution and washing with dilute (0.1 N)

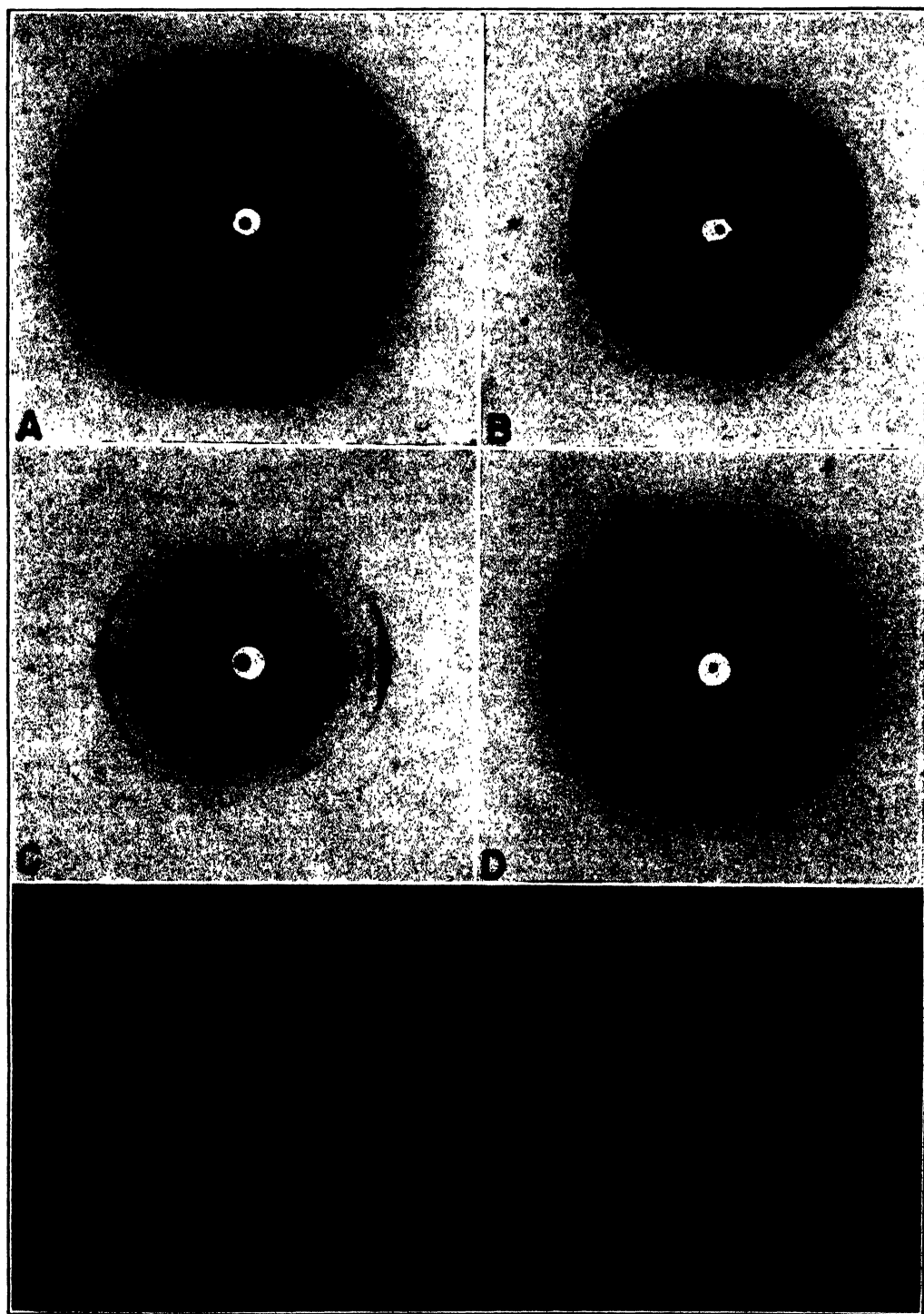


FIGURE 1. X-ray diffraction patterns of (A) residue centrifuged from liquid cytoplasm, (B) same material as (A) after purification, (C) fibers formed by stretching viscous cytoplasm, (D) disintegrated membrane after treatment with 18 per cent sodium hydroxide, and (E) photomicrograph of cellulose particles and fibrils in viscous cytoplasm.

hydrochloric acid and water. The X-ray diagram of the final residue is shown in Figure 1 B. In addition to the few Laue spots due to salts, there is present the characteristic pattern of crystalline cellulose.

VISCOUS CYTOPLASM

A number of *Valonia* cells were emptied of their liquid contents and the membrane cut into four quarters with a sharp razor. These quarters were carefully washed by dipping them in distilled water. The viscous cytoplasm lining the inner membrane was then removed by fastening to a support one end of the membrane and scraping with a spatula. The sample of cytoplasm obtained in this way was divided into two parts, one of which was dried in the form of films and the other as fibers. A photomicrograph of the viscous cytoplasm is shown in Figure 1 E.

Films. The viscous cytoplasm was spread in the form of a thin film on a glass slide and dried at room temperature. The tough dried film, which adhered to the glass slide, was cut into strips 2 mm. wide which were removed from the glass with a razor blade. Several of these strips were mounted horizontally to build up a mount for X-ray examination. X-ray diagrams of this mount with the X-ray beam perpendicular and parallel to the plane of the film are shown in Figure 2 A and B, respectively. Corresponding X-ray diagrams of the natural membrane are shown in Figure 2 C and D.

The parallel X-ray diagrams (Fig. 2 B and D) show the 101 lines present as strong arcs of about equal orientation, while the 101^- lines are only faintly present as weak arcs which are at right angles to the 101 arcs. This type of X-ray diagram indicates a selective orientation (8) which is further shown in the perpendicular diagrams (Fig. 2 A and C) where the 101 line is now absent and the 101^- present. The perpendicular diagrams of the artificial and natural membranes, however, differ in that the artificial membrane (Fig. 2 A) shows a random orientation while the natural membrane (Fig. 2 C) shows the characteristic cross orientation.

Fibers. The viscous cytoplasm was placed in the form of a strip on a glass slide, and after the strip had dried sufficiently to support itself it was removed from the glass, stretched slowly as drying progressed, and finally placed across the open end of a glass tube. Upon drying, the ends of the strips, which extended down the outside of the tube, adhered to the glass so that a tension was set up in that portion of the strip which was stretched across the open end of the tube. The stretched portion dried to form a strong taut fiber. X-ray diagrams of the fiber are shown in Figure 1 C.

Examination of Figure 1 C shows that the 101 , 101^- , and 002 lines all exist as arcs on the equator. This means that the b axes (direction of cellulose chains) are oriented parallel to the direction of stretch and that they have a random orientation around this axis [uniaxial orientation (8)].

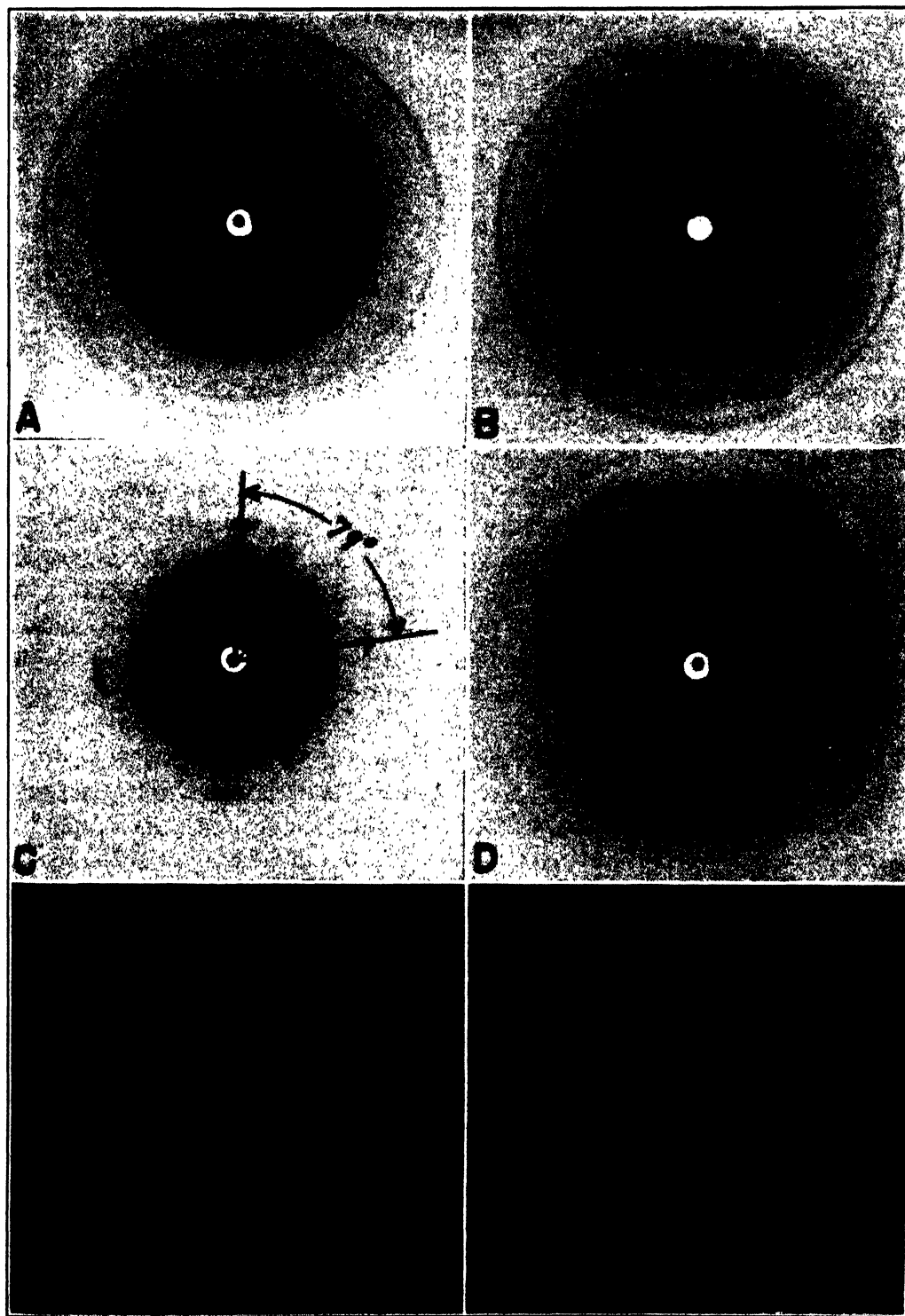


FIGURE 2. X-ray diffraction pattern of membrane formed from viscous cytoplasm (A) perpendicular and (B) parallel to membrane surface; (C) and (D) are corresponding X-ray diagrams, and (E) and (F) photomicrographs of the mature membrane.

MATURE MEMBRANE

The present investigation confirms the cross spiral orientation, shown in the perpendicular X-ray diagram (Fig. 2 C), and the selective orientation, shown in the parallel X-ray diagram (Fig. 2 D), which have been observed by other investigators (1, 7, 11, 12). In addition, comparative X-ray and microscopic studies show that the X-ray orientation may be closely correlated with the orientation of the cellulose particles which are arranged end to end to form the fibril. The close correlation between the X-ray angle and the fibril angle is shown by a comparison of C and E in Figure 2. The selective orientation shown in the parallel X-ray diagram (Fig. 2 D) cannot be detected in the microscope because of the circular shape of the fibril cross section. The selective orientation shown in the X-ray diagram, however, is in agreement with the laminated structure shown in the photomicrograph of the cross section (Fig. 2 F).

The effect of *Valonia* cell size upon the cross spiral angle was investigated. A series of 15 uniformly-shaped cells, varying in size from 0.27×0.39 cm. to 3.6×4.2 cm. for their minor and major axes, was selected. The relation of size to age for these cells is not known but it is assumed that the size is related to age. X-ray diagrams of all the cells were taken on a section of the membrane opposite the holdfast. The angles measured on the X-ray diagram all fall between 73° and 85° and there is no correlation between the angle and the size of the *Valonia* cell. The angle variation observed from cell to cell is no greater than that observed in different sections of the same cell. The spiral angle appears to be more closely related to the shape of the *Valonia* cell rather than to the size, since diagrams taken at various positions on very irregular cells show a wider variation in angle than do similar diagrams made on cells which more closely approach a sphere.

Action of swelling agents. The native membrane of *Valonia* is rather inert toward many chemical reagents which normally swell or disperse most cellulosic membranes. For example, 70 per cent sulphuric acid, cuprammonium hydroxide, 18 per cent sodium hydroxide, etc. produce no visible swelling, and after washing out the swelling agent the X-ray diagram is still that of native cellulose. If the membrane, however, is first boiled in water and treated with dilute hydrochloric acid it is then swollen by swelling agents, and after washing out the reagent it gives the characteristic X-ray diagram of mercerized cellulose as shown in Figure 1 D. This inertness of the *Valonia* membrane is probably due to the compact form of the membrane since microscopic observations show isolated fibrils to be affected by swelling agents.

DISCUSSION OF RESULTS

LIQUID CYTOPLASM

The X-ray identification of crystalline cellulose in the liquid cytoplasm of *Valonia* is in agreement with the observation of Farr (3) that there are

present in the liquid cytoplasm cellulose particles, whose size, shape, double refraction in polarized light, and reaction to sulphuric acid and iodine are like those of the particles from the young cotton fiber. The cellulose diffraction rings from *Valonia*, however, differ from those of the young cotton fiber in that the diffraction rings are sharper and more clearly resolved, indicating that the crystallinity of the cellulose is more perfect in *Valonia* than in cotton. Sponsler (11) has observed that the diffraction rings produced by the mature *Valonia* membrane are sharper and more clearly resolved than those obtained from other cellulose fibers.

It is interesting to note that the X-ray diagram of the unpurified residue (Fig. 1 A) is similar to that obtained by Preston and Astbury (7, Fig. 6, Plate I) for the "pole" area of the *Valonia* cell. They attributed this diagram to a cross section of a cylindrical holdfast located at the pole. They state further that the holdfast is "filled with small granules which appear to be plastids surrounded by a comparatively thick layer of starch."

Since sharp, well defined cellulose diffraction lines may be observed in the original negative of Figure 1 B, it is most likely that the cellulose pattern is merely masked by the pattern of the other constituents present in the material of Figure 1 A which are later removed by the purification process to give the diagram shown in Figure 1 B. The masking here is probably similar to that observed in young cotton fibers (9).

VISCOUS CYTOPLASM

Previous X-ray studies (8) have shown that whenever a swollen sample of cellulosic material is constricted in one direction, either by dehydration or by pressure, then the cellulose crystallites orient themselves with the 101 plane normal to the direction of constriction. On the basis of this behavior, Preston and Astbury (7) have suggested that "it is probably unnecessary to invoke anything more complicated than the simple act of drying" in order to explain the observed selective orientation of the 101 crystallographic planes parallel to the *Valonia* membrane surface. The present work would appear to offer experimental confirmation of this suggestion. A comparison of the parallel X-ray diagram of the membrane artificially prepared from the *Valonia* cytoplasm (Fig. 2 B) with the diagram of the native membrane (Fig. 2 D) shows that the selective orientation in the artificial membrane is just as perfect as that in the natural membrane.

The orientation in the plane of the two membranes, however, is quite different. In the artificial membrane (Fig. 2 A) the crystallites have a random orientation while in the native membrane (Fig. 2 C) they have the characteristic cross orientation. This would seem to suggest that the selective orientation in both artificial and native membranes may have been produced by a common factor such as dehydration, but that other factors besides dehydration are involved in the production of the cross

orientation of the native membrane. The fact that parallel orientation can be produced by the application of tension (Fig. 1 C) would indicate that tension could be a factor in producing the parallel cross orientation of the native membrane.

The production of a selective orientation by drying viscous cytoplasm composed of fibrils and particles (see Fig. 1 E) would seem to indicate that a whole fibril can be rotated during drying so that the 101 crystallographic plane is parallel to the surface. In previous work on bacterial cellulose membranes (8) it was impossible to determine definitely the nature of the crystalline aggregate (crystallite) which moved as a unit, but there was "some evidence that bead-like strands of bacteria move as units during plastic flow." The present results, however, indicate more clearly that the crystalline aggregate which moves as a unit is the whole cellulose particle or many particles united as a fibril. It is well known that relatively short molecules possessing polar groups or that large molecular aggregates which are anisodimensional can be oriented, but it appears unique to have as large a unit ($1\ \mu$ in diameter) as the cellulose particle rotated by molecular forces. The parallel orientation of the fibrils by stretching could be due to the shape of the long thread-like fibrils, but there is nothing visible about the shape of the fibril which would explain selective orientation.

MATURE MEMBRANE

The cross spiral and the selective orientation observed in the X-ray diagram of the mature *Valonia* membrane and its close correlation with the observed particle or fibril orientation makes possible certain conclusions regarding the structure of the cellulose particle. As pointed out elsewhere (10), it is possible to account completely for the dispersion from a perfectly parallel oriented X-ray diagram for *Valonia* on the basis of the observed deviation of the fibrils from a parallel orientation in the two spiral directions, as may be observed by a comparison of C and E in Figure 2. Since the rotation of the particle around its long axis cannot be observed in the microscope it is impossible to draw as close a correlation with the parallel X-ray diagram, but it seems quite certain that the length of the 101 arc (Fig. 2 D) can be explained largely on the basis of the fibril rotation.

Particle structure. From the present data two conclusions can be drawn regarding the structure of the cellulose particle. First, the cellulose chain molecules (direction of *b* axis of unit cell) are arranged parallel to the long axis of the cellulose particle. Whether a single cellulose chain runs the entire length of the particle ($1.5\ \mu$) or only part of the way, is not known, but it is fairly certain that the cellulose chains, regardless of their length, can have little or no deviation from a perfect orientation parallel to the long axis of the particle. Second, the various crystallographic planes of the unit cell extend continuously in the same direction throughout the particle.

This means that, in addition to the parallel orientation, the cellulose chain molecule and also the glucose units which build up the chain have a three dimensional regularity which extends throughout the particle. In other words, the crystalline regularity of the particle approaches that of a single crystal. It is impossible to say definitely that the cellulose particle does not contain still smaller aggregates, but if such is the case then the present data impose the condition that the aggregates have a perfect three dimensional orientation within the particle, and the particles may still be classified as a single crystal rather than a crystal aggregate (2).

SUMMARY

1. The marine alga, *Valonia ventricosa* Agardh, has been examined by X-ray diffraction analysis with special reference to the inner liquid cytoplasm, the surrounding layer of viscous cytoplasm, and the outer limiting cellulosic membrane.

2. The liquid cytoplasm upon purification shows the presence of cellulose, the crystallinity of which is more perfect than that of the cellulose obtained from other cellulosic membranes.

3. The viscous cytoplasm is composed largely of crystalline cellulose which may be dried in the form of either films or fibers. The films show a selective orientation of the 101 crystallographic plane parallel to the surface, but a random orientation in the plane of the surface. The fibers show a uniaxial orientation parallel to the fiber axis.

4. The outer membrane possesses a selective orientation of the 101 plane parallel to the surface, but a cross spiral orientation of approximately 80° in the plane of the membrane, confirming the observations of other investigators. No correlation was found between the angle of the cross spiral orientation and the size of the *Valonia* cell.

5. The selective orientation in the dried films and the parallel orientation in the stretched fibers indicate that dehydration and tension may play a part in producing the orientation found in a mature *Valonia* membrane.

6. The X-ray orientation is compared with the fibril orientation and a close correlation is indicated between the X-ray crystallite and the microscopic cellulose particle. This correlation enables one to conclude that the cellulose chain molecules are arranged parallel to the long axis of the particle and that the crystalline regularity of the particle approaches that of a single crystal.

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FORMATION OF MICROSCOPIC CELLULOSE PARTICLES IN COLORLESS PLASTIDS OF THE COTTON FIBER¹

WANDA K. FARR

The observed formation of *mercerized* cellulose particles of microscopic dimensions in the chloroplasts of *Halicystis* (2, 4) constituted a basis for the reinvestigation of cotton fiber protoplasm during the period of formation of *native* cellulose particles of similar dimensions. Microscopic studies of developing fibers over a period of many years had failed to furnish information concerning early stages of cellulose particle development. They appeared suddenly and full-formed in the protoplasm of the living fibers. Neither the attributes of a crystallization phenomenon nor of any recognized biological process of formation were in evidence. A possible similarity in the method of formation of cellulose particles in *Halicystis* and in the cotton fiber was not indicated. The cotton fiber contains no chloroplasts, and the absence of starch formation throughout its entire period of development (1) suggested that colorless plastids are either absent or non-functional in this highly specialized cell.

In *Halicystis* the formation of uniform-sized cellulose particles (1.1×1.5 microns) takes place through the fragmentation of cellulose rings of varying diameter but equal thickness. These rings are produced successively in the outer regions of the plastid plasma during the normal growth of the chloroplast. The membrane of the mature chloroplast finally bursts and its entire contents, including the cellulose particles and the green plasma, are deposited in the course of the formation of a new lamella of the cell membrane. The various stages of cellulose ring and cellulose particle formation can be followed microscopically with comparative ease, although the dark green plasma tends to obscure such minute structural details. It seemed to be unlikely that the counterpart of this phenomenon had been overlooked in the cotton fiber.

MATERIAL AND METHODS

The reexamination of developing cotton fibers (*Gossypium hirsutum* L.) was undertaken with improved methods. The fibers were removed from the seeds with extreme care and mounted in filtered juices which had been expressed from other fibers in the same boll. This mounting medium, while less clear than either water or a sugar solution with similar osmotic properties, was found to be preferable to either of these for maintaining the fiber contents in good condition for a period of a half hour or more. In order

¹ A contribution from the former Cellulose Department of the Chemical Foundation, Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.

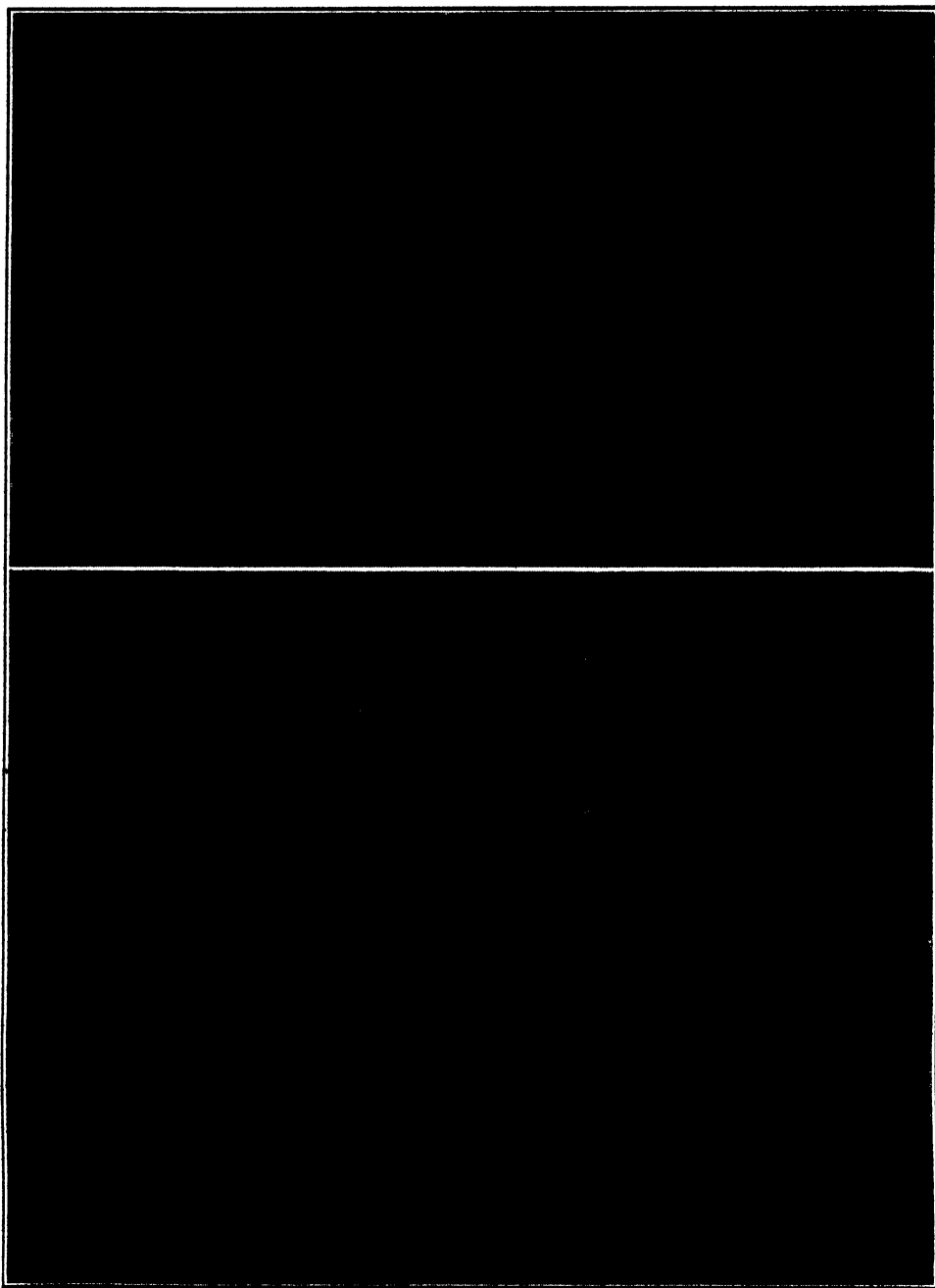


FIGURE 1. A. In the protoplasm of young cotton fibers disc-shaped cellulose-forming plastids varying over a wide range in diameter are barely visible. The relative refractive indices of the plastids and the cytoplasm in which they are floating obscure their contents and cause them to resemble vacuoles. ($\times 820$). B. A young cotton fiber from a 10-day-old boll, slightly crushed. The separate cellulose particles and the cellulose-forming plastid in the exuded protoplasm, along with the cellulose particles in the fibrils already deposited in the cell wall, are swelling and coloring blue in the presence of H_2SO_4 and I_2KI —a reaction characteristic of cellulose ($\times 1700$).

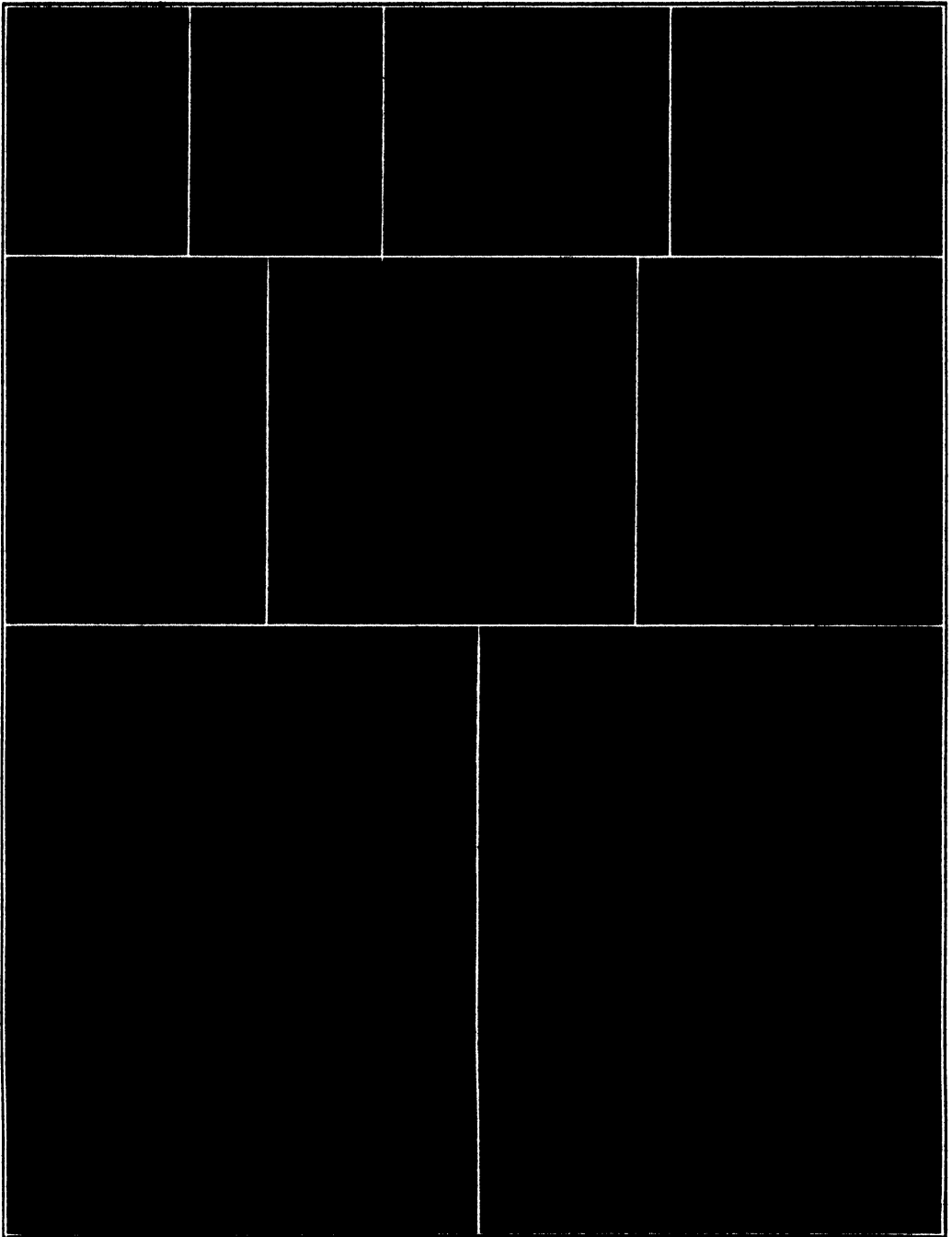


FIGURE 2. Stages in development of cellulose-forming plastids from the cotton fiber show cellulose particle formation through the fragmentation of cellulose rings, of equal thickness but varying diameter, produced successively at the interface of the plastid plasma and the plastid membrane. The membrane of the mature plastid finally bursts and the cellulose particles within and outside of plastids covered with the plastid plasma are discharged into the protoplasmic matrix of the cell ($\times 1580$).

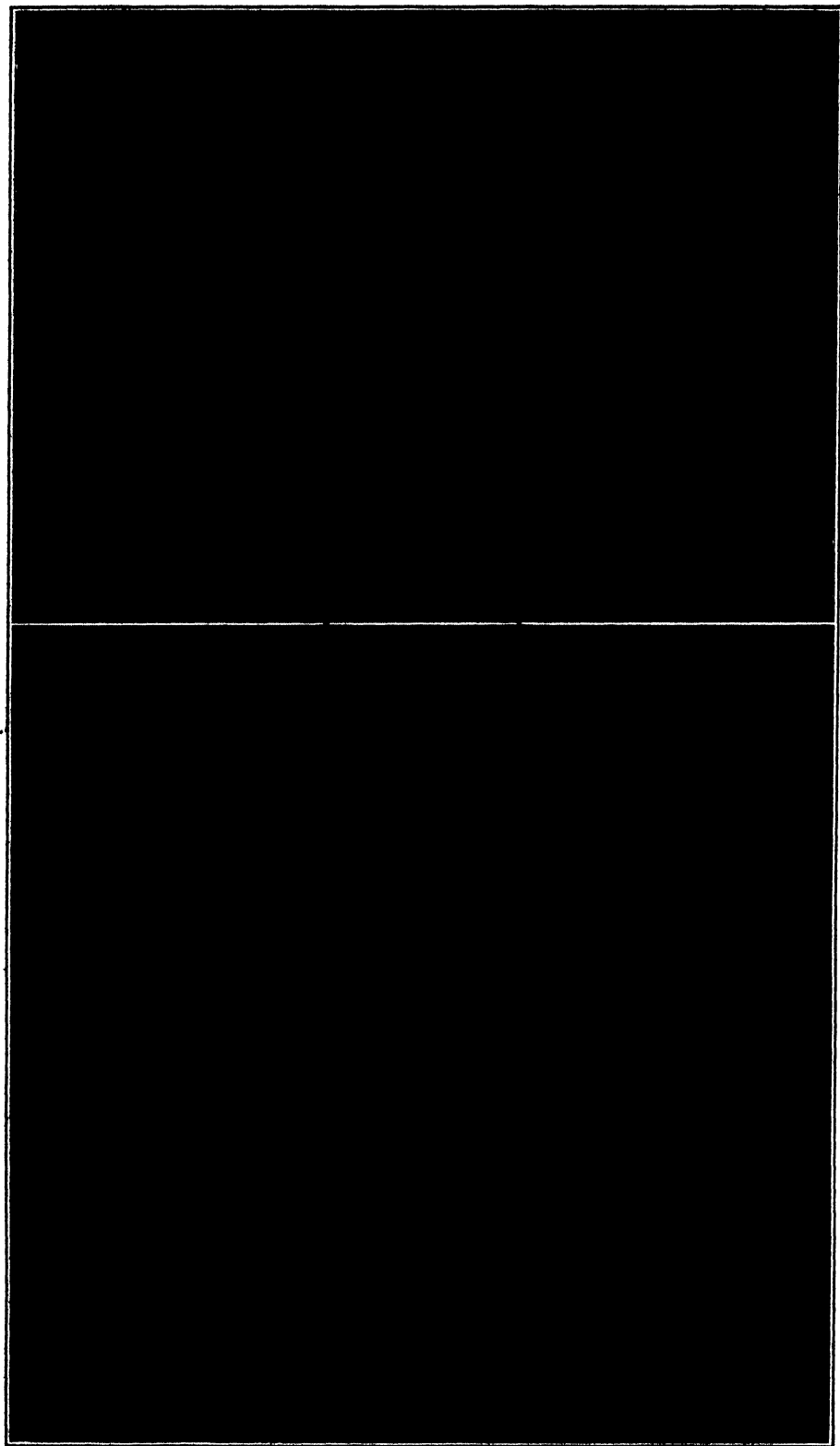


FIGURE 3. A. Cross section of a young cotton seed showing the cells of the epidermal layer in the process of elongation to form cotton fibers and certain cells of the outer integument of the seed filled with starch ($\times 420$). B. A starch-forming plastid from an integument cell and a cellulose-forming plastid from a cotton fiber photographed together to show their comparative visibility ($\times 1100$).

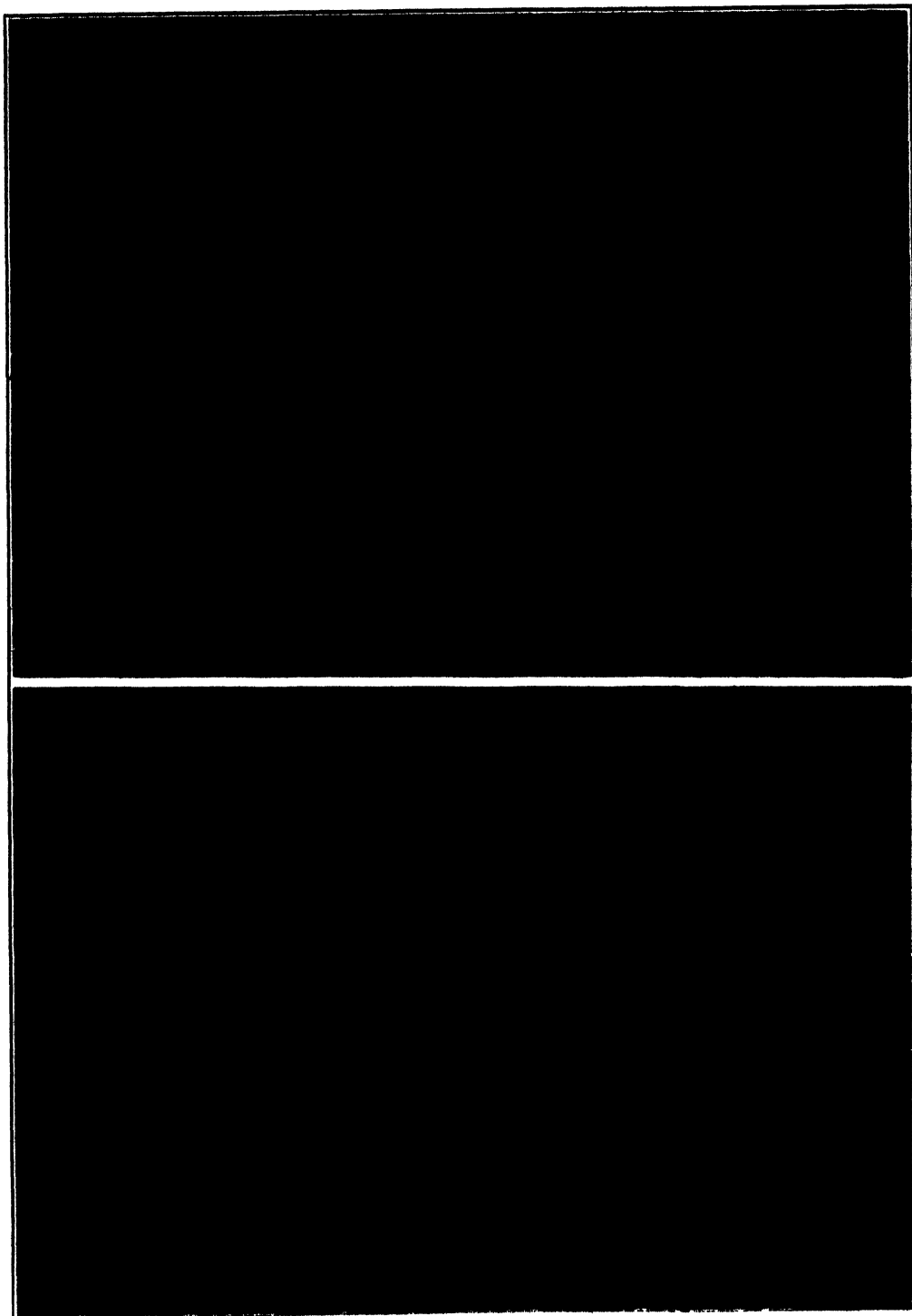


FIGURE 4. A. Expressed protoplasm from a young cotton fiber shows large numbers of cellulose plastids and the fiber nucleus ($\times 785$). B. Expressed protoplasm from starch-forming cells of the outer integument show large numbers of starch grains in various stages of development ($\times 1430$).

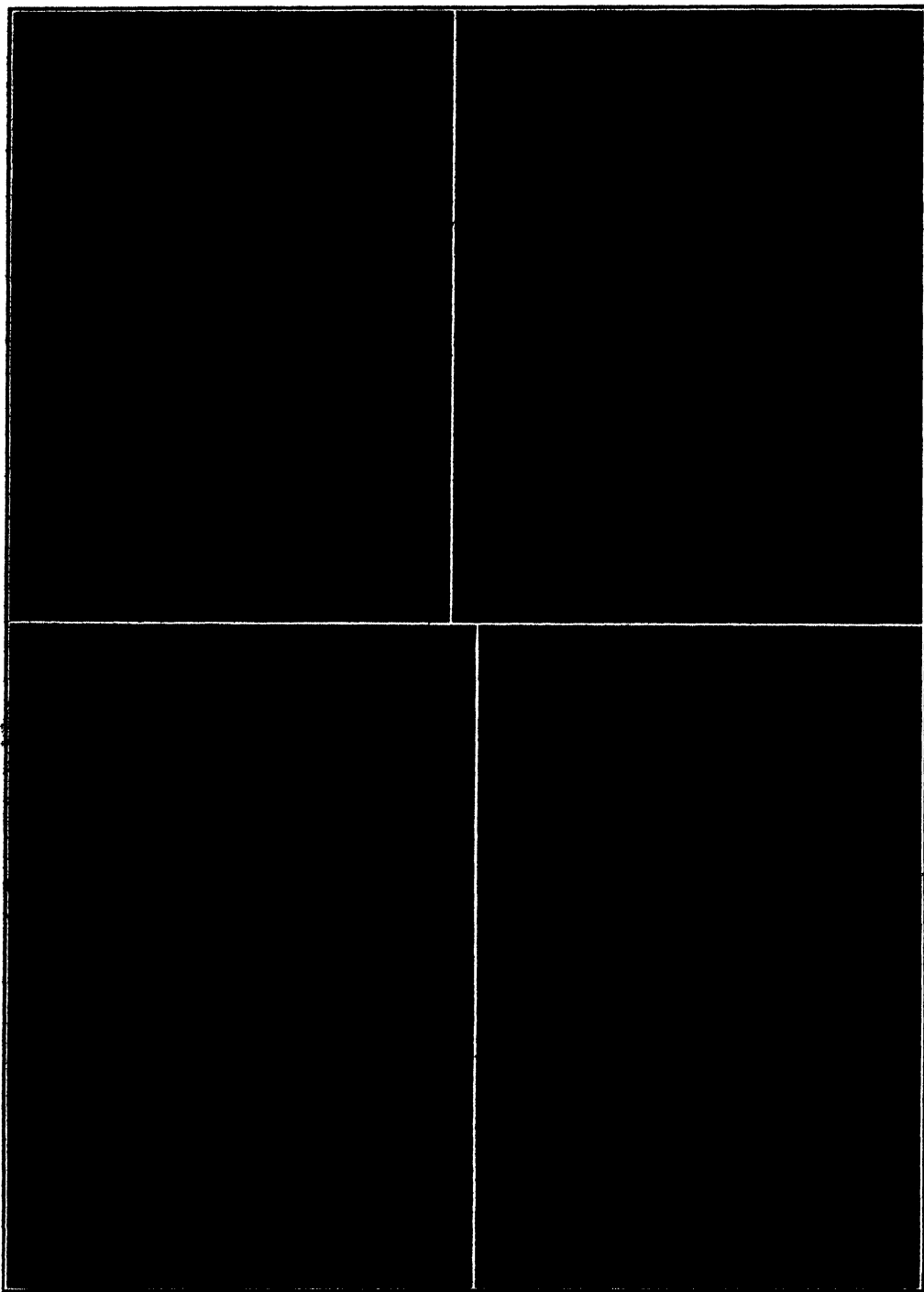


FIGURE 5. A. An early stage of the H_2SO_4 and I_2KI reaction of a small cellulose plastid from a cotton fiber ($\times 1460$). B. As the plastid swells the membrane frequently bursts, allowing the plastid plasma to escape. Cellulose particles are blue and the plastid plasma yellow ($\times 1460$). C. A later stage of reaction of a larger cellulose-forming plastid with H_2SO_4 and I_2KI ($\times 1460$). D. An earlier stage of reaction of the plastid shown in 5 C. The last formed cellulose ring is still in evidence at the surface of the plastid plasma ($\times 1460$).

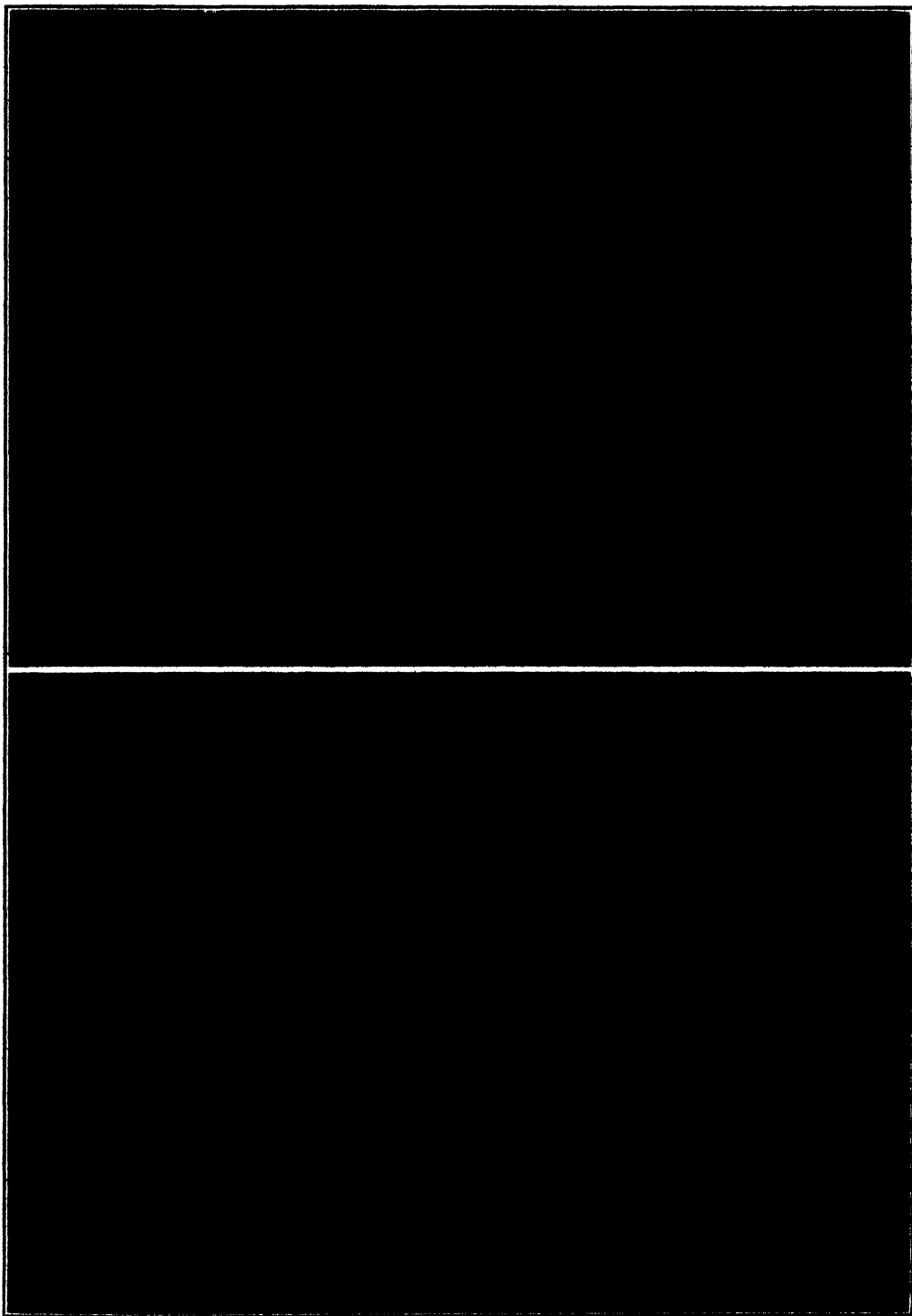


FIGURE 6. A. Separate cellulose particles and cellulose rings in various stages of disintegration, surrounded by the plastid plasma, as they flow from a ruptured plastid ($\times 1500$). B. Subsequent alignment of the cellulose particles in single rows to form cellulose fibrils ($\times 1500$).

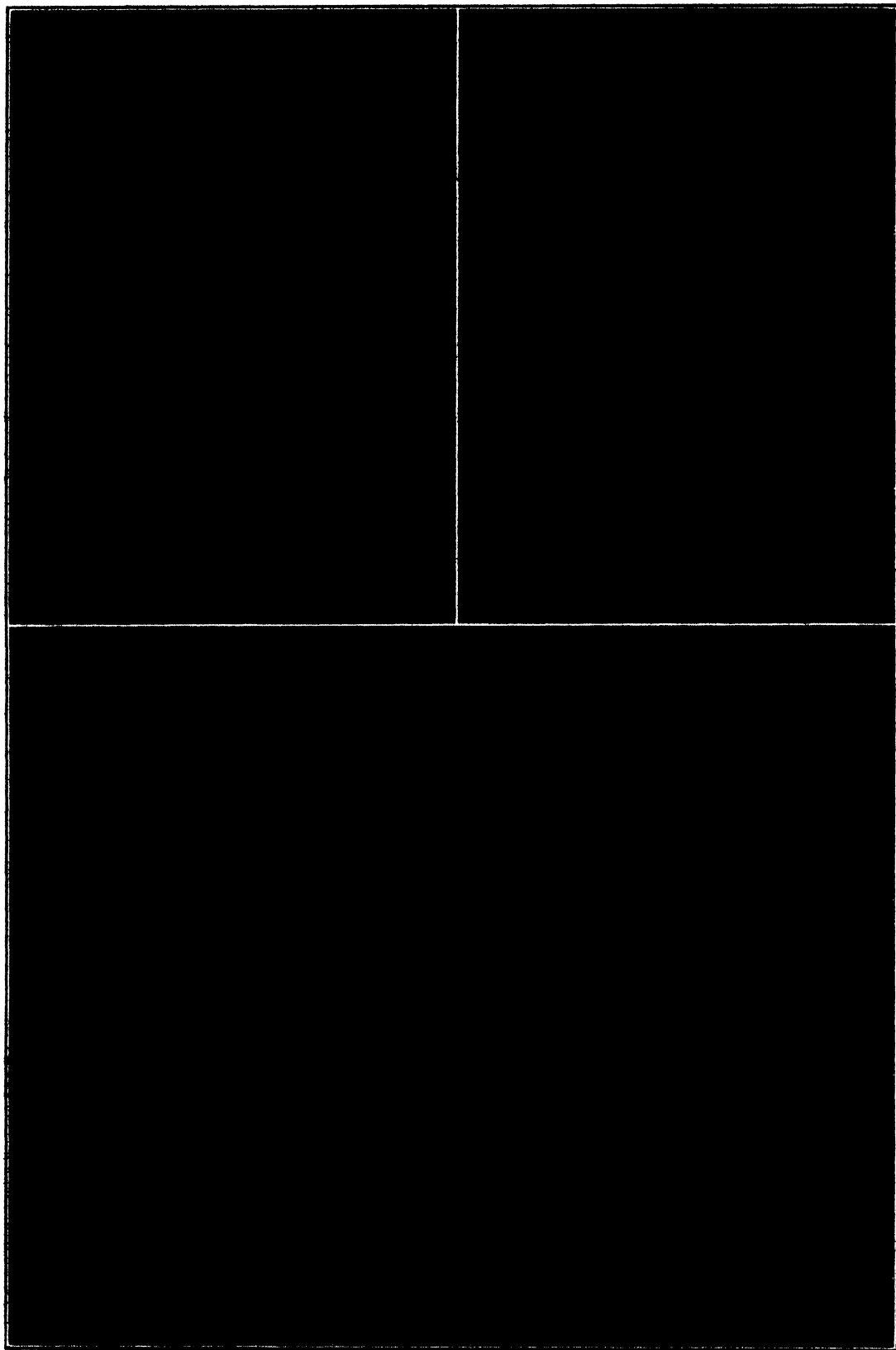


FIGURE 7. (For description see legend on opposite page.)

to prevent excessive evaporation from the microscopic mount, the cover glass was sealed on three sides with paraffin of a low melting point (45° C.). Microscope slides of colorless glass not more than 0.6 mm. in thickness were selected and the thinnest of the No. 0 cover glasses alone were used. The light intensity of the low-voltage, ribbon-filament, microscope lamp was lowered by means of a rheostat in order to increase the visibility of any small protoplasmic structures of low refractive index. The range of refractive indices which were taken into consideration in making this adjustment is indicated in the following table:

Refractive Indices

Water 1.33

Protoplasm 1.33 to 1.34

Starch (cotton) 1.513

Cellulose (3) particle 1.565 Lengthwise

1.530 Crosswise

This effort to obtain experimental conditions in which any hitherto invisible structures in the fresh protoplasm could be discerned was quickly rewarded. In young cotton fibers, tips of two of which are shown in Figure 1 A, structures, circular in outline and varying widely in diameter, were faintly but definitely visible. Floating, as they were, in the fiber cytoplasm they had the appearance of *vacuoles* filled with clear sap. Careful focusing suggested, however, that their contents were more or less dense and this belief was strengthened by the observation that other cytoplasmic inclusions which were moving past them in a lower focal plane were completely obscured—a microscopic phenomenon not associated with the passage of cytoplasmic structures beneath real vacuoles.

In order to observe these new structures more closely, the wall of a fiber was broken with a dissecting needle and the protoplasmic contents allowed to flow out into the mounting medium. Their form was then seen to be disc-like, not spherical, and their contents not only dense but also granular. All stages of cellulose ring and cellulose particle formation were rapidly identified in the various stages of development of these colorless plastids (Fig. 2). The successive stages of ring formation and disintegration reveal the fact that the mechanism of *native* cellulose particle formation in the colorless plastids of the cotton fiber is essentially similar to the mechanism of *mercerized* cellulose particle formation in the chloroplast of *Halocystis*.

FIGURE 7. A. Separate cellulose particles in the protoplasm of a young cotton fiber (×1450). B. Early stage of fibril formation in a young cotton fiber (×1450). C. Cell membrane of the cotton fiber after the deposition of cellulose fibrils in spiral arrangement (×1450).

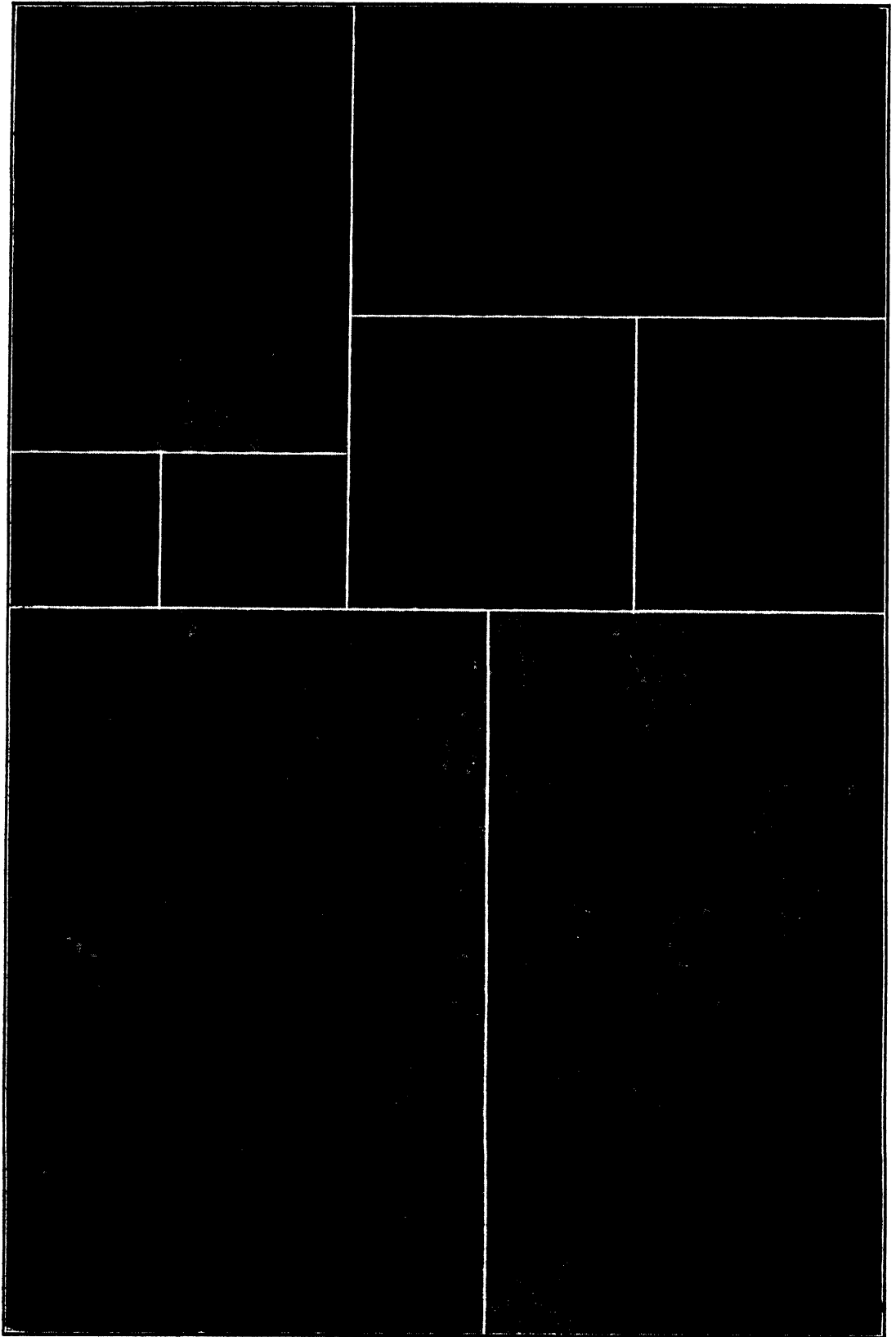


FIGURE 8. (For description see legend on opposite page.)

In the developing cotton fiber the cellulose-forming plastid is rendered nearly invisible by the similarity in its refractive index and that of the cytoplasm which surrounds it. Measurements show that both are between 1.33 and 1.34. While the cellulose particles, with refractive indices of 1.565 (lengthwise) and 1.530 (crosswise), are held within the plastid membrane they are very effectively obscured. When they are freed by the bursting of the membrane, as shown in Figure 2 I, they attain a degree of visibility which made possible their identification in 1934 (3). Their complete development within a specialized organ such as a plastid accounts fully for their sudden appearance, full-formed, in the cytoplasm, prior to the assumption of their final rôle in the formation of the cell membrane.

In the course of the identification of a protoplasmic structure such as the cellulose-forming plastid it is necessary that it be carefully compared with other protoplasmic structures in which any form of resemblance may exist at any stage of development. In Figure 3 a cellulose-forming plastid from the protoplasm of a young cotton fiber and a colorless, starch-forming plastid from a cell of the outer integument of the cotton seed have been photographed together. The comparative obscurity of the cellulose-forming plastid as well as the very different physical aspects of crystalline carbohydrate synthesis in which the two plastids are engaged are thus clearly shown. The cellulose-forming plastids, in large numbers and in many different stages of development, are compared with the single nucleus of the cotton fiber from a mount of expressed fiber protoplasm in Figure 4 A. Figure 4 B illustrates the contrast in the general appearance of expressed protoplasm from the starch-forming cells of the outer integument of the young cotton seed (1).

The microchemical identification of the cellulose particles in the cellulose-forming plastids of the cotton fiber is shown in Figure 5. When the I_2KI solution is applied, the plastid plasma becomes deep yellow in color. No differential coloration of either the cellulose particles or the well-formed cellulose rings occurs until the strong sulphuric acid is added (3) to the mount. As the acid comes into contact with the cellulose particles they begin to swell and their blue color is in sharp contrast with the yellow color of the plastid plasma (Fig. 5 A). As the swelling reaction continues the

FIGURE 8. M. Stem cells of the cotton plant showing chloroplasts and colorless cellulose-forming plastids ($\times 650$). N. Cellulose-forming plastids in young leaf cells ($\times 810$). O. A young leaf cell with a nucleus, chloroplast, and cellulose-forming plastid ($\times 810$). R. Expressed protoplasm of leaf cells showing both starch-forming and cellulose-forming plastids ($\times 1100$). S and T. Cellulose formation contrasted with starch formation in plastids from cotton fibers ($\times 1470$). U. Expressed protoplasm from leaf cells shows chloroplasts and cellulose-forming plastids ($\times 1290$). W. Expressed protoplasm from boll wall cells shows small chloroplasts with single starch grains and cellulose-forming plastids in many stages of development ($\times 810$).

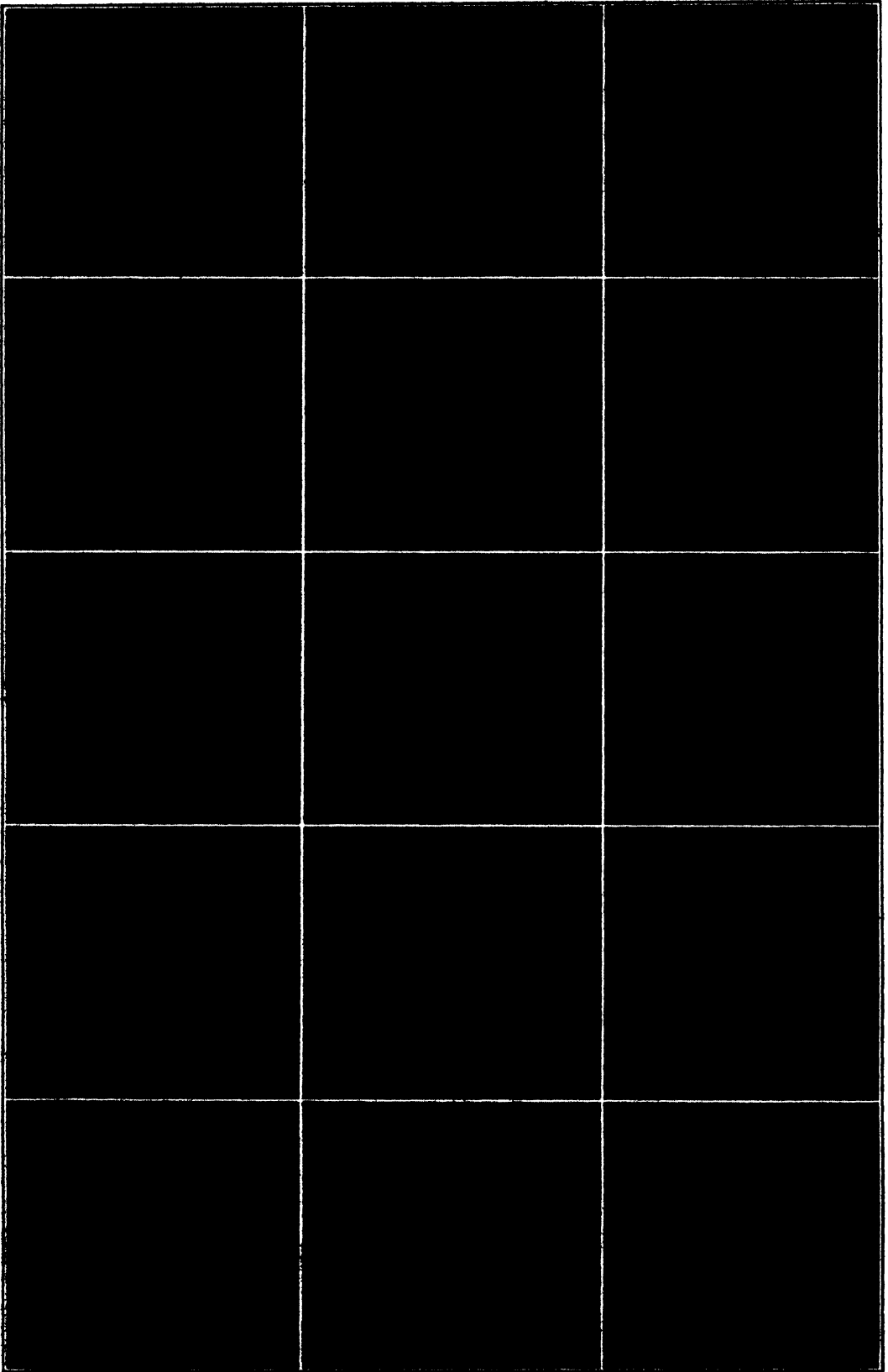


FIGURE 9. (For description see legend on opposite page.)

plastid membrane frequently bursts, as shown in Figure 5 B, allowing the plastid contents to pass through the opening. The same type of reaction takes place in both small and large plastids (Fig. 5 B and C). The well developed unfragmented rings within the plastid have a tendency to fragment more readily than the *Halicystis* rings. When the sulphuric acid was added to the mount containing the plastid shown in Figure 5 D, the cellulose particles were barely visible in an unbroken ring. Contact with the reagent caused them to separate very quickly.

If the reaction of the plastid with sulphuric acid and iodine is permitted to continue beyond the stage shown in Figure 5 C, the rapidly swelling cellulose particles finally spread through the yellow plasma which surrounds them producing a blue coloration of the entire plastid mass. In Figure 1 B a young cotton fiber, taken from the boll ten days after the date of flowering, has been crushed in order to express some of the protoplasm. The addition of sulphuric acid and I_2KI brings about the swelling and blue coloration of the cellulose particles in the recently deposited fibrils in the cell wall, of the separate cellulose particles in the expressed protoplasm, and an advanced stage of particle swelling and coloration in a cellulose plastid, as described above.

The phenomenon shown in Figure 2 I constitutes the connecting link between this recently acquired knowledge of the method of formation of cellulose particles in the colorless plastid of the cotton fiber and our previous knowledge of the method of formation of the cell membrane of the cotton fiber by cellulose particles and their associated cementing materials (3). A more detailed study of the plastid contents which pour out through the ruptured membrane is shown in Figure 6 A. It is composed of a few disintegrating rings and many separate cellulose particles, all imbedded in the plastid plasma. These same particles are found later aligned end to end in single rows to form cellulose fibrils as shown in Figure 6 B. The important steps in cellulose fibril and cell membrane formation are summarized in Figure 7 A, B, and C.

The formation of mercerized cellulose particles in the chloroplast of *Halicystis* and of native cellulose particles in the colorless plastid of the cotton fiber by processes which are essentially similar serve to indicate the significance of these observations in connection with the synthesis of cellulose in living plant cells. Generalizations concerning the process of cellulose

FIGURE 9. Column A. Stages in development of the chloroplast of *Halicystis* showing cellulose ring and cellulose particle formation ($\times 1540$). Column B. Stages in starch formation in the chloroplasts of the cotton plant ($\times 1540$). Column C. Cellulose particle formation takes place in the colorless plastids of the cotton fiber by a process of successive ring formation and fragmentation essentially similar to the mechanism of cellulose formation in *Halicystis* ($\times 1540$).

formation throughout the plant kingdom are not warranted, however, upon the basis of such limited observations. Among the many immediate problems created by these new findings no one is more important than the determination of the method of cellulose formation in plant cells in which both starch and cellulose are being formed. In the leaf, stem, and boll-wall cells of the cotton plant, starch is produced in chloroplasts. The process of cellulose formation in these same cells is shown in Figure 8. In the stem cells illustrated in Figure 8 M both chloroplasts and colorless cellulose-forming plastids are in focus. The chloroplasts are usually found near to the surface of the protoplast; the cellulose-forming plastids lie in the interior regions of the protoplast. Figure 8 N shows plastids in the process of cellulose particle formation in young leaf cells; Figure 8 O, a young leaf cell with a chloroplast, nucleus, and cellulose-forming plastid in one focal plane; Figure 8 R, expressed protoplasm from a leaf cell with both starch and cellulose formation in progress in different plastids; Figure 8 S and T contrast sharply the mechanisms of starch formation and cellulose formation in the cotton plant; and Figure 8 U and W show both cellulose-forming plastids and starch-forming plastids in expressed protoplasm of boll-wall cells and leaf cells respectively.

SUMMARY

The results of this investigation are summarized in Figure 9.

Native cellulose particles are formed in the colorless plastids of living cotton fibers by a process which is essentially similar to the method of formation of *mercerized* cellulose particles in the chloroplasts of *Halicystis*. These physical aspects of cellulose formation have no apparent points in common with the process of starch formation either in chloroplasts or in colorless plastids in the cotton plant.

Within very young plastids, whether colorless or pigmented, no structural features have been observed which will indicate the type of crystalline carbohydrate to be produced. The cells of the leaves, stems, and boll walls of the cotton plant carry on the formation of these two closely related carbohydrates, starch and cellulose, simultaneously, in separate plastids.

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A PREPARATION FROM YEAST THAT IS ACTIVE IN BREAKING THE REST PERIOD OF BUDS

JOHN D. GUTHRIE

The discovery of Bennett and Skoog (3) that yeast extracts will break the rest period of buds of peach and pear has resulted in speculation as to what constituent of yeast is responsible for this activity. It was suggested by Guthrie (6, 7) that glutathione might be the active substance. However, Bennett, Oserkowsky, and Jacobson (2) in a recent paper have shown that yeast extracts are much more active than would be expected from their glutathione content, and that the extracts retained their activity after the removal of glutathione. They think that the effect of glutathione is due to the presence of an active impurity rather than to glutathione itself. This could be proved by obtaining a sample of glutathione that had no stimulative effect on resting buds. Until this is done, an equally valid explanation of their results and those of the present paper would be that glutathione is one of the constituents of yeast extracts that has dormancy-breaking action, but is not the most active one. The purpose of the present paper is to record the mode of separation and chemical properties of a preparation from yeast that was found to be much more active than glutathione in breaking the rest period of buds. Since the work on this preparation had to be terminated because of the acceptance of another position, some of the conclusions are based on limited data and are more in the nature of suggestions for those who may wish to continue the work on this problem.

EXPERIMENTAL RESULTS

Dormancy-breaking action of the preparation. A preparation from yeast that had been prepared in February 1938 for another purpose was tried on resting buds of pear (*Pyrus communis* L.). This preparation was the one designated as C in the description of mode of preparation given later in this paper. It proved to be very active in amounts of 10 mg. and definitely more so than the same amount of glutathione. An extract from yeast was also included in this experiment, the details of which follow:

The preparation from yeast was tested on branches of pear trees that had grown outside in pots all summer and were put in the greenhouse about November 1, 1940. The injections were made by the methods used by Bennett and Skoog (3). An empty calcium chloride tube was connected to the cut end of the twig with a piece of rubber tubing and the solution to be tested introduced into the tube. The solution was usually drawn into the tree within a few hours. The yeast preparation was compared with a yeast

extract made by adding alcohol to 50 g. of fresh yeast until the total volume was 250 cc., heating to boiling, cooling, centrifuging, and evaporating 200 cc. of the supernatant liquid almost to dryness *in vacuo* in a bath at 50° C. The material was taken up in 200 cc. of water and filtered. Five cc. of this extract representing 1 g. of yeast were injected into each of five pear branches. This was compared with 10 mg. of the yeast preparation and with 10 mg. of glutathione, each dissolved in 5 cc. of water, and injected into five pear branches. The results were: 1 g. yeast induced 6, 5, 2, 4, 2 buds to grow or a total of 19; 10 mg. of the yeast preparation induced 8, 3, 5, 10, 5 buds to grow or a total of 31; 10 mg. of glutathione induced 2, 2, 2, 1, 1 buds to grow or a total of 8; and water alone induced 2, 2, 2, 1, 2 buds to grow or a total of 9. This experiment shows that glutathione was not active in amounts of 10 mg. However, another test made at the same time showed it to be active when 50 mg. were used. These results agree with the conclusions of Bennett, Oserkowsky, and Jacobson (2) that yeast contains one or more substances that are much more active than glutathione in breaking the rest period of buds. The procedure used for making the yeast preparation should offer a good starting point for work directed toward the isolation or identification of these substances.

Yeast extract also breaks the rest period of potato tubers. The extract used was prepared by mixing 100 g. of yeast with 500 cc. of alcohol, heating to boiling, cooling, and centrifuging. The supernatant, 450 cc., was evaporated *in vacuo* in a bath at 50° C. to about 5 cc. and then made up to 150 cc. with water. After filtration it was used to treat potatoes by placing 12 one-eye, approximately cubical, pieces in a Petri dish with the eyes up and pouring in enough solution to come about two-thirds of the way up the sides of the pieces. The treatment was for four days at 10° C., after which the pieces were planted, and the time for 6 pieces out of 12 to show sprouts above ground was determined. Those treated with the undiluted extract described above were 50 per cent above ground in 22 days. For the same extract diluted one-third, the time was 25 days, and for a one-ninth dilution the time was 40 days. Controls treated with water were 50 per cent above ground in 44 days. Those treated with 200 mg. of glutathione in 100 cc. were 50 per cent above ground in 43 days. This amount of glutathione is one-fifth to one-tenth that found effective in breaking the dormancy of potato tubers in previous experiments (7). It was chosen since it represents the approximate glutathione content of the yeast extracts used. This experiment also shows that the major part of the rest period breaking action of yeast extracts is due to some substance or substances other than glutathione.

Procedure for obtaining the preparation from yeast. The starting material for this preparation was the supernatant liquid left from the preparation of glutathione. The yeast, 3.2 kg. of Fleischmann's baker's, was crumbled

in an 8-liter battery jar. A mixture of 175 cc. of 89 per cent alcohol, 55 cc. of concentrated sulphuric acid, and 130 cc. of ether was added. After ten minutes it was stirred, 1500 cc. of water added, and allowed to stand 30 minutes. The sulphuric acid was partly precipitated with 120 g. barium hydroxide, $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$, in 300 cc. of hot water and allowed to stand 30 minutes. The material was then centrifuged. The supernatant had a volume of 2.8 liters. It was brought to approximately half normal by addition of sulphuric acid and the glutathione precipitated with 0.9 g. cuprous oxide. The yeast was extracted a second time with 2 liters of 1 per cent sulphuric acid, the supernatant adjusted to approximately half normal and the glutathione precipitated with 0.45 g. cuprous oxide. After standing overnight at about 5° C. the cuprous salt of glutathione was centrifuged out, and the supernatants from several batches of yeast were used to make the following preparations.

The supernatant liquid from 36 kg. of yeast after removal of the cuprous salt of glutathione was precipitated by the addition of 50 cc. of 10 per cent mercuric sulphate in 10 per cent sulphuric acid per kg. of yeast or a total of 1.8 liters of the reagent. After standing several days the precipitate (A) was centrifuged out and washed three times with water. After standing about a week another precipitate (B) came down from the supernatant liquid. After separation and washing of this precipitate the supernatant liquid was precipitated further by the addition of 0.6 l. of 10 per cent mercuric sulphate in 5 per cent sulphuric acid. A precipitate (C) was centrifuged out after about a week and washed three times with water.

These three precipitates were suspended separately in water and decomposed with hydrogen sulphide. After removal of the mercuric sulphide precipitate the supernatant was freed from hydrogen sulphide with a stream of nitrogen. The supernatant was then almost neutralized to litmus by the addition of hot, saturated barium hydroxide, and the precipitate centrifuged out, washed three times with water, and discarded. The supernatant was then precipitated with an excess of saturated cupric acetate, 250 cc., 100 cc., and 100 cc. being used respectively for precipitates A, B, and C. After standing a week or longer at about 10° C. the copper precipitates were centrifuged out, washed with water, and decomposed with H_2S . After removal of the copper sulphide the supernatant was freed from hydrogen sulphide with a stream of nitrogen. The supernatants were then evaporated to small volume *in vacuo* in a bath at 40° C. and precipitated by the addition of excess alcohol. After standing several days at 5° C. the precipitates were filtered out with suction on hardened paper, dried in a vacuum desiccator over sulphuric acid, and weighed. Precipitate A weighed 6.9 g., B 2.4 g., and C 1.2 g. After about three years the B and C preparations were used in the experiments described in this paper. Both were active

in breaking the dormancy of buds, but most of the experiments were made with preparation C.

Properties of the yeast preparation. The preparation C from yeast, the mode of preparation for which is described above, contained 17.4 per cent nitrogen by the micro method of West and Brandon (12). It gave only a faint blue color with Folin's phenol reagent (10, p. 213) or with sodium sulphite and the uric acid reagent of Folin and Marenzi (5), indicating that little or no tyrosine or cystine was present. The ninhydrin reaction was faint, indicating that it contained but small quantities of the amino acids that give this test. Knoop's test for histidine (8, p. 69) and the Sakaguchi test for arginine (10, p. 188) were negative. The preparation gave a heavy precipitate with phosphotungstic acid. The Kossel test and the Murexide test (9, p. 112) were faint. The faint Kossel test is hard to explain in the light of subsequent findings in which adenine was identified as one of the constituents. Some interfering substance may have been present. The sulphur content was 2.6 per cent. The preparation contained about 5 per cent barium from the barium hydroxide used in its preparation. No precipitate was observed with picric acid or metaphosphoric acid. A very strong test for pentose was obtained with Bial's reagent (11, p. 284) and with phloroglucinol and hydrochloric acid. The organic phosphorus content by the method of Briggs (4) was 5.2 per cent. The presence of pentose and phosphorus suggested the presence of a nucleotide. The properties of preparation B were similar to those of preparation C.

It was also found that the preparation C from yeast activated the reduction of methylene blue by glutathione and the juice of potato tubers. Thus in experiments conducted *in vacuo* in Thunberg tubes, 2 mg. of preparation C, plus 5 mg. glutathione, plus 5 cc. of potato juice at pH 6.9 reduced 2 cc. of methylene blue, 25 mg. per liter, in one minute, while without preparation C the reduction time was 13 minutes. The potato juice alone reduced the methylene blue in about two hours.

Unpublished experiments by Dr. Helen Purdy Beale showed that preparation C inactivated tobacco mosaic virus (*Marmor tabaci* var. *vulgare* Holmes) when mixed with the virus in concentrations of 2 mg. per 2 cc. 8 minutes before inoculation. The lesion count on *Nicotiana glutinosa* L. was 22 compared with 203 for the water control. Preparations A and B likewise inactivated the virus.

Attempt at further purification. After considerable preliminary experimentation it was found that the yeast preparation could be fractionated by precipitating with lead acetate and then precipitating the lead acetate supernatant with copper acetate. The lead acetate precipitate contained the substances giving the strong pentose test and contained phosphorus, while the copper acetate precipitate contained a large amount of adenine. The details of such fractionation follow:

One gram of the preparation C from yeast was dissolved in 22.75 cc. of water and 7.5 cc. of N/10 sulphuric acid. After one-half hour a small precipitate of barium sulphate was centrifuged out, washed with 10 cc. of water, and discarded. Saturated lead acetate, 5 cc., was added to the supernatant and washings. After one-half hour the lead precipitate was centrifuged out and washed three times with water. Saturated copper acetate, 10 cc., was added to the supernatant and washings. After four hours the copper precipitate was centrifuged out and washed three times with water. The washings and supernatant were discarded.

The lead acetate precipitate obtained as described above was suspended in water and decomposed with hydrogen sulphide. The lead sulphide was centrifuged out and washed three times with water. After removal of the hydrogen sulphide with a stream of nitrogen the supernatant and washings were evaporated *in vacuo* to about 1 cc. in a bath at about 45° C. The liquid was then transferred with about 4 cc. of water to a centrifuge tube and precipitated with 50 cc. of absolute alcohol. After standing at about -7° C. overnight the precipitate was taken up in about 3 cc. of water with gentle warming. A few drops of absolute alcohol were added and after 2 hours at room temperature a small amount of brown gum was removed on the end of a stirring rod. Absolute alcohol, 50 cc., was then added rapidly and the solution placed at about -7° C. overnight. The precipitate was centrifuged out, washed with 15 cc. of absolute alcohol and 15 cc. of ether and allowed to dry. It weighed 240 mg., gave a strong Bial's test for pentose, and contained 7.3 per cent phosphorus. When neutralized 5-mg. portions were injected into four twigs of peach (*Prunus persica* Sieb. & Zucc.), a total of 25 buds grew. With water controls six buds grew per four twigs.

The combined alcohol and ether washings and supernatant liquid were evaporated to dryness *in vacuo* and washed from the flask into a centrifuge tube with about 20 cc. of absolute alcohol. After standing overnight the precipitate was centrifuged out, washed with absolute alcohol and ether, and allowed to dry. It weighed 92 mg. It contained 9.2 per cent of phosphorus. When 4-mg. portions were injected into three twigs of peach, a total of 11 buds grew. With water controls four buds grew per three twigs.

The precipitate obtained with copper acetate after lead acetate as described above was suspended in water and decomposed with hydrogen sulphide. The copper sulphide was centrifuged out, washed with about 7 cc. of water containing three drops of acetic acid, and with water. After removal of the H₂S with a stream of nitrogen, the supernatant and washings were evaporated *in vacuo* in a bath at 45° C. and then in a conical centrifuge tube by the method of Williams and Spies (13, p. 141) to about 0.5 cc. About 6 cc. of absolute alcohol were added. After standing overnight at about -7° C. the precipitate was centrifuged out, taken up with about 1

cc. of water and again precipitated with about 6 cc. of absolute alcohol. After standing overnight at about -7° C. the precipitate was centrifuged out, washed with 2 cc. of alcohol, and dried by the method of Williams and Spies. Later this dried precipitate was taken up with three drops of concentrated hydrochloric acid, precipitated with 1 cc. of alcohol containing a little hydrochloric acid, and dried. It gave lath-shaped crystals like adenine dihydrochloride on evaporation with dilute hydrochloric acid on a microscope slide. When injected in neutralized 4-mg. portions into three peach twigs, 22 buds grew. On water controls four buds grew per three twigs.

The supernatant and washings of the alcohol precipitation described immediately above were evaporated to dryness, taken up with two drops of concentrated hydrochloric acid, and precipitated with 1.5 cc. of absolute alcohol. After several hours at about -7° C. the precipitate was centrifuged out, washed with absolute alcohol containing a little hydrochloric acid, and dried. It weighed 34 mg. When injected in neutralized 4-mg. portions into three peach twigs, 23 buds grew. On water controls four buds grew per three branches. The nitrogen content of this preparation was 35.2 per cent, the calculated percentage for adenine dihydrochloride is 33.7 per cent. It gave typical crystals of the gold chloride double salt of adenine with gold chloride and hydrochloric acid. This indicated that it was composed chiefly of adenine.

Action of adenine on resting buds. In order to see if adenine was effective in breaking the rest period of buds, adenine sulphate (Eastman Kodak), neutralized with dilute sodium hydroxide, was injected in amounts of 4 mg. in 2 cc. volume into six peach twigs, while six controls were injected with an equivalent amount of sodium sulphate in 2 cc. volume. The results were: 16 buds grew on the twigs injected with adenine and 7 buds grew on the controls. In a similar experiment 26 buds grew on five pear twigs injected with 4 mg. of neutralized adenine dihydrochloride (prepared from the sulphate), while 15 buds grew on controls injected with an equivalent amount of sodium chloride. Although it is possible that the sample of adenine contained an active impurity, these experiments indicate that adenine has some action in breaking the rest period and should be further investigated.

Since the preparations from yeast that are active in breaking the rest period contain adenine, pentose, and phosphorus, and since adenine itself appears to have some stimulative action, it would be of interest to investigate the effect of adenine-containing nucleotides on resting buds, especially those showing co-enzyme activity (1, p. 71). It may be mentioned that the adenylyl methylthiopentose of Suzuki, Odake, and Mori (11) was found to have little or no stimulative action on resting buds. This substance was supplied by the laboratory of the late Dr. P. A. Levene of the Rockefeller Institute for Medical Research.

SUMMARY

The results of Bennett, Oserkowsky, and Jacobson, which showed that the action of yeast extracts in breaking the rest period is due to some substance or substances other than glutathione, were confirmed. A procedure is given for obtaining a preparation from yeast that is much more active than glutathione. This preparation contained adenine, pentose, and phosphorus. It also hastened the reduction of methylene blue by potato juice plus glutathione. Experiments indicated that adenine had some dormancy-breaking action. It is suggested that the effect of adenine and adenine-containing nucleotides on resting buds be investigated further.

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DORMANCY IN SEEDS OF *IMPATIENS BALSAMINA* L.

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INTRODUCTION

That some seeds exhibit dormancy when freshly-harvested has been known for some time. This condition is reported to have been found in cotton (8), cucurbits (7), tobacco (5), seed of crested wheatgrass (2), bluegrass (3), and lettuce (4, 10, 12). Niethammer (6), in dealing with the germination behavior of several kinds of seed, places *Impatiens balsamina* in the group which requires a resting period of at least four months. Her results are based on known harvest dates and tests made immediately and at intervals after harvest.

In the experiments reported in this paper, the object was to determine the length of the dry storage period necessary after harvest date for completion of after-ripening of *Impatiens balsamina* L., and also to see if seeds harvested at different times were different in their degree of dormancy or germination capacity. It was hoped to discover whether a certain length of time in dry storage was necessary for after-ripening or all seeds became germinable at the same time of year regardless of harvest date. Experiments were also conducted to find out what effect pretreatment in moist condition at a low temperature (5°C.) had on subsequent germination at 25°C.

The term after-ripening is used in this paper in its broadest sense, that of preparation of the seed for the resumption of growth,—in this case by means of dry storage. After-ripening is often used in a more limited sense, indicating the overcoming of dormancy by treatment in a *moist* condition, usually at a low temperature.

EXPERIMENTAL RESULTS AND DISCUSSION

PRELIMINARY TESTS

Seeds of *Impatiens balsamina* were received from Dr. Donald W. Davis, Department of Biology, College of William and Mary, Williamsburg, Virginia, on April 12, 1937. The seeds had been collected at The Forest Research Institute, Dehra Dun, U. P. India. When placed as soon as received in a moist medium at a daily alternating temperature of 15° to 30° C. or in a greenhouse held at approximately 21° C., the seeds failed to germinate. Low temperature pretreatment in a moist condition was only slightly effective, producing but a few seedlings. However, high germination percentages over a wide range of temperatures resulted from a test of samples of the same seed lot started in December 1937. The lower constant temperatures, 15° C. or 20° C., or a daily alternating temperature of 10° to 20° C. yielded 20 to 54 per cent germination. At constant temperatures of

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25° C. or 30° C., or daily alternations of 10° to 30° C., 15° to 30° C., or 20° to 30° C., 60 to 92 per cent germination was obtained. The highest germination percentage was obtained when seeds were placed at a constant temperature of 25° C. Moist granulated peat moss was the medium used for these controlled temperature tests. Some of the seeds were also planted in soil in the greenhouse, which resulted in 73 per cent seedling production. Low temperature pretreatment was found to be of no advantage. Seeds planted in flats and placed in a cold frame in December 1937 rotted before the following spring. The results of December tests of seeds received in April indicated after-ripening in dry storage, the seeds having been held dry in the laboratory for the intervening period.

Some of the resulting seedlings of the above test were raised to maturity in the greenhouse and seeds were collected from the plants on various dates. Seeds collected in April and May 1938 gave 100 per cent germination the following November while those collected in August and September gave only 58 per cent in the November test. These tests were made at 25° C. in a medium of moist granulated peat moss. When the seeds which were collected in August and September were tested a year later, they gave 100 per cent germination indicating dormancy had been overcome by the dry storage period at room temperature. These tests were limited by the scarcity of seeds, only 25 seeds being available for each sample. Tests were not made soon enough after harvest to determine the minimum effective time for dry storage.

Several seedlings from the 1938 test were grown in the field for 1939 harvest. Collection dates were carefully recorded and tests made immediately and at weekly intervals after harvest. Collection of the seeds began in July 1939 and lasted through October of the same year. The seeds were stored dry in open containers in the laboratory. For the germination tests, the seeds were mixed with moist granulated peat moss and placed in a germinator held at constant 25° C. From 18 to 24 weeks were required to reach full germination capacity for seeds placed in the germinator immediately after harvest. After 13 weeks of dry storage the time required for completion of germination had dropped to 9 to 17 weeks (varying with the different collection lots). Due to lack of seeds, after 13 weeks of dry storage no further tests were made until the seeds had remained for 26 weeks in the laboratory, at which time tests indicated complete after-ripening had been obtained; that is, 80 to 100 per cent germination resulted from the seeds remaining 3 to 5 weeks in the germinator. Here, as in the 1938 experiment, scarcity of seed limited the number of tests, the samples consisting of from 5 to 50 seeds.

In another experiment seeds from the 1939 collection were used to determine the effect of temperature and humidity on after-ripening in dry storage. The experiment was started in September 1939 and seeds were

stored at 20° and 30° C. in desiccators in which the relative humidity was controlled by saturated salt solutions according to the method of Spencer (9). Sodium chloride was used to produce 76 per cent relative humidity and calcium nitrate to produce approximately 50 per cent relative humidity. Seeds were also stored over calcium oxide which was placed in desiccators at both temperatures and an additional lot of seed was stored open at 30° C. A control seed lot was placed open in the laboratory.

Germination tests consisting of samples of 20 seeds each were made at 25° C., the seeds being mixed with moist granulated peat moss. The seeds were tested after one-half, one, three, five, six, and seven months of storage under the conditions just described. After one-half month's storage, the highest germination percentage was obtained by seeds stored over calcium oxide at 30° C. After one month's storage, seeds stored at 20° C. in all conditions gave lower percentages than those stored at 30° C. After storage for three months, all lots gave high percentages, 100 per cent being obtained by seeds stored over calcium oxide at 30° C., open at 30° C. and in the laboratory. Seeds stored longer than three months at 70 per cent relative humidity at 30° C. began to deteriorate from mold and gave only 8 per cent germination after storage for seven months. This was the only lot which was harmed by the storage conditions. The time required for complete germination decreased gradually with increasing time of storage up to six months. None of the temperature and humidity conditions tried proved to be of more benefit for after-ripening than dry storage in the laboratory. This is in agreement with Wharton's (12) data on lettuce seed which show that high temperature storage of dry seed may be effective in breaking dormancy. Further work done on lettuce seed in Arizona (4) also indicates the desirability of storing "new" seed at relatively high temperatures and in open containers to break their dormancy.

FURTHER TESTS

With these preliminary experiments as a background, a new experiment was begun in 1940 with the setting out of the parent plants which were seedlings produced from the 1939 crop of seeds.

Flowering began in the following June and the first collection of seeds was made on August 14. The last collection was made on October 16, and seeds were collected thrice weekly during this interval. Each week's harvest was combined to form one lot and ten lots in all were collected and numbered consecutively 1 to 10. As green fruits turned a tannish color they were considered ripe and only ripe fruits were collected. Each pod of *Impatiens balsamina* has five valves which coil elastically and eject the seed in dehiscence. The fruits were ready to dehisce at the time of harvest and broke open when touched. The seeds were then spread out to dry in the laboratory. Each collection of seeds was separated before drying into two

sizes, those held by a sieve with an opening of 2.00 mm. and a sieve with an opening of 1.19 mm. Except where otherwise stated, the experiments below were carried out with the set of larger seed. This size separation was done so as to give comparable results of different collection lots in case the germination percentages of large and small seeds varied. As soon as the seed of the last harvest in any one week had had a day or two to dry out, the week's collection was combined and stored open in the laboratory, since this storage condition had previously been found favorable for after-ripening.

Germination tests were made for each lot immediately after each week's collections were combined and at weekly intervals thereafter through 25 weeks of storage. Each test consisted of two samples of 100 seeds each, one put directly in the germinator held at constant 25° C. (which proved the most favorable temperature in preliminary tests), the other at 5° C. for a pretreatment period of two weeks before being placed at the germination temperature. The seeds were mixed with granulated peat moss kept moist throughout the experiment and kept in bottles. As soon as the root appeared, seedlings were counted and removed. The bottles containing the seeds and peat moss were examined once a week during the experiment.

Dry storage effects. The beneficial effect of a dry storage period has been reported by Simpson (8) for freshly-harvested cottonseed and by Malskaia (5) for freshly-harvested seed of *Nicotiana rustica* L. The experimental results described in this paper place *Impatiens balsamina* in the class with cotton and tobacco in that a period of dry storage was found necessary for freshly-harvested seeds to obtain full germination capacity.

Data in Figure 1 show dry storage effects on two of the ten lots collected, the first and last collections made. These collections were made August 14-19 (Lot 1) and October 14-16 (Lot 10) respectively. Those seeds which were placed in moist granulated peat moss at the germination temperature of 25° C. immediately after harvest were retarded both as to germination capacity and as to germination energy, neither of the two lots reaching 60 per cent after 20 weeks in the germinator. After storage for four weeks in the laboratory, both germination percentage and rate increased considerably. A great improvement was found when seeds stored for nine weeks were tested. Eighty-two per cent germination was obtained for both lots, although seeds of the August collection required four weeks longer to reach this percentage than did seeds of the October collection. Still further improvement resulted when seeds were stored for 16 weeks. Here again the germination energy of the October collection seemed slightly superior to that of the August collection. Storage for 25 weeks was found to improve seeds of Lot 1 but showed no improvement over storage for 16 weeks for seeds of Lot 10. All of the ten lots collected showed substantial gain in germination percentage and rate from 16 weeks of dry storage, but beyond this period results varied with the different lots. From 16 to 25 weeks of dry storage at room temperature was required for overcoming dormancy of

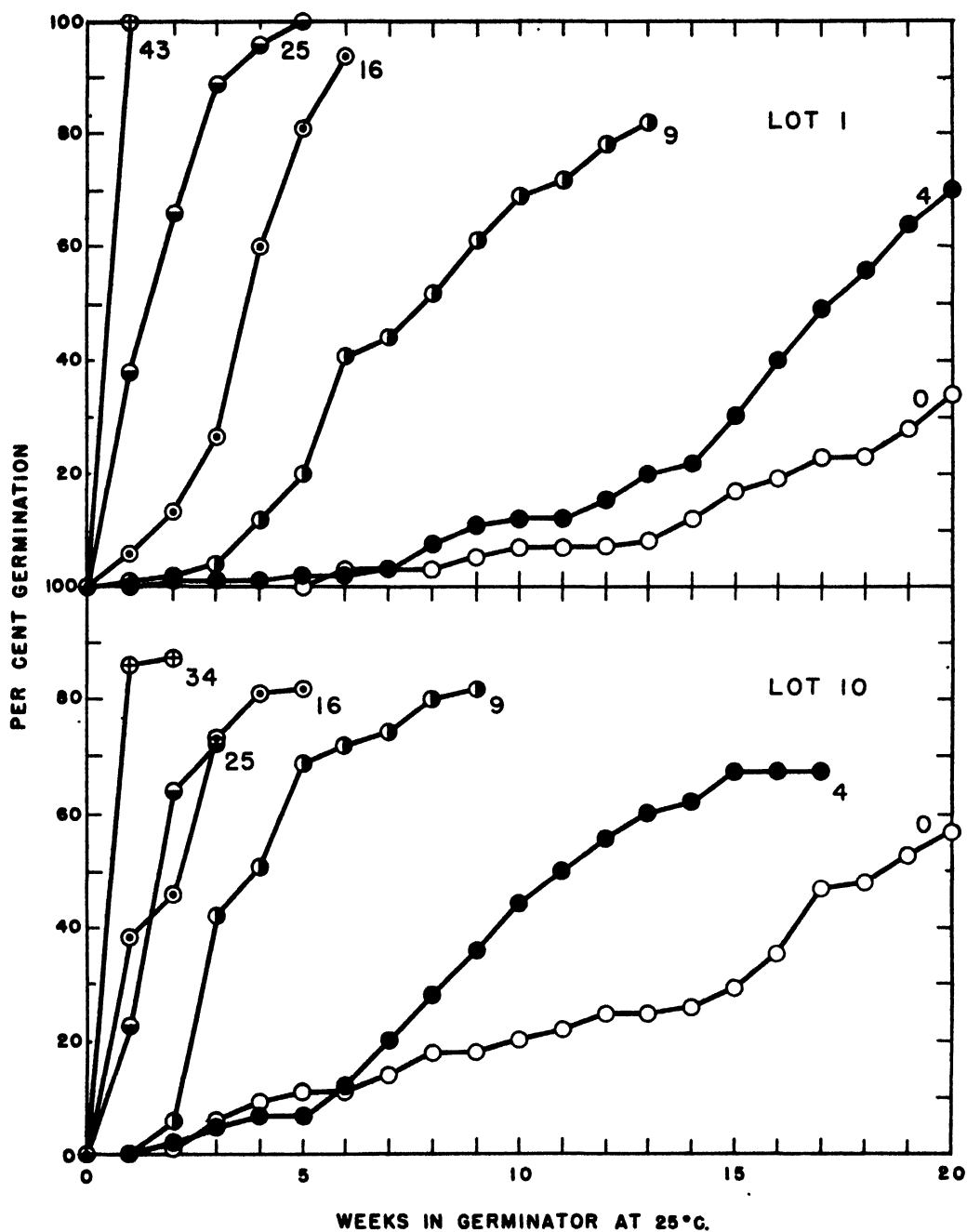


FIGURE 1. Effect of weeks of dry storage at room temperature and date of harvest on after-ripening. Lot 1 was collected August 14-19 and Lot 10, October 14-16.

fresh seed. When after-ripening was complete two to three weeks in the germinator were generally required for obtaining a substantial germination percentage, the time varying with the different seed lots. When this percentage was obtained in two to three weeks, the seed was considered no longer dormant.

A final test was made on all of the collection lots in June 1941. This test occurred 43 weeks after the harvest of Lot 1 and 34 weeks after the harvest of Lot 10. As is shown by the data in Figure 1, one week in the germinator yielded 100 per cent germination for Lot 1 and 86 per cent for Lot 10.

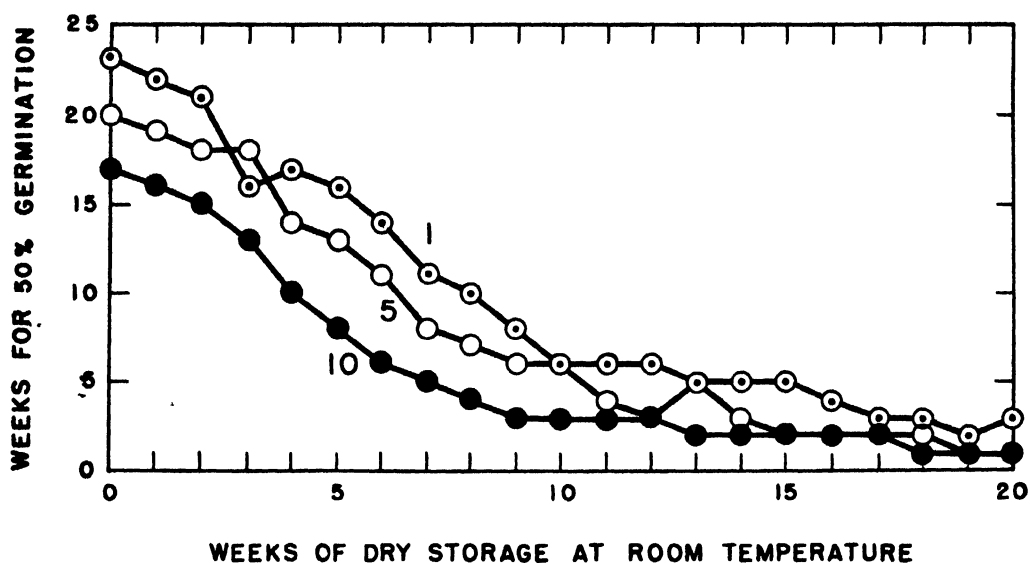


FIGURE 2. Decrease in number of weeks required to obtain 50 per cent of germination capacity with increase of number of weeks of dry storage. Lot 1 was collected August 14-19; Lot 5, September 9-13; and Lot 10, October 14-16.

Malskaia (5), in his work on tobacco seed, found that during the three to five months following harvest, the germination capacity was unstable and became stable only at the time of spring sowing. This may apply to the findings on *Impatiens balsamina*, especially on Lot 10 shown in the graph, which seemed to have reached a maximum after 16 weeks of dry storage; further storage up to 25 weeks showed no improvement in germination percentage or energy.

In general, the later collections of seeds seemed to be slightly inferior in germination capacity to the earlier collections. The maximum final percentages obtained by these later collections were between 85 and 90, while the earlier lots produced 95 to 100 per cent germination. This inferiority seemed to be offset, however, by a tendency of the seeds harvested later in the season to be less dormant than those gathered during the first four or five weeks. This tendency may be seen in Figure 1 but is more clearly defined in Figure 2. Since the lots varied greatly in their germination capac-

ity, the standard of measurement for data was the number of weeks required to reach 50 per cent of the total germination capacity. The three lots indicated in Figure 2 were collected approximately a month apart, in August, September, and October of 1940. The August harvest required 23 weeks to reach 50 per cent of its germination capacity after 0 weeks of dry storage as compared with 20 weeks for the September harvest and 17 weeks for the October harvest. This tendency seemed to endure up to ten weeks of storage after which time there was slight difference between the lots.

In connection with these results it is of interest to note the findings of Hite (3) who reported that ripening of bluegrass seed occurred at a more rapid rate on the plant than during storage. Seed collected June 29 reached normal germination in a much shorter time than those collected June 1.

A weak dormant period of about four weeks is reported for cucurbits by Odland (7). He found differences in germination from seeds of different fruits in the case of the pumpkin. If the fruit was allowed to remain on the vine until "over-ripe" the seed germinated promptly, but if the fruit was picked at the stage generally considered ripe the germination was delayed several weeks. In the experiment discussed in this paper an effort was made to collect seeds all of which were at the same stage of maturity. Hence, unlike the findings of Odland (7), the results with *Impatiens* cannot be explained by "ripeness" or "unripeness" of seed when collected.

Thompson (10) discovered that lettuce seed from the fourth or fifth harvest was not more than one-half as large as seed from the first harvest, but its germination was 100 per cent higher. There is a general belief that second harvest seed is inferior to that harvested first. This may be true as far as size and weight are concerned but Thompson found the second harvest seed superior in germination. This difference in seed size between early and late harvests was not found in *Impatiens*. Nor was any evidence found to show a larger percentage of small seeds present in later collections. In order to determine the germination capacity of the small seeds (those which would not pass through a sieve with an opening of 1.19 mm.), a duplicate test was run as for the larger seeds which were used exclusively in this experiment. No difference was noted either in germination capacity or energy between the large and the small seeds.

Samples of all seed collections tested the June after harvest were found to be completely after-ripened thereby indicating that for the gardener who wants to plant seeds of *Impatiens balsamina* in the spring for summer flowering there is no problem involved. But for the geneticist or others who want seedlings as quickly as possible after harvest, it is important to know that fresh seed exhibit a dormancy which can be partially overcome by pre-chilling in a moist condition and completely overcome by several months' dry storage at laboratory temperature.

Effect of moist low temperature pretreatment. Although the preliminary tests on *Impatiens balsamina* had shown no indications of a beneficial effect

derived from pretreatment of the seeds in a moist medium at low temperature, the fact is well established for many kinds of seeds that dormancy may be overcome in part or wholly by this treatment. Davis (1), in working on dormancy in *Ambrosia*, emphasized the close relation between moisture content of the embryo and the temperature employed in after-ripening. He reported that at low or high temperature the moisture content determines whether after-ripening will take place. If the temperature is low, the seeds must be moist; if high, the seeds must be dry. If the seeds were kept moist at a high temperature, after-ripening was counteracted by the effects of high respiration.

Toole (11) reported that prechilling moist seed of timothy, which is slightly dormant when freshly harvested, increased germination but this treatment alone did not bring about complete germination. Experiments were conducted on *Impatiens balsamina* to determine whether prechilling would be of benefit in hastening after-ripening and hence germination.

A two-week period of low temperature was arbitrarily decided upon as the pretreatment period and later experiments proved a longer period of one month to be of no further benefit. For each sample of seed placed directly in the germinator held at 25° C. a duplicate sample was placed at 5° C. Moist granulated peat moss was the medium. At the termination of two weeks, the sample was removed from the low temperature and placed at the germination temperature of 25° C. These samples were treated in a similar manner to those placed directly at 25° C.; upon examination once a week germinated seeds were counted and removed.

The beneficial effect on fresh seed of prechilling can be seen if Figure 3 is examined. The two lots used in these graphs are those collected in August (Lot 1) and September (Lot 5) approximately a month apart. For both lots of freshly-harvested seed, low temperature pretreatment gave an improvement in germination capacity and energy. After several weeks of dry storage, six and four for Lots 1 and 5 respectively, a period at 5° C. proved to be still of great value in hastening germination. After 12 weeks of dry storage for Lot 1 and after eight weeks for Lot 5, the pretreatment at low temperature was of only slight advantage to germination. After five weeks in the germination temperature, seeds of Lot 1 stored for 13 weeks gave approximately the same germination percentage regardless of pretreatment. Seeds of Lot 5 stored for nine weeks gave better germination percentage at a more rapid rate than those pretreated at 5° C.

It was found for most of the lots that once the effect of prechilling ceased to be of benefit, it actually hindered both germination percentage and speed. In many cases the seeds became moldy in the low temperature and had to be discarded. In no instance did germination occur within two weeks in the germinator at 5° C. The number of weeks of dry storage after which prechilling proved of no benefit varied with each seed lot, ranging

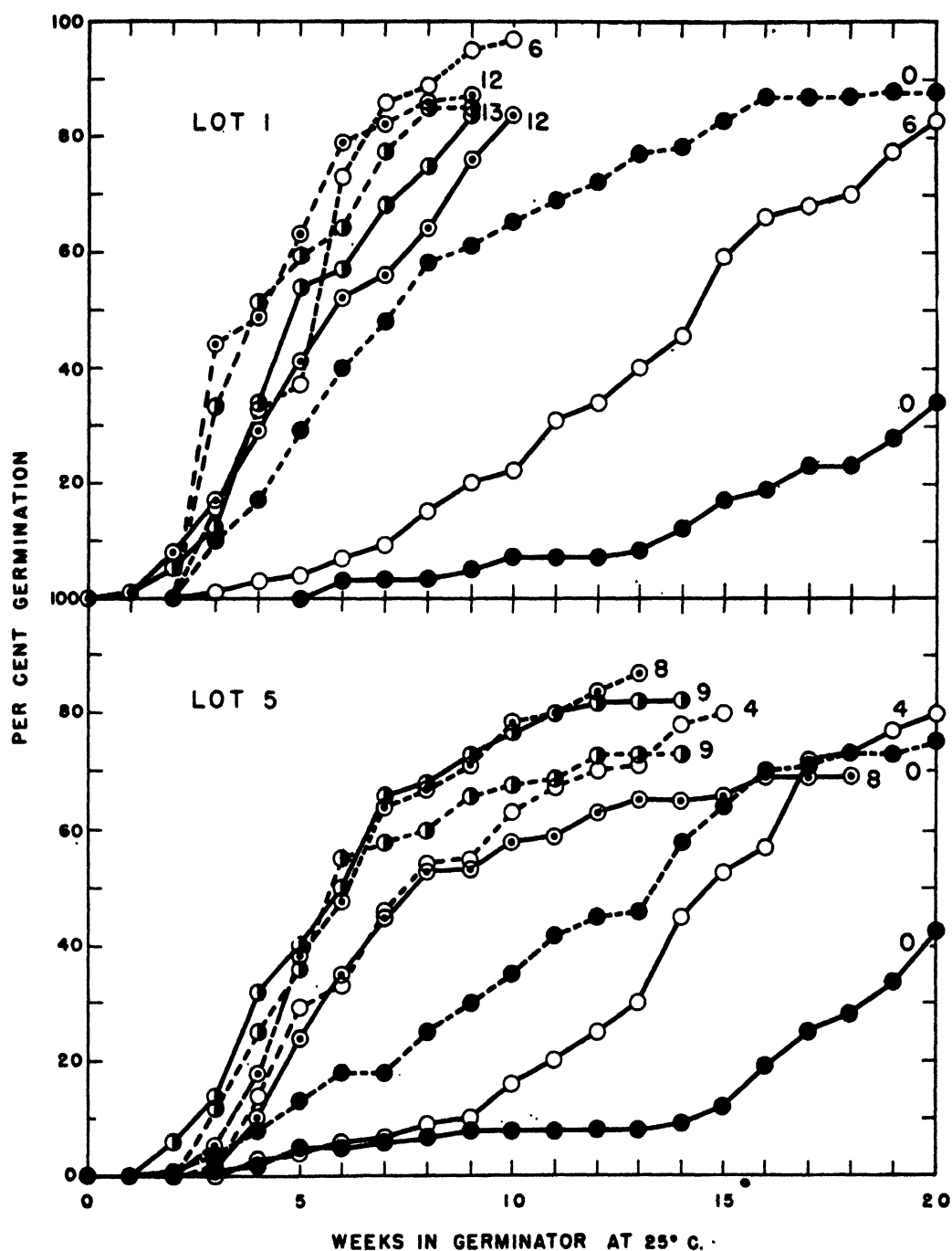


FIGURE 3. Effect of pretreatment for 2 weeks in a moist medium at 5° C. on germination after indicated number of weeks in dry storage at room temperature. Dotted lines, seeds pretreated; solid lines, seeds not pretreated. Lot 1 was collected August 14-19 and Lot 5, September 9-13.

from 8 to 14 weeks. Data were examined in an effort to determine whether seeds of early harvests required low temperature pretreatment after a longer period of dry storage than did those of late harvests. No such indications were found.

SUMMARY

Seeds of *Impatiens balsamina* are dormant when freshly harvested and require from four to six months of dry storage at room temperature for after-ripening. From two to three weeks at the germination temperature were required for completion of germination, when full after-ripening had been attained. Twenty-five degrees Centigrade was found to be the most favorable temperature for germination. Germination of fresh seeds was accelerated by pretreatment for two weeks in a moist condition at 5° C. before the seeds were placed at the germination temperature. Prechilling ceased to be effective after the seeds had been stored dry for two to three months and in some cases proved harmful after that time.

There was a tendency for seeds harvested later in the season to be inferior in germination capacity, but less dormant as measured by time required for the completion of germination. Seed size was not responsible for this difference.

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EFFECT OF TEMPERATURE ON THE AVAILABILITY OF INSOLUBLE NITROGEN

M. M. McCool

It was observed during the progress of our studies on the agronomic value of synthetic insoluble nitrogen that this element in the soluble form in urea was more effective in the promotion of plant growth than it was in the insoluble form (1). The indications were, however, that this relationship was affected by the temperature under which the growth comparisons were made. Accordingly, the rate of nitrate formation from urea, insoluble nitrogen in Urea-Ammonia Liquor-37 (UAL-37) base, and cottonseed meal at different temperatures has been measured. In addition, the effect of time of application of the two latter to soil on the growth rate of millet (*Echinochloa frumentacea* Link) has been investigated.

MATERIALS AND METHODS

The methods of procedure which were followed in measuring the effect of temperature on the rate of nitrate formation were the same as those given previously (1), with the exceptions that 100 grams of Sassafras sandy loam to which were added 5 grams of soil from a productive garden and 30 milligrams of nitrogen composed the cultures, and the entire mass of soil in each culture was dispersed. The cultures were placed in ventilated incubators which were maintained at 10°, 20°, 27°, and 35° C. respectively. After 30 and 60 days the nitrate nitrogen in triplicate cultures was determined. In measuring the plant growth response millet was planted in Gloucester loam which was taken from a local area, screened, and while moist placed in glazed jars of two-gallon capacity. The basic treatment consisted of 6 grams of superphosphate, 16 per cent P_2O_5 , 0.5 gram of potassium chloride, and sufficient dolomite to bring the pH value to 6.0. Three-tenths gram of nitrogen was added to the cultures where the plans called for its addition. The random system was followed in arranging the cultures on the bench in the greenhouse. The cottonseed meal and UAL-37 base were leached with distilled water to remove the soluble nitrogen therefrom. The growth period extended from February 24 to May 14, 1941. The minimum temperature of the greenhouse was 21° and the maximum 27° C.

RESULTS

The results obtained from the studies on nitrification are represented by Figure 1. It is apparent from these results that urea is nitrified most rapidly of the lot of carriers employed. It was the only one from which nitrates

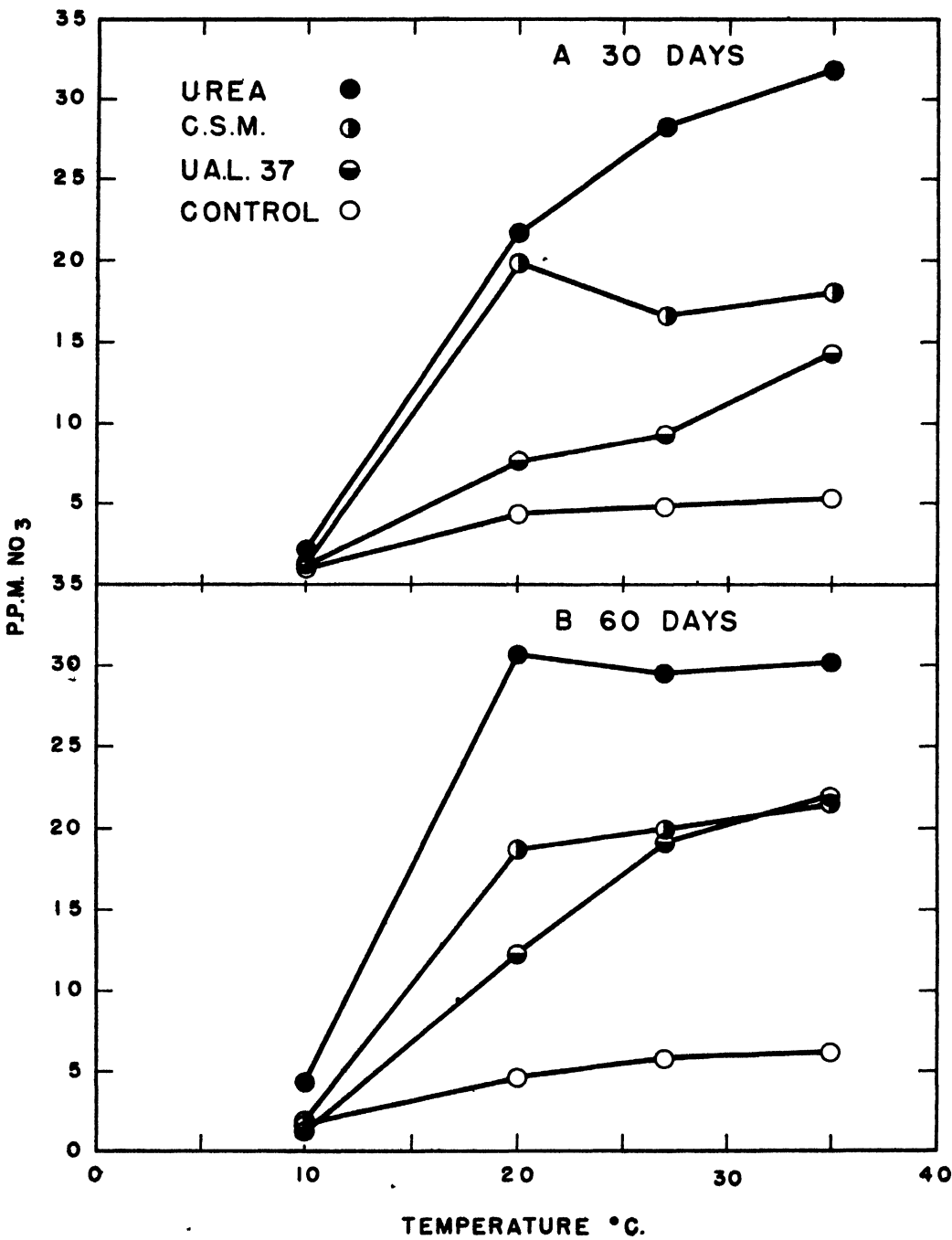


FIGURE 1. Effect of temperature on nitrate formation. Results expressed as parts per million NO₃ in the soil.

were formed in this soil at 10° C. At 20° C. urea and cottonseed meal had approached their maximum production of nitrates after 30 days but the UAL-37 base had not done so. The cultures to which the latter was added did not give up the maximum amount in the chambers maintained at 27° and 35° C. until tested at the close of the 60-day period. It thus appears that the nitrification of the insoluble nitrogen in UAL-37 base is relatively slower at each of the temperatures than is that in cottonseed meal, the difference being greatest at 20° and 27° C. It should be noted that Smith (2) determined the rate of nitrate formation from ammonium sulphate, urea, ground fish, and activated sludge in jars of Norfolk sandy loam when held under different ranges of temperature. He reported urea to nitrify most rapidly under all ranges of temperature.

TABLE I

EFFECT OF TIME OF APPLICATION OF INSOLUBLE NITROGEN ON GROWTH RATE OF MILLET.
RESULTS EXPRESSED AS DRY WEIGHT IN GRAMS

Cultural treatment*	Time of application in days before seeding			
	0	17	34	68
Cottonseed meal	14.2, 15.3, 10.8	14.3, 13.4, 12.6	14.1, 15.0, 15.4	16.9, 14.7, 17.6
UAL-37 base	15.4, 12.6, 13.9	14.5, 14.8, 12.3	14.2, 14.4, 11.9	16.1, 15.4, 17.3
Analysis of Variance				
	Degrees of freedom	Sum of squares	Mean square	
Effect of application date	3	28.97	9.66	
Cottonseed meal, UAL-37	1	0.10	0.10	
Comparison of cottonseed meal and UAL-37—different periods	3	3.31	1.10	
Error	16	31.28	1.95	
Total	23	63.66		

* Yield control, no nitrogen—3.6, 3.75, 2.65 g.

Cottonseed meal and UAL-37 base were mixed with the soil at seeding and 17, 34, and 68 days prior to it. The yields of millet together with the analysis of the data are given in Table I. The yields were increased slightly by adding the materials to the soil 34 days prior to seeding and significantly so when they were added 68 days before the millet seeds were planted. There is no evidence of any difference between the cottonseed meal and the UAL-37 base.

SUMMARY AND CONCLUSIONS

The rate of nitrate formation from urea, cottonseed meal, and UAL-37 base was determined after 30 and 60 days in Sassafras soil incubated at 10°, 20°, 27°, and 35° C. The urea was the most active and was the only

one which nitrified at 10° C. The UAL-37 base was the least active and most strikingly so at 20° C.

Cottonseed meal and UAL-37 base were added to soil cultures at seeding and 17, 34, and 68 days prior to the seeding of millet. The yields were greatly augmented over the control in all cases. The addition of the materials 68 days before seeding gave a significant increase in yields over the yields found when the materials were added at shorter intervals before seeding.

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POTATO VARIETIES: SUGAR-FORMING CHARACTERISTICS OF TUBERS IN COLD STORAGE, AND SUITABILITY FOR PRODUCTION OF POTATO CHIPS

F. E. DENNY AND NORWOOD C. THORNTON

In the previous test (2) with tubers of the 1939 crop 11 varieties were included, and the temperatures employed were 5°, 10°, and 15° C. The experiment was continued with tubers of the 1940 crop, including in addition to the same 11 varieties 14 others; and, since the previous results had shown that 5° was too low and 10° too high for continuous storage, if chips of good color were to be obtained, tests at 10° were largely discontinued, but those at 5° were included, and the interval between 5° and 10° was divided, lots being stored at 7° and 8.2°.

Two other factors not studied in the previous experiment were brought into the present series of tests: (a) time after harvest at which tubers were placed in cold storage, with starting dates on October 25 and December 24; (b) the duration of the storage at a given temperature, four (and in a few cases, five) storage periods being employed, these varying from about 10, 17, or 18 days to as much as 144 days.

In addition to this main experiment, there were various side experiments in which information was obtained on such factors as: the effect of small differences in temperature (e.g. at 5°, 6°, and 7°), rate of de-sugaring when tubers were transferred from low to high temperatures, comparison of tubers of the same variety from different localities or grown under different proportions of fertilizer constituents.

Sugar analyses for both reducing sugar and sucrose, and potato chip samples were obtained from each lot of each variety under the various conditions of storage, although some of the combinations of the factors had to be omitted because of an insufficient stock of tubers of some of the varieties. In addition, data (not presented in this paper) were obtained for many of the varieties over long periods of storage at 10° C. and at room temperature.

In all, about 1000 analyses (for each of the two sugars) were obtained, the data including combinations of 25 varieties, three storage temperatures, four duration periods at each temperature, and two different stages after harvest at which storage was started. The de-sugaring tests involved removal of tubers of several varieties from cold storage at three different intervals after storage started.

Since such a wide range of conditions was covered with so many varieties, and with so many analyses, it is believed that the results give a general

picture of the situation, and have sufficient validity to justify the rather detailed presentation which is attempted in this report.

MATERIALS AND METHODS

The potato (*Solanum tuberosum* L.) tubers were grown in the Institute gardens and, except as mentioned in the text, were harvested August 5 to 14, 1940. The yield was low due to a prolonged period of hot weather in July. The planting stock was obtained from a seed-supply firm, and the variety names used in this paper are those on the labels as received from the seedsman. The variety names are subject to some variation in the literature. The spelling for Spaulding Rose is usually Spaulding's Rose, and our stock may be the same as Spaulding's Rose 4 listed in the text by Thompson (5). The variety Eureka may be the same as Early Eureka (see Thompson 5, p. 370).

After harvest, the small tubers (those with side-to-side diameter less than 1.75 inches) and those having cuts or bruises were discarded, and the remainder was divided into samples of 15 to 16 tubers each, with an equal distribution among them as to size of tubers. Each sample was placed in a cheesecloth bag and was labeled, the sample from the various varieties for each storage condition was accumulated in a burlap bag to which was attached a tag showing the samples included and the dates on which removal from storage or transfer to another storage condition was to be made.

The burlap bags containing the samples were placed in storage rooms at the selected temperatures. The rooms were under thermostatic control and a reading was made each day (except Sundays) to give assurance that the proper temperature was being maintained. The storage temperatures were not measured by air temperatures but by the temperatures shown by a thermometer immersed in 250 cc. of water placed in a 500 cc. Erlenmeyer flask. Tests indicated that the rate of temperature change in the liquid was nearly the same as the change in a medium-sized potato tuber, as shown by a thermometer inserted into a tuber subjected to the same temperature change.

The methods used in obtaining juice, preparing the juice for the sugar analyses, the analytical procedure, the slicing of tubers for chips, and the details of cooking in the preparation of chips are the same as those described in a previous paper (2).

PLAN OF EXPERIMENTS

Samples were placed in cold storage at two different stages after harvest (which, as previously stated, was in the interval August 5 to 14), the first starting period being October 25, and the second December 24, the tubers for the last series remaining in burlap bags at room temperature (approx. 25° C.) from harvest until December 24. Sugar analyses were made at har-

vest, or shortly after, at the start of each starting period, and at each interval at which tubers were removed from storage. The temperatures of storage were 5°C ., 7°C ., and 8.2°C ., these being used for the following reasons: 5°C . because increase in sugar content of tubers was known to occur readily at this temperature, and this permitted a study of the sugar-forming characteristics of the various varieties; and 7° and 8.2° because it was hoped that at one of these temperatures the reducing sugar values of some or of all of the varieties would remain low enough to permit the production of potato chips of good color. The particular value 8.2° was not deliberately selected, and happens to be the temperature resulting from an approximate thermostat setting between 8° and 8.5° ; and since there was no way of knowing whether better results would be obtained at 8° or 8.5° , the thermostat setting was not disturbed. The results show that this was a favorable temperature for demonstrating temperature effects upon sugar content, upon potato chip color, and upon the relation of the variety of potato to these factors.

From the lots at each temperature and each starting period samples were removed at intervals; sugar analyses were made and chips were prepared. Since it was expected that the sugar increase would be slower at the higher temperatures, the duration of these intervals between two removal dates varied with the temperature, being shorter for 5° and progressively longer for 7° and 8.2° .

As is shown in the tables, samples of certain varieties were not included in the tests at certain of the storage temperatures. This is because there were not enough tubers of some varieties to permit that many samples. In such cases, the available samples were spread over the range of conditions as far as the supply would go, the placing of samples of varieties with a deficient number of tubers being arranged among the varieties so as to give representation to all of the conditions of the test. A complete set was obtained for the 5° storage at both starting dates, but six to nine of the varieties lacked representation at 7° and three to six of them at 8.2° .

In addition to this main experiment there were a number of side experiments on various phases of the problem. The details of each of these tests are described when the results of the experiment itself are presented.

RESULTS

The results for the main experiment are given separately for reducing sugar and for sucrose. In each case there is a table showing the separate original values for each of the determinations made (578 in each of Tables III and VIII). From these master tables were prepared summary tables in which the analyses were brought together by aggregating comparable items, to show the general effect of temperature, duration of storage, effect of time of starting storage, and characteristics of different varieties with

regard to each of these factors. Figure 1 shows the values for all of the varieties combined into one average value for each of the storage conditions. The method of combining the determinations to obtain this average value is described in the paragraph "Experimental errors."

EXPERIMENTAL ERRORS

There were no duplicate samples for any of the individual determinations in the main experiment. To have carried out the experiment in duplicate would have restricted seriously the range of conditions that could have been tested, and it seemed that the important consideration at this stage of the problem was to cover the range as widely as possible, and thus make sure of an orientation in the field.

TABLE I

ANALYSIS OF VARIANCE OF THE VALUES IN TABLES III AND VIII FOR THE 13 VARIETIES WITH DATA FOR EACH OF THE VARIOUS STORAGE CONDITIONS INCLUDED IN THE EXPERIMENT

Source of variation	Degrees of freedom	Reducing sugar		Sucrose	
		Sums of squares	Variance	Sums of squares	Variance
Total	311	4589.67	—	8177.84	—
Between varieties	12	653.29	54.44	3703.64	308.64
" starting dates	1	136.01	136.01	306.23	306.23
" temperatures	2	1854.81	927.41	2102.02	1051.01
" removal dates	3	866.83	288.94	86.00	28.67
Starting dates X temperatures	2	60.74	30.37	5.87	2.94
" " X removal dates	3	66.52	22.17	119.48	39.83
Temperatures X " "	6	450.75	75.13	123.41	20.57
Starting dates X temperatures X removal dates (residue)	6	32.02	5.34	98.42	16.40
Varieties X temperature	24	115.68	4.82	436.68	18.20
" X starting dates	12	41.38	3.45	668.35	55.70
" X removal dates	36	148.67	4.13	114.09	3.17
Error	204	162.97	0.80	413.65	2.03

Nevertheless, although no duplicates were run, the experimental error was obtainable by the analysis of variance. For this purpose not all of the values in Tables III and VIII were available, due to the necessity of omissions of samples for which there was an insufficient supply of tubers.

However, there were 13 of the varieties for which measurements covering all of the experimental conditions were available. These were: Chipewa, Russet Rural, Heavyweight, Carman No. 3, Rural New Yorker, Number Nine, Irish Cobbler, Early Rose, Katahdin, Eureka, Green Mountain, Delaware, and Spaulding Rose. Data for these for both reducing sugar and sucrose permitted setting up two 13 X 24 tables which gave the values shown in Table I. It is seen that the error variance for reducing sugar was 0.80, and for sucrose was 2.03. These values were used to test the significance of differences between totals for comparable items in various portions

of the tables, as will be described when the data in the tables are discussed in later paragraphs.

Although duplicate determinations were not made in the main experiment, a side test of the variation among samples was carried out by removing quadruplicate samples of some of the varieties at some of the storage conditions, and making the sugar determinations in the usual way. The results are shown in Table II and the variances found were 0.76 for reducing sugar and 1.83 for sucrose, in good agreement with the values found in the main experiment.

TABLE II

SAMPLING AND ANALYTICAL ERROR AS SHOWN BY ANALYSES OF QUADRUPPLICATE SAMPLES

Variety	Temp.	Values obtained on quadruplicate samples, milligrams per cc. of juice								Sums of squares of deviations from the mean of each quadruplicate	
		Reducing sugar				Sucrose				Reducing sugar	Sucrose
Irish Cobbler	5°	15.0	13.7	12.9	13.9	9.2	7.9	8.2	7.1	2.28	2.26
	7°	4.1	4.0	3.8	5.3	3.1	2.6	2.8	2.5	1.38	0.21
Russet Rural	5°	7.3	6.5	5.3	4.5	4.3	3.7	4.2	4.8	4.64	0.61
	7°	0.0	0.0	0.6	0.9	1.9	1.6	1.8	1.4	1.11	0.15
Chippewa	7°	4.1	4.1	4.7	2.6	27.5	30.4	23.0	27.6	2.41	28.11
Carman No. 3	7°	1.7	2.7	0.9	2.4	1.8	2.0	1.0	2.8	1.93	1.64
Pooled sum of squares										13.75	32.98
Degrees of freedom = 3 × 6 =										18	18
Variance =										0.76	1.83

The analyses that formed the basis for Table I included only those from the 13 varieties that were held in all of the storage conditions. A basis for including the data for all of the varieties, even though simultaneous analyses of each from all of the conditions of the experiment were not available, was found in the following procedure. The weighted average of all of the measurements at 5° for all of the varieties for both starting dates was prepared by multiplying the average value between two removal dates by the number of days between the removal dates. The sum of these products divided by the sum of the days for the intervals gave a weighted value for that variety at 5° over that period of storage. For example, the procedure for the variety Neverblight in line 1 of Table III is as follows:

$$\frac{\left(\frac{0.0+5.4}{2}\right)(32-18) + \left(\frac{6.7+5.4}{2}\right)(59-32) + \left(\frac{4.0+6.7}{2}\right)(93-59)}{(32-18) + (59-32) + (93-59)} = 5.1$$

When the values for the two starting dates were found, and these also combined so as to give a weighted mean, the final value was the average for that variety for storage at 5°, and represented the capacity of that variety to form reducing sugar during storage at 5°. This process was repeated for each of the 25 varieties.

Then a new table (not shown in this report) similar to Table III was prepared, in which each value in the table was expressed as a percentage of the weighted mean for that variety. Each column (corresponding to the columns in Table III) then consisted of a series of percentages. These columns had average values; the separate items in a column had deviations from this average; from these the squares of the deviations and the sums of the squares of the deviations were formed; and from these by pooling both the sums of squares and the $(n-1)$ values, an average standard deviation was obtained. The same procedure was carried out for the sucrose values. The standard deviation for the reducing sugar obtained in this way was 15.09 per cent and for sucrose it was 18.39 per cent. These are expressed as percentages of the average value at 5° and to change these percentage values into milligrams of sugar per cc. of juice, for comparison with the error variance values in Table I, they were multiplied by the average sugar value at 5° for the 13 varieties represented in Table I. The average values at 5° were 6.61 mg. of reducing sugar and 10.71 mg. for sucrose. These values multiplied by 0.1509 and 0.1839 give 1.00 mg. and 1.97 mg. as standard deviations respectively for reducing sugar and sucrose. The square of each value gives the variance in each case and is 1.00 for reducing sugar and 3.88 for sucrose.

These error values are somewhat larger than those computed by the two other methods, but they are sufficiently similar to give confidence that this procedure for combining the observations on the different varieties under the various storage conditions is permissible, and gives a dependable composite value. For this reason the average values obtained by pooling all of the variety percentages for the various temperatures and starting dates were used in preparing the curves in Figure 1.

DATA FOR REDUCING SUGAR

The analyses for reducing sugar are shown in Table III. One item, the one for Early Rose at 8.2° under the column heading 144, is enclosed in parentheses. The flask holding the liquid for this sugar determination was broken accidentally and the true value could not be obtained. The value in parentheses is an estimated value and was inserted to facilitate the computations. It is seen that a considerable error in the estimate of this value could have been made without causing any important change in the values which would be obtained in the various estimates.

The values 0.0 in the table mean that the analysis value was less than

0.1 mg. but this does not mean that such juices had no sugar. In many cases, it is known that the samples so listed had traces of sugar, as shown by a trace of cuprous oxide on the asbestos mat in the sugar determination. To have obtained a measure in such cases would have required that the amount of juice taken for a sample be increased or some other sugar-method be used, but in any event, the amount would have been very low, and would have been of no significance in connection with the objects of these experiments.

Effect of Temperature

The over-all effect of temperature on the reducing sugar in the juice is shown in Table IV which was prepared by aggregating the comparable

TABLE IV
REDUCING SUGAR: SUMS AT DIFFERENT STORAGE TEMPERATURES

No. of varieties simultaneously represented	Starting dates	Sums of reducing sugar values for all four removal dates at each temperature			No. of items summed	Diff. req. for sign.
		5°	7°	8.2°		
13	Oct. 25	408.4	142.8	57.2	52	18.2
	Dec. 24	279.0	84.7	38.7	52	18.2
		687.4	227.5	95.9	104	25.8
14	Oct. 25	431.6	147.5	—	56	18.9
	Dec. 24	294.7	88.7	—	56	18.9
		726.3	236.2	—	112	26.8
19	Oct. 25	619.9	216.0	—	76	22.1
16	Dec. 24	328.7	92.8	—	64	20.2
19	Oct. 25	668.7	—	110.2	76	22.1
22	Dec. 24	579.0	—	111.3	88	23.7

items in Table III. Only analyses representing simultaneous determinations in strictly comparable groups were summed. For example, in Table III there were 13 varieties that had complete data for 5°, 7°, and 8.2° at both starting periods; there were 14 varieties (including the 13 just mentioned and one other) that had complete data for 5° and 7° at both starting periods; there were 19 varieties for 5° and 7° for the October 25 series, etc. As is shown by the table headings in Table IV, the values in columns 3, 4, and 5 are the summed values for all four removal dates at each temperature, and for all of the varieties sampled simultaneously, with the number of varieties involved being indicated in column 1.

The values in columns 3, 4, and 5, Table IV, show the large effect of small differences in storage temperature upon the reducing sugar content,

causing, for example (see line 1), a decrease from 408.4 at 5° to 142.8 at 7°, and then a further decrease to 57.2 at 8.2°. The other items in Table IV are in general agreement with this difference. Considered as a whole, the data show that the reducing sugar values at 7° are about 35 per cent and at 8.2° about 16 per cent of those at 5°. It is true that these computations are not made at exactly the same number of days after the start of storage, but if the reader will turn to Figure 1 and interpolate on the curves for 5°, 7°, and 8.2° to get the values for the 7° and 8.2° curves at the points corresponding to the final value for 5°, i.e. after the same number of days from the start of storage, the ratios of the values at these points will be found to be about the same as those indicated above.

By comparing the values for the two different starting periods, it is seen that the general temperature effect has been about the same whether the storage started October 25 or December 24, but this statement must be read carefully, because in a table to follow it is shown that the *rate of increase* of reducing sugar at a given temperature is markedly influenced by the starting date.

The data in columns 6 and 7, Table IV, are included to assist the reader in deciding whether the differences shown are sufficiently large to be significant. The figure 52 in column 6, line 1, in the body of the table, is the number of items summed to give the values 408.4, 142.8, and 57.2, i.e. there were analyses for 13 varieties at each of four removal dates. The value 18.2 is computed from this expression: $\sqrt{0.80 \times 52 \times 2 \times 2} = 18.2$ (3, page 49), in which the value 0.80 is the variance of each determination of reducing sugar (see Table I), 52 equals the number of items included in each sum, the 2 under the square root sign is the multiplier for the difference between two sums, and the final 2 is the multiplier to furnish odds of 19 to 1 (probability = 0.05), when the degrees of freedom are in excess of about 30, and which in our case are 204 (see Table I).

The differences in each comparison are well over the requirement (e.g. 84.7 - 38.7 = 46.0, to take the smallest difference). Perhaps, in this table, the right-hand columns are hardly necessary, but the same value called "Diff. req." in this table will be found in other tables in which its use may be required to furnish an estimate of significance, and the computation given above will serve to show how the value was established for each case in which it appears in a table.

Influence of Starting Date on the Rate of Reducing Sugar Increase

When tubers which are low in reducing sugar, either because they have been harvested recently, or because they have been exposed to high temperatures (10° C. or higher) for a sufficiently long period, are placed at low temperatures, such as 5° and 7° and to a less marked extent at 8.2°, the reducing sugar increases. Table V has been prepared from the data in Table

III by summing comparable values, to show the rate of this increase, and how it is influenced by the temperature, and especially by the time after harvest at which the storage was started.

The values in Table V were assembled in the following way: e.g. there were 13 varieties in Table III which furnished analyses for all three tem-

TABLE V
REDUCING SUGAR: EFFECT OF STARTING DATE UPON THE SUMS AT DIFFERENT
INTERVALS DURING STORAGE

No. of varieties simultaneously represented	Starting date	Temp.	Sums of reducing sugar values for all of the varieties				No. of items summed	Diff. req. for sign.
			Removal stage					
			1st	2nd	3rd	4th		
13	Oct. 25	5°	14.9	105.8	149.1	138.6	13	9.1
		7°	4.1	34.8	59.4	44.5	13	9.1
		8.2°	3.3	15.7	20.8	17.4	13	9.1
			22.3	156.3	229.3	200.5	39	15.8
	Dec. 24	5°	16.2	66.3	95.6	100.9	13	9.1
		7°	4.0	18.0	25.2	37.5	13	9.1
		8.2°	0.7	4.1	9.8	24.1	13	9.1
			20.9	88.4	130.6	162.5	39	15.8
19	Oct. 25	5°	28.3	162.1	220.2	209.3	19	11.0
		7°	5.5	55.4	90.7	64.4	19	11.0
			33.8	217.5	310.9	273.7	38	15.6
16	Dec. 24	5°	18.3	79.8	112.0	118.6	16	10.1
		7°	4.0	18.1	29.0	41.7	16	10.1
			22.3	97.9	141.0	160.3	32	14.3
25	Oct. 25	5°	49.8	234.4	307.2	288.8	25	12.7
	Dec. 24	5°	44.3	151.0	211.0	222.4	25	12.7
19	Oct. 25	8.2°	8.6	27.4	36.3	37.9	19	11.0
22	Dec. 24	8.2°	6.6	17.8	27.2	59.7	22	11.9

peratures, 5°, 7°, and 8.2°, and for both starting dates, October 25 and December 24. The first removal stage was at 18 days at 5°, and the sum of the reducing sugar values for these 13 varieties, at the 18-day stage at 5° for the October 25 starting date, was 14.9. There were 19 varieties with data for both 5° and 7° at the October 25 starting date, and the reducing sugar sum for these 19 at 5° in the October 25 series was 28.3.

Perhaps the most interesting fact in Table V is the difference shown by the two starting dates in the behavior of the reducing sugar on storage at

low temperatures. The values for the October 25 starting date (lines 1, 9, and 15) rise rapidly to a maximum value at the third removal stage, 59 days, and then show decreasing values at the fourth removal, 93 days. The values for the December 24 starting date (lines 5, 12, and 16), however, rise much more slowly and continue to rise even to the last removal date, 94 days. The final values (for 93 and 94 days) also are lower for the December 24 starting date, 138.6 and 100.9. There is a similar situation for the 7° items (see lines 2 and 6 in Table V), except that the final value for December 24, i.e. 37.5, is not significantly lower than that for October 25, i.e. 44.5, the difference being 7.0 and not reaching the required difference for the sums of 13 items, i.e. 9.1 (see right-hand column).

When the sums for all three temperatures at October 25 and December 24 are considered, the final value for October 25, i.e. 200.5, is significantly higher than that for December 24, i.e. 162.5, because the difference 38.0 is greater than 15.8 which is the minimum required difference between sums of 39 items. Furthermore, the October 25 values show a drop from the third to the fourth removal stage, $229.3 - 200.5 = 28.8$, and the December 24 values show a rise between the same intervals, $162.5 - 130.6 = 31.9$, both values being greater than 15.8.

The values for the storage at 7° in the October 25 series also show a decrease from a maximum at the third removal interval to a lower value for the fourth (see lines 2 and 10, Table V); and likewise the December 24 series at 7° shows a rise continuously and even between the third and fourth intervals.

The entries for 8.2° storage do not show a significant decrease from the third to the fourth interval for the October 25 series, and the December 24 series shows a gain, and a very large one, between the last two intervals. This may be due to the onset of rapid sprouting which occurred at about that time.

In addition to emphasizing a fact already brought out in a previous section, that there are large differences between the sums for 5°, 7°, and 8.2°, Table V also furnishes additional information on the point. It shows the length of the storage period required for this difference to become apparent. The required difference between 5° and 7° was reached at the first removal stage (17 to 18 days), e.g. $14.9 - 4.1$; $16.2 - 4.0$; etc., all such differences being larger than the minimum difference listed in column 9. The required difference between 7° and 8.2° was reached at the second removal interval.

Graphical Representation of the Temperature and Starting-Date Effects

After having examined the summed values in Tables IV and V and having noted the significance of the differences between comparable sets of data, it is profitable then to examine Figure 1. The graphs for reducing sugar are shown in the top half of the figure. These curves represent the

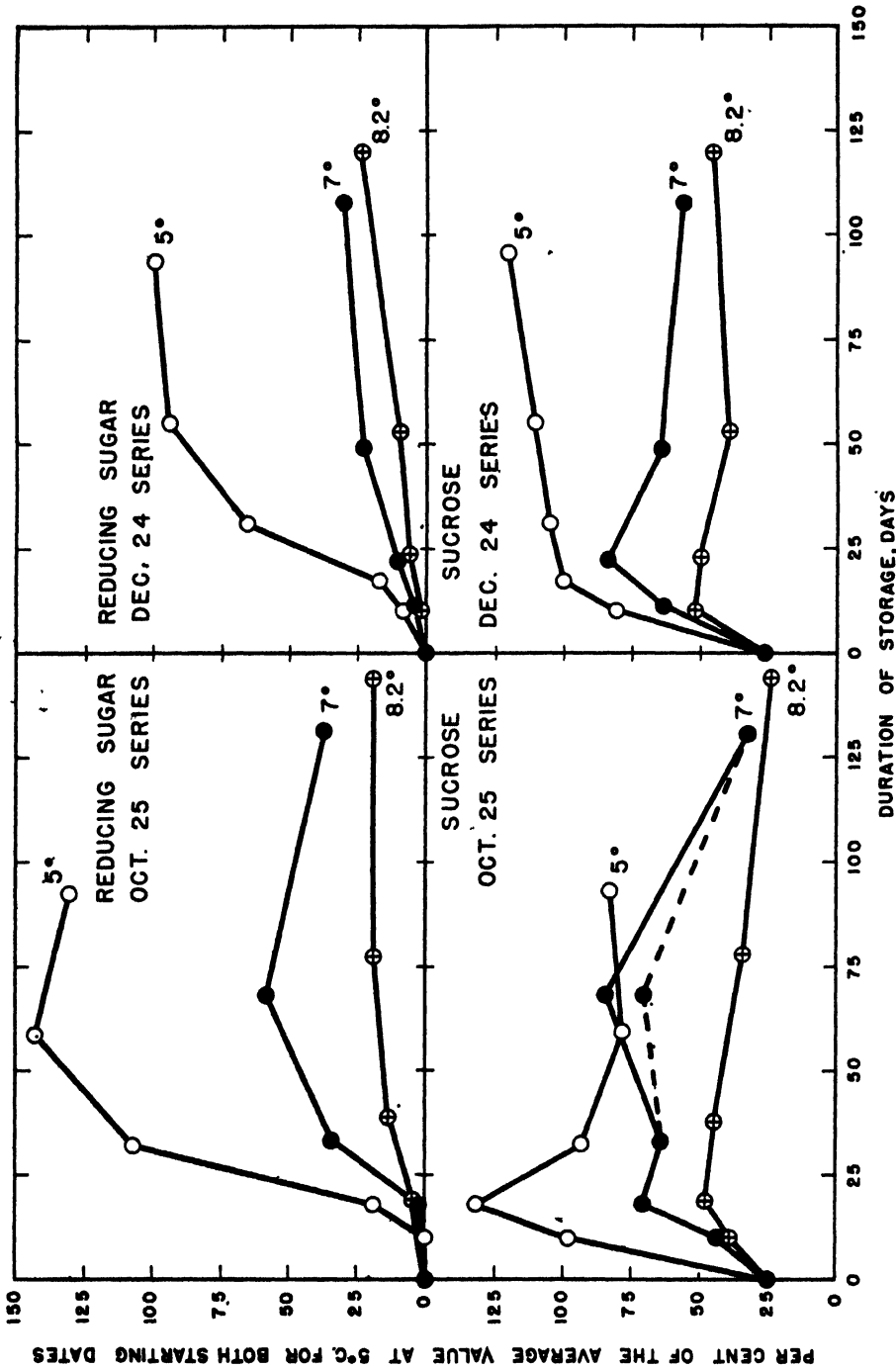


FIGURE 1. Average values for the combined analyses of all 25 varieties at all removal dates for each temperature and each starting date, expressed as a percentage of the weighted 5° values including both of the starting-date series. The broken line portion of the 7° sucrose curve in the October 25 series shows the course of the curve if the abnormally high value for Russet Burbank at the 68-day interval (see Table VIII) is omitted.

combined data for all of the varieties under all of the conditions tested. Note, again, as was mentioned in the discussion of the tables, the large differences between the reducing sugar values at the three temperatures; the temporary maxima in the 5° and 7° curves at the third removal interval with a lowering of the reducing sugar values at the fourth interval in the curve for the October 25 series, but not for the December 24 series, the curves for this starting date showing a progressive rise from the start to the end of the test at these temperatures.

An estimate of the dependability of the plotted points for reducing sugar in Figure 1 can be made in the following way. As shown in a paragraph in the sub-heading "Experimental errors," the standard deviation of a single determination was 15.09 (expressed as per cent of the average value at 5°). There were 16 to 25 entries for each point for each storage duration of 17 days or more, and, therefore, the standard error of the average of these values (but not including the 10-day items) will be in the range from $15.09 \div \sqrt{25}$ to $15.09 \div \sqrt{16}$, or from 3.0 to 3.8 units of the plotting scale at the left edge of Figure 1.

Characteristics of the Various Varieties

For convenience in comparing the varieties in regard to the reducing sugar values after storage at different temperatures, Table VI was prepared by summing the analyses for all four removal dates at each temperature and for each starting date. For example, the value 16.1 in column 2, line 1 in Table VI was obtained by adding the values 0.0, 5.4, 6.7, and 4.0 in Table III, columns 4, 5, 6, 7, line 1, and the value 12.2 is the sum of 1.0, 3.7, 3.7, 3.8, in Table III, columns 17, 18, 19, 20, line 1. These two values, 16.1 and 12.2, represent the capacity of this variety to accumulate reducing sugar at 5°. It was the sum of the values in these columns in Table VI which established the order of varieties in Tables III, VI, VIII, and XI. But, although this establishes a basis for the arrangement of the varieties, it does not mean that each variety in the list forms sugar less readily than the variety just below it, or that the sugaring capacity of a variety is greater than the one just preceding it in the list. The error variance previously referred to (Table I) will help to determine the limits beyond which the differences with respect to these values may be regarded as significant.

Since each one of the items in Table VI is made up by summing four values, the difference required between entries in the table is given by the value of the expression $\sqrt{0.80 \times 4 \times 2 \times 2}$, which equals 5.1. If comparable pairs were added such as 16.1 + 12.2, then eight items enter into the sum and the value 8 would replace 4 in the above expression and the required difference between sums of two comparable pairs would be 7.2 (see bottom of Table VI).

The varieties in Table VI form a series, with each member differing from the adjoining variety by only a small amount, and, only in the case of one pair (Blue Mercer and Warba, column 3) by as much as the required difference of 5.1. If a variety is selected it will be noted that usually only at a considerable distance in the series will a value be found that differs from that of the variety selected by 5.1. Nevertheless, it is possible to divide the series roughly into groups.

TABLE VI
REDUCING SUGAR: SUMS FOR DIFFERENT VARIETIES

Variety ↓	Sums of reducing sugar values for all four removal dates					
	5°		7°		8.2°	
Temperatures→						
Starting dates→	Oct. 25	Dec. 24	Oct. 25	Dec. 24	Oct. 25	Dec. 24
Neverblight	16.1	12.2	—	0.9	0.0	—
Chippewa	17.1	12.4	8.9	6.0	3.0	2.3
Russet Rural	20.5	12.3	4.6	0.5	0.9	0.0
Heavyweight	22.1	14.5	7.3	4.3	0.0	0.0
Carman No. 3	22.1	16.5	4.0	2.9	0.0	0.3
Blue Victor	23.2	15.7	4.7	4.0	—	—
White Rural	25.1	14.2	7.6	—	—	0.0
Rural New Yorker	24.7	16.6	7.4	3.4	0.3	0.0
Number Nine	24.0	18.9	4.9	2.5	0.0	0.0
Sir Walter Raleigh	32.3	16.3	7.2	—	—	0.0
Irish Cobbler	33.5	19.1	11.0	0.2	2.6	0.0
Early Rose	31.0	22.0	8.2	6.6	2.7	1.6
Early Six Weeks	34.3	21.8	—	3.2	0.0	—
Katahdin	30.5	26.6	14.2	8.8	3.3	3.1
Early Ohio	40.0	27.2	14.8	—	—	1.3
Eureka	43.7	24.9	15.7	7.2	8.4	3.7
Russet Burbank	40.8	28.1	12.3	—	—	2.5
Green Mountain	42.5	28.8	17.2	13.5	9.9	10.8
Delaware	44.9	30.8	16.9	15.2	12.2	10.0
Bliss Triumph	46.2	31.0	—	—	18.4	17.0
Pride of Multnomah	54.8	30.9	—	—	7.9	5.7
Spaulding Rose	51.8	35.6	22.5	13.6	13.9	6.9
Blue Mercer	54.7	37.0	—	—	9.7	10.9
Warba	50.1	55.4	26.6	—	—	19.7
Axtell's Bugless	54.2	59.9	—	—	17.0	15.5

Differences required:

Between any two items (each representing the total for 4 analyses in Table III)—5.1; between the sums of two comparable items (these values representing the total for 8 analyses in each comparable series)—7.2; between the sums of three comparable items (12 analyses in each series)—8.8.

Examining first the data for 5° in columns 2 and 3, it is seen that the first nine varieties are considerably lower than the others, especially for the October 25 starting date. By comparing this list of varieties from Neverblight to Number Nine inclusive with the table in the text by Thompson (5, p. 370-371) in which various varieties have been classified into groups on the basis of shape and color of tubers, sprouts, and flowers, it is found

that all of these except Chippewa and Blue Victor belong to the Rural group. (White Rural is not listed by Thompson, but we are assuming that it belongs to the Rural group.) Thus, the chemical data obtained from sugar analyses of tubers stored at different temperatures line up with the external characteristics which formed the basis of the original classification by Stuart (4).

One of the varieties in the Rural group as given by Thompson, i.e. Sir Walter Raleigh, shows sugar values higher than the other varieties in the Rural group in the data for the October 25 starting date. For the December 24 date and for both starting dates at 7° and 8.2° , Sir Walter Raleigh lines up with the other varieties in the Rural group, leaving in doubt the question whether there is a real difference between any of the varieties in the Rural group in the response to storage temperature.

In the list from Irish Cobbler downward to Katahdin and possibly including Early Ohio are a number of varieties intermediate with regard to reducing sugar values, ranging over into the Rural group at the top and into the remaining varieties below them. There are insufficient data for Early Six Weeks and Early Ohio to place them more accurately in the series.

From Eureka downward in Table VI all of the varieties (except possibly Russet Burbank), at all temperatures, and for both starting dates, are high in reducing sugar. These varieties are Green Mountain, Delaware, Bliss Triumph, Pride of Multnomah (except at 8.2°), Spaulding Rose, Blue Mercer, Warba, and Axtell's Bugless.

Chippewa, although it has sugar values as low as any in the Rural group at 5° storage, is differentiated from the Rural group by its response during storage at 7° and 8.2° , being distinctly higher over that range than any of those in the Rural group.

Irish Cobbler, although having more sugar at 5° in the October 25 series than members of the Rural group, compares favorably with them at other portions of the table. This variety gave results quite different for the two starting dates. When start of storage was delayed until December 24, reducing sugar values were much lower, and at 7° and 8.2° remained at values comparable to those of the Rural group.

Blue Victor, listed by Thompson in the Peerless group, showed sugar characteristics similar to varieties in the Rural group, but no supply of tubers of other varieties in the Peerless group was available, at the time, to test further the question of similarities between the Rural and Peerless groups.

There are some other comparisons of varieties within groups that may be made with the results in Table VI. The list given by Thompson (5) shows Green Mountain and Delaware in the same group, the Green Mountain group. The data in Table VI show a remarkably good agreement between these two, at all temperatures, and both starting dates.

Early Rose and Spaulding Rose are listed as being in the same group, the Rose group, but are placed in two different sections of the group, Early Rose in Section 1 and Spaulding Rose in Section 2. A corresponding difference is found in Table VI for these two varieties, Early Rose having significantly lower amounts of reducing sugar.

Another comparison is between Irish Cobbler and Eureka. If our Eureka is the same as the Early Eureka in the Thompson table, these two are in the same group. The data in Table VI, however, indicate a difference between them. When the data are summed separately for 5°, 7°, and 8.2°, the differences are respectively 16.0, 11.7, and 9.5, all exceeding the required difference 7.2, and when the data are summed for all three temperatures and both starting dates the difference is 37.2, a value much higher than the required difference 8.8. Possibly upon further study of these two varieties a division into sections could be made in the Cobbler group, as was done with the Rose group.

Other pairs within groups are Russet Burbank and Pride of Multnomah; Early Six Weeks and Early Ohio; Bliss Triumph and Warba. The first two appear in Thompson's list in two different sections in the group, and our analyses are in accord with that separation. As for the other two pairs, our data are too incomplete for a satisfactory decision. It may be that Early Six Weeks and Early Ohio are significantly different. Any difference between Bliss Triumph and Warba, by the analyses available, depends upon the fact that the Warba value for December 24 at 5° (i.e. 55.4) was greater than the corresponding value for October 25 (i.e. 50.1) and since in the entire list only this variety and Axtell's Bugless showed greater reducing sugar values for the December 24 starting date than for that of October 25, it seems that further tests would be needed for a decision as to any dissimilarities between Bliss Triumph and Warba with regard to reducing sugar values under different storage conditions.

Varietal Response in Different Years

In the 1939-40 series of experiments (2), 11 varieties were included and the reducing sugar values after 62 days at 5° with a starting date of December 1 are given on page 300 of the article. These same 11 varieties were included in the 1940-41 experiment, and it seemed of interest to attempt to compare the values for the same varieties in the two tests in different years.

There was no series starting on December 1 in the 1940-41 experiments, but there were series starting October 25 and December 24. By getting the sums of these 11 varieties at each removal interval for each starting date from the values in Table III and plotting them, it was found that an interpolation for December 1 could be made and that the removal dates of 59 days for the October 25 series and 55 days for the December 24 series occurred

at places on the curves at which the curves had flattened out to nearly straight lines. The 1940-41 values for each of the 11 varieties at a December 1 starting date and a 62-day removal from 5° were then approximated by straight line interpolation. The reducing sugar values for the 11 varieties for the experiments in the different years are shown in Table VII. Since the values are paired, the differences can be tested by Student's method

TABLE VII
COMPARISON OF RESULTS WITH THE SAME VARIETIES IN
EXPERIMENTS IN TWO DIFFERENT YEARS

Variety	Reducing sugar values, mg. per cc. of juice	
	1939-40 experiment, 62 days at 5° starting on Dec. 1	1940-41 experiment. By interpolation to get the value for 62 days at 5° starting on Dec. 1
Russet Rural	4.1	5.8
Chippewa	6.6	4.5
Carman No. 3	7.3	6.8
Irish Cobbler	8.3	9.2
Early Ohio	9.6	12.0
Katahdin	9.8	10.6
Russet Burbank	11.1	11.6
Blue Victor	13.0	7.2*
Green Mountain	13.5	12.7
Spaulding Rose	13.7	14.2
Bliss Triumph	20.4	12.7

* The corresponding value for Blue Mercer under these conditions at this date was 13.3.

(3, p. 20). The mean difference is 1.01 and the standard deviation of the mean difference between the 11 entries is 1.00, which indicates no significant difference in the series as a whole.

That the behavior of these 11 varieties has been similar in the two different years is also shown by use of the method of "Correlation from Ranks," using a formula originally developed by Pearson, and the use of which has recently been illustrated by Crist (1). If the rank of the 11 varieties in 1939 was 1, 2, 3, etc. as shown in column 2 in Table VII, the rank of these same varieties taken in the same order in 1940 was: 2, 1, 3, 5, 8, 6, 7, 4, 9, 11, 9. The repetition of 9 and omission of 10 is in accordance with Crist's scheme, and is due to the tie in column 3 between Green Mountain and Bliss Triumph. The deviations between ranks of the varieties are, therefore, (1-2), (2-1), (3-3), etc. and the sum of the squares of these deviations is 33. The rank correlation is computed as follows:

$$\text{Corr.} = 1 - \frac{6\Sigma(d)^2}{n(n^2-1)} = 1 - \frac{(6)(33)}{11(11^2-1)} = 1 - \frac{198}{1320} = 0.850$$

The probable error of this rank correlation coefficient was computed by the

formula given in the same paper by Crist, and was found to be ± 0.079 . Consequently, the correlation in the rank of the varieties in the two years is highly significant.

The varieties that do not show values agreeing satisfactorily in the two years are Blue Victor and Bliss Triumph. Now in 1940-41, data were obtained not only for Blue Victor but also for the variety Blue Mercer, and the value for Blue Mercer, after interpolation for a December 1 starting date and a 62-day period at 5°, was 13.3, which agrees well with the 1939-40 value given for Blue Victor. The tubers of these two varieties are very similar and unusual for potato tubers, being coarsely blotched with blue and yellow. It is possible, therefore, that the 1939-40 lot was mislabeled Blue Victor, and was in fact Blue Mercer. A planting of these two varieties has been made for the 1941-42 tests.

Bliss Triumph, although showing higher values in 1939-40 than in 1940-41, in another respect was similar in both years, being relatively high in each. It is to be included in future experiments to determine whether there is a difference in the response of tubers of this variety in crops of different years.

Potato Chips

A sample of potato chips was prepared for each of the lots listed in Tables III and VIII. The results of the present tests were in agreement with those of the previous year (2) in showing that the reducing sugar value could be taken as a satisfactory indication of potato chip color. This year, again, the value 5.0 mg. of reducing sugar per cc. of juice was found to be a good dividing line; lots showing values greater than this gave chips too brown in color, grading upward from this, with increasing shades of darkness, until at values of 10.0 or more the chips became unsightly. Even at 5.0 the color was at the higher limit, and in the range 3.0 to 5.0 the color would not be good according to the standard set by many, if not most, of the potato chip manufacturers and distributors. Lots showing reducing sugar values of 3.0 or lower, however, gave chips very light brown or tan in color, ranging down to nearly white.

The relation of chip color to the various factors studied in this test, such as varieties, time of starting storage, temperature, and the duration of the storage, can best be studied by merely examining the entries in Table III, and looking in the various columns and lines for reducing sugar entries below 5.0, and especially for those below 3.0.

Thus, at 5°, and for the October 25 starting date, seven of the varieties failed to furnish good chips after only 18 days of storage, and all of the varieties had passed the 5.0 limit by 32 days; the late starting date (Dec. 24), however, retarded the rate of reducing sugar increase to such an extent that only one variety had passed the limit by the 17th day of storage, and many varieties gave chips of fair color after 31 days at 5°.

Quite a different situation is encountered when the values in the columns headed 7° are examined. With storage starting on October 25, the chips for all varieties from the top of the table downward to Eureka (and Russet Burbank from the varieties below Eureka in the list) gave chips with either good or fair color for the entire sampling period of 131 days. By delaying the start of storage until December 24 (see columns 21-24 in Table III), a still further improvement was obtained so that at 7° such varieties as Eureka, Katahdin, and Spaulding Rose were brought to values below the limit.

When the analyses for the 8.2° storage are examined it is found that only Axtell's Bugless, Warba, Bliss Triumph, and possibly (or probably) Green Mountain developed reducing sugar beyond the upper limit, and so were unsuitable for production of chips of good color.

For each temperature and each starting date the length of the storage period is also a factor, and shorter periods may be satisfactory for a variety which during longer periods accumulates too much sugar. However, it is seen from Table III that, in general, an interval of about 40 to 60 days is sufficient to permit the variety to reach a sugar value which is approaching the equilibrium at that temperature, a fact which is also shown for the varieties in general in Table V (comparing the entries under the third removal date with entries for the second and fourth).

The discussion of the factors for potato chip color in the four preceding paragraphs has been mainly on the basis of a limit of 5.0 mg. per cc. of juice. If the problem is now re-examined on the basis of a 3.0 mg. limit, which would be a limit for a high standard as to color, it is seen that in the October 25 series many varieties failed to meet the requirement at 7° , and even some varieties of the Rural group which tend to maintain low amounts of reducing sugar, showed values above the 3.0 mark at the 68-day removal period. Early Rose also could be placed in this group at that temperature and that starting date.

In the December 24 series, however, the values at 7° are brought down to much lower levels, and reducing sugar concentrations below 3.0 were maintained during the entire experimental period of 108 days by all of the varieties in the list from Neverblight to Early Six Weeks, inclusive. Eureka, Green Mountain, and Katahdin are within the limit up to and including the 49-day removal date.

A still further reduction in the concentration of reducing sugar was obtained by storage at 8.2° but even at this temperature some of the varieties failed to maintain a sufficiently low reducing sugar value, and so furnished chips of poor color. Among them are Axtell's Bugless, Warba, Bliss Triumph, Delaware, Green Mountain, and Spaulding Rose, although the last-named may have been below the limit when the starting date was December 24.

Other factors to be considered in connection with the requirements for potato chips are: sprouting at the different temperatures and with different starting dates, shrinkage, ease of slicing, etc. There was no sprouting at 5° at any removal period, some sprouting at 7° at the last removal period, and at this stage there was considerable sprouting at 8.2° . But in no case in this report were the tubers sprouting beyond the limit for producing potato chips, and slices for frying were made without difficulty. At the last removal date at 8.2° , the tubers of some of the varieties, especially Chippewa, were soft, but they could not be regarded as unsuitable at that time. It is believed, however, that 8.2° represents the upper limit of storage temperature for many of these varieties. From the standpoint of commercial production of potato chips, an important consideration is the amount of dry weight shrinkage. Our data do not cover this phase of the problem.

No apparatus was available for a mechanical measurement of chip color and the color ratings were made merely on the basis of individual judgment. The limits as to color were those based on the examination of potato chip samples collected from retail stores, and upon suggestions made by a number of persons engaged in the commercial production of potato chips. Furthermore, at a meeting of an association of potato chip manufacturers, some samples of various lots obtained as a result of these tests were exhibited, and in the ensuing discussion it appeared that the limits we had set corresponded quite well with the opinions expressed by many of those present. Perhaps their standard was more restricted, since samples near our upper limit were regarded by them as distinctly too dark. Another difficulty in rating the color is due to variation in the color of chips from different tubers in the same lot. Occasionally there are a few chips in a set of 32 chips from 16 tubers that are considerably darker than the others. This is probably accounted for by the variation in reducing sugar among the tubers. Out of a sample of 100 tubers about 67 will have a reducing sugar value between the values mean ± 1.0 times the standard deviation (*S.D.*) of a single tuber. Of the other 33, one-half of the number or, let us say, 17 will have a value higher than mean plus $1.0 \times S.D.$ (and the others will be below the value mean minus $1.0 \times S.D.$). In a sample of 16 tubers, $0.16 \times 17 = 2.7$ tubers, say 2 or 3 tubers, will have values higher than mean plus $1.0 \times S.D.$ If the variance of the reducing sugar of a sample of 16 tubers is 0.80 mg., the *S.D.* is $\sqrt{0.80} = 0.9$ mg., and the *S.D.* of a single tuber is $\sqrt{16 \times 0.9} = 3.6$ mg. Thus, if the mean is 3.0 mg., 2 or 3 tubers in a sample of 16 tubers may show reducing sugar values higher than 6.6 mg. per cc. This would give a distinctly darker color to chips from such tubers as compared with chips from tubers with the average values of reducing sugar. But while we are showing how these chips from tubers with sugar values much higher than the average can be obtained in a given sample, we must not neglect the fact that about two-thirds of all of the tubers will

TABLE VIII
SUCROSE VALUES SHOWING RESPONSE OF TUBERS OF POTATO VARIETIES TO CONDITIONS OF STORAGE

Variety	At har-vest	Storage started Oct. 25, 1940												Storage started Dec. 24, 1940											
		5° C.				7° C.				8.2° C.				5° C.				7° C.				8.2° C.			
		At start				At start				At start				At start				At start				At start			
		Removal intervals Days				Removal intervals Days				Removal intervals Days				Removal intervals Days				Removal intervals Days				Removal intervals Days			
		18	32	59	93	18	33	68	131	19	38	78	144	17	31	55	94	11	22	49	108	10	23	53	120
Neverblight	3.9	9.6	8.3	6.6	6.5	—	—	—	—	3.5	4.2	2.9	1.9	7.8	8.9	10.3	8.1	4.5	6.0	5.5	4.2	—	—	—	—
Chippewa	2.4	1.7	13.0	10.3	8.7	9.9	—	—	—	3.9	2.9	2.5	3.3	16.6	17.5	20.6	25.1	13.9	14.8	10.3	9.8	11.2	12.9	7.5	12.5
Russet Rural	0.9	2.1	8.8	5.9	5.5	5.5	4.3	3.2	2.3	2.6	2.8	2.4	0.0	6.8	6.2	6.1	7.7	2.4	4.2	2.3	3.3	2.5	1.9	0.4	0.9
Heavyweight	1.5	1.8	9.2	5.7	4.9	4.1	3.9	3.1	3.5	3.1	2.9	1.7	0.6	5.2	5.6	6.6	5.1	2.9	4.0	2.5	1.9	2.5	2.1	0.6	0.7
Carman No. 3	2.1	1.4	9.8	6.2	5.4	5.3	3.7	4.1	3.7	2.2	2.7	2.5	1.2	5.3	5.4	7.4	7.6	2.9	3.9	3.0	2.5	2.4	1.9	1.6	3.0
Blue Victor	1.7	0.8	7.0	7.2	3.7	4.5	3.2	4.9	6.5	2.5	—	—	—	1.4	9.7	8.7	10.4	11.0	5.5	7.3	5.8	5.6	—	—	—
White Rural	1.2	0.8	8.8	5.1	3.1	4.0	2.9	2.9	3.2	1.9	—	—	—	1.6	4.8	6.4	5.4	7.3	—	—	—	—	—	—	—
Rural New Yorker	2.5	1.0	7.6	4.4	4.4	4.9	2.7	3.5	3.3	1.4	2.3	2.7	2.3	0.2	4.7	5.4	4.9	4.4	2.1	4.3	2.4	1.2	1.6	1.7	0.9
Number Nine	4.4	1.7	8.5	5.5	5.1	4.4	5.3	3.6	6.6	2.1	2.2	2.9	2.2	0.2	4.7	5.1	6.7	5.9	2.7	3.5	3.2	2.3	2.1	1.6	1.2
Sir Walter Raleigh	2.2	1.5	8.7	5.6	4.6	4.0	4.2	2.9	2.9	1.8	—	—	—	0.4	5.0	4.3	4.4	5.3	—	—	—	—	1.7	2.7	1.6
Irish Cobbler	2.5	0.7	11.4	10.5	10.7	10.8	6.2	6.1	10.1	3.5	3.2	4.4	3.8	2.4	14.0	18.1	18.7	23.0	13.2	15.6	13.9	12.2	8.6	7.7	6.7
Early Rose	3.7	—	14.0	15.0	11.7	11.8	8.0	8.3	5.1	4.0	6.3	5.4	3.7	1.6	13.8	14.6	13.3	12.9	8.6	10.5	8.6	6.5	5.7	5.8	6.8
Early Six Weeks	2.4	2.5	14.3	8.7	7.7	8.6	—	—	—	—	5.7	5.0	4.2	2.7	2.5	8.0	8.0	8.2	9.7	5.5	6.7	6.4	4.8	—	—
Katahdin	2.1	1.1	9.1	7.5	5.0	8.3	6.2	5.2	4.8	3.4	3.7	3.5	3.0	1.4	6.7	7.1	8.9	8.2	7.2	5.8	6.2	5.4	—	—	—
Early Ohio	3.0	2.5	13.9	10.8	8.4	9.0	7.8	6.4	6.8	3.8	—	—	—	2.4	15.6	11.6	9.6	11.2	—	—	—	—	3.9	4.0	3.0
Eureka	2.6	0.2	12.7	8.4	6.8	7.5	5.7	8.9	16.9	4.0	6.2	5.8	4.2	0.4	15.6	17.1	18.6	23.7	15.4	15.3	14.5	15.4	8.5	9.0	4.5
Russet Burbank	1.5	1.2	13.5	11.3	6.5	6.3	9.5	9.4	23.4	2.7	7.8	7.5	5.1	0.1	8.3	8.1	6.4	5.0	—	—	—	—	4.4	3.9	3.0
Green Mountain	3.7	3.8	24.3	20.2	14.3	15.1	13.8	12.1	18.0	4.1	8.2	5.9	4.9	3.7	17.6	19.4	21.2	19.5	12.3	15.3	14.2	8.8	9.5	8.7	8.6
Delaware	3.8	4.5	20.2	16.1	15.7	16.2	10.7	9.9	12.4	5.2	5.7	5.0	3.1	6.3	16.7	18.8	24.5	30.8	10.1	12.9	8.6	9.5	7.4	7.7	6.1
Bliss Triumph	6.1	1.8	15.7	10.2	9.0	9.4	—	—	—	—	7.4	4.8	2.5	2.0	1.6	7.1	6.7	6.1	5.2	—	—	—	9.0	9.4	9.9
Pride of Multnomah	3.1	4.0	13.2	8.3	6.0	7.0	—	—	—	—	6.0	5.0	4.3	4.7	1.9	10.7	10.4	8.7	11.1	8.0	10.1	9.7	4.2	5.0	6.2
Spaulding Rose	2.5	2.6	10.4	5.3	5.5	7.6	8.2	4.7	5.4	2.9	3.8	2.9	1.6	1.6	6.1	7.2	8.8	10.5	—	—	—	—	5.0	6.2	4.9
Blue Mercer	1.9	1.7	6.2	4.4	6.2	6.6	—	—	—	—	—	—	—	3.4	11.9	10.7	10.2	15.1	—	—	—	—	4.1	3.8	2.0
Warba	3.2	3.0	14.5	9.3	3.9	8.6	9.8	7.2	8.6	4.3	—	—	—	3.7	11.7	12.8	15.3	18.3	—	—	—	—	7.0	7.3	6.2
Axtell's Bugless	3.9	2.0	17.3	9.9	11.6	13.2	—	—	—	—	6.3	4.2	4.0	4.4	—	—	—	—	—	—	—	—	8.7	6.5	5.2

Milligrams of sucrose per cc. of juice

have sugar values within the limits, mean $\pm 1.0 \times S.D.$ and these two-thirds will have a preponderance of effect in establishing the tint of the combined chips from all of the tubers from a given lot brought together into a single package.

DATA FOR SUCROSE

Although, as stated in previous reports (2, 6) and confirmed by the present tests, the sucrose content is not a factor in the color of potato chips, analyses for sucrose were made, and for each sample reported upon in Table III there is a sucrose value to correspond to it in Table VIII. The varieties in the sucrose table are listed in the same order as that given for the reducing sugar table, and it is seen, at once, that the order of varieties for increasing amounts of reducing sugar is not at all the order of these same varieties for sucrose. The differences among the varieties with respect to sucrose will be referred to in a later paragraph.

Effect of Temperature

A summary of the temperature effect by adding the sucrose values for various varieties with strictly comparable analytical values is shown in Table IX. The values in columns 3, 4, and 5 show that increasing the tem-

TABLE IX
SUCROSE: SUMS AT DIFFERENT STORAGE TEMPERATURES

No. of varieties simultaneously represented	Starting dates	Sums of sucrose values for all four removal dates at each temperature			No. of items summed	Diff. req. for sign.
		5°	7°	8.2°		
13	Oct. 25	496.0	303.2	185.9	52	29.1
	Dec. 24	617.4	404.3	272.5	52	29.1
		1113.4	707.5	458.4	104	41.1
14	Oct. 25	518.4	320.3	—	56	30.2
	Dec. 24	657.2	428.5	—	56	30.2
		1175.6	748.8	—	112	42.6
19	Oct. 25	678.3	459.8	—	76	35.1
16	Dec. 24	726.2	472.1	—	64	32.2
19	Oct. 25	724.0	—	283.5	76	35.1
22	Dec. 24	984.0	—	447.4	88	37.8

perature by 2 degrees, i.e. from 5° to 7°, decreased the sucrose values so that the 7° values are about 60 to 70 per cent of those at 5°; and that increasing the temperature to 8.2° brought about a still further reduction to

values at 8.2° about 40 per cent of those at 5°. The differences between 7° and 8.2° are also definite, the values at 8.2° being about 65 per cent of those at 7°. These percentage changes in sucrose over this temperature range are not as great as the percentage changes in reducing sugar in the same temperature range.

Changes at Intervals during Storage at 5°

Rapidity of change. Upon being transferred from room temperature to 5° the response to increase in sucrose was more rapid than that of increase in reducing sugar. This is shown readily merely by inspection of the analyses in columns 3 and 4, 16 and 17, in Tables III and VIII. Even by the first removal date, 17 to 18 days after start of storage, the sucrose values had increased markedly. In reality the increase was more rapid than is indicated in Table VIII, as is shown by analyses which were obtained from 16 varieties in the October 25 series, and from 9 in the December 24 series on samples that were taken on the 10th day after start of storage. These are not entered in Table VIII but are included in the measurements that formed the basis for Figure 1. The results at this early sampling period show that the sucrose values on the 10th day after starting storage at 5° were about 75 per cent of the values on the 17th to 18th day. On the other hand, there were no increases in the reducing sugar values during the 10-day period at either starting period. This does not mean that the reducing sugar increase is always slow in starting. It is slow if there is a delay after harvest before putting the tubers into cold storage, but if the tubers are cold-stored soon after harvest a rapid increase in reducing sugar results (see Table XIII).

Attainment of maximum. After the rapid start in the increase in sucrose in storage at 5°, the subsequent progressive change depended upon the time after harvest at which the cold storage was started. This is shown by Table X. Comparing first the 5° values for October 25 and December 24, it is seen that the samples from the October 25 series, lines 1, 9, 15, reached a maximum sucrose value at the first removal interval, i.e. after 17 to 18 days of storage, and then the values receded. The samples from the December 24 series, lines 5, 12, 16, on the other hand, showed gradually increasing values and did not reach the highest value in the series until the fourth (last) removal date, 93 to 94 days after the start of storage. This total effect, however, as is shown in later paragraphs, is caused by the behavior of only certain of the varieties, and is not a characteristic of the entire list of varieties.

Changes during Storage at 7°

The effect upon sucrose values by storage at 7° was much less distinct than at 5°. These values are shown in lines 2 and 10, Table X, for the

October 25 series, and although there is a distinctly low value at the fourth removal interval, 131 days after the start, the time at which the maximum was reached is uncertain.

The successive values for the December 24 series at 7° are shown in lines 6 and 13, and on comparing the differences between intervals with the required difference in the right-hand column it is found that the sucrose value at the second interval is significantly higher than those at either the third or fourth interval. This indicates a maximum value at that stage, even though the curve is rather flat over the first three intervals.

Changes during Storage at 8.2°

The sucrose values for various removal intervals during storage at 8.2° are shown for the October 25 series in Table X in lines 3 and 17. In both comparisons the differences between the values for the first and fourth removal intervals exceed the required difference value in the right-hand

TABLE X
SUCROSE: SUMS AT DIFFERENT STAGES OF REMOVAL FROM STORAGE

No. of varieties simultaneously represented	Starting date	Temp.	Sums of sucrose values for all of the varieties				No. of items summed	Diff. req. for sign.
			Removal stage					
			1st	2nd	3rd	4th		
13	Oct. 25	5°	159.9	121.0	103.7	111.4	13	14.5
		7°	86.3	79.4	97.6	39.9	13	14.5
		8.2°	57.7	54.4	42.6	31.2	13	14.5
			303.9	254.8	243.9	182.5	39	25.2
	Dec. 24	5°	139.4	149.9	156.6	171.5	13	14.5
		7°	101.7	120.2	99.4	83.0	13	14.5
		8.2°	70.9	71.5	58.5	71.6	13	14.5
			312.0	341.6	314.5	326.1	39	25.2
19	Oct. 25	5°	226.3	170.3	133.9	147.8	19	17.6
		7°	123.7	113.1	149.0	56.9	19	17.6
			350.0	283.4	282.9	204.7	38	24.9
16	Dec. 24	5°	164.9	175.5	185.5	200.3	16	16.1
		7°	117.2	140.2	117.1	97.6	16	16.1
			228.1	315.7	302.6	297.9	32	22.8
25	Oct. 25	5°	302.6	220.1	184.5	199.1	25	20.1
	Dec. 24	5°	245.5	262.1	276.2	309.0	25	20.1
19	Oct. 25	8.2°	90.1	80.5	60.9	52.0	19	17.6
22	Dec. 24	8.2°	118.5	115.2	94.8	118.9	22	18.9

column, and since a rather regular series of values is obtained, it seems likely that a temporary maximum for the sucrose values is shown. The analyses for the 8.2° storage in the December 24 series are shown in lines 7 and 18. No differences between intervals equal to the value in the right-hand column are shown. The sucrose value in the December 24 series rose within 10 days to an equilibrium value at 8.2° and then remained essentially stationary at that value for more than 100 days. It is true that there is a significantly low value at the third removal date, but one would hardly accept it as a characteristic behavior without further tests.

Graphical Representation of the Temperature and Starting-Date Effects

The curves in the lower half of Figure 1 show the changes in the sucrose values under the different storage conditions. These are based on the combined values for all of the varieties.

Again an estimate of the dependability of the plotted points may be made by referring to the proper paragraph under the heading "Experimental errors" and noting that the standard deviation for sucrose was 18.39 (expressed as percentages of the 5° average, which are the units for the plotting scale in Fig. 1). Since there were 16 to 25 entries for all points for storage durations of 17 days or more (but not for the 10-day points), the standard error for the averages of 16 to 25 items would be from $18.39 \div \sqrt{25}$ to $18.39 \div \sqrt{16}$, i.e. from about 3.7 to 4.6 units in the plotting scale at the left edge of Figure 1.

The general situation for sucrose is the same as that described in the preceding paragraphs. There is a difference in the sucrose curves for 5° between the October 25 and December 24 series, the former showing a temporary maximum and then a rapid fall, while the latter shows a continuous rise. This rise in the curve for the December 24 series is partly deceptive, however, since the effect is dependent on the response of only eight of the varieties (see Table XI, columns 2 and 3). These eight (named in a paragraph that follows) increased markedly during the 5° storage following the December 24 starting date, while all of the other varieties but two (Russet Burbank and Pride of Multnomah) reached sucrose maxima nearly exactly the same as that reached at 5° for those in the October 25 series, and then stayed there.

It will be noted that the 7° curve for sucrose in the October 25 series has a portion drawn with a dotted line. This was inserted because of the preponderant effect of the value 23.4 mg. of sucrose for the variety Russet Burbank in Table VIII in the column under 7° and 68 days. The expected value at this point is 5.3 mg. and the difference between the value as entered and the expected value is 18.1 mg. which is 9 times 2.02 which is the standard deviation of the difference between two measurements ($\sqrt{2.03} \times \sqrt{2} = 2.02$). If this entry is omitted the graph would follow the

dotted portion of the graph over that interval. However, it is only in connection with this graph that any attempt has been made to make allowance for this unusual value. In all other parts of the paper, the entry 23.4 mg. was included in the computations, and was allowed to contribute its share toward the sums and toward the items of variation which entered into the analyses, and which were used to compute the values of the experimental errors.

TABLE XI
SUCROSE: SUMS FOR DIFFERENT VARIETIES

Variety ↓	Sums of sucrose values for all four removal dates					
Temperatures →	5°		7°		8.2°	
Starting dates →	Oct. 25	Dec. 24	Oct. 25	Dec. 24	Oct. 25	Dec. 24
Neverblight	31.0	35.1	—	45.1	12.5	—
Chippewa	42.8	79.8	23.0	48.8	12.6	44.1
Russet Rural	25.7	26.8	11.6	12.2	7.8	5.7
Heavyweight	23.9	22.5	11.8	11.3	8.3	5.9
Carman No. 3	26.7	25.7	13.6	12.3	8.6	8.9
Blue Victor	22.4	39.8	17.1	24.2	—	—
White Rural	21.0	23.9	10.9	—	—	5.4
Rural New Yorker	21.3	19.4	10.9	10.0	7.4	5.3
Number Nine	23.5	22.4	17.6	11.7	7.3	6.1
Sir Walter Raleigh	22.9	19.0	11.8	—	—	6.4
Irish Cobbler	43.4	74.4	25.9	54.9	14.5	35.2
Early Rose	52.5	54.6	25.4	34.2	18.6	24.0
Early Six Weeks	39.3	33.9	—	23.4	17.6	—
Katahdin	29.9	30.9	19.6	24.6	12.9	14.6
Early Ohio	42.1	41.4	24.8	—	—	18.6
Eureka	35.4	75.0	35.5	60.6	20.1	36.5
Russet Burbank	37.6	27.8	45.0	—	—	13.9
Green Mountain	73.9	77.7	48.9	50.6	24.4	38.1
Delaware	68.2	67.3	38.2	41.1	23.4	27.2
Bliss Triumph	45.2	90.8	—	—	22.0	45.8
Pride of Multnomah	37.1	25.1	—	—	16.7	15.6
Spaulding Rose	28.8	40.9	21.2	32.0	20.0	20.9
Blue Mercer	23.4	32.6	—	—	9.9	12.5
Warba	36.3	47.9	29.9	—	—	27.9
Axtell's Bugless	52.0	58.1	—	—	18.9	27.8

Differences required:

Between any two items (each representing the total for 4 analyses in Table III)—8.1; between the sums of two comparable items (these values representing the total of 8 analyses in each comparable series)—11.4; between the sums of three comparable items (12 analyses in each series)—14.0.

Characteristics of the Various Varieties

Table XI is a summary of the sucrose analyses in Table VIII for various varieties, and was prepared by adding the values for each of the four removal periods for each temperature and each starting date.

It will be noted that although the varieties of the Rural group and Blue Victor are low in both reducing sugar and sucrose, and although such varieties as Green Mountain and Bliss Triumph are high in both sugars,

there are some contrasts with regard to the content of these two sugars in the case of certain other varieties. For example, Chippewa, although low in reducing sugar, is very high in sucrose, and Blue Mercer is high in reducing sugar and low in sucrose.

There was a difference also in the response of varieties to the development of sucrose as a result of different starting dates. This is shown in Table XI. Comparing the values for 5° for each variety for the two starting dates, it is seen that most of the varieties show about the same totals for both October 25 and December 24. The agreement is in many cases quite surprising in view of the different effects in other respects caused by the difference in the starting dates. This agreement, therefore, emphasizes the exceptions found in columns 2 and 3. Note that large increases were found for the December 24 over the October 25 series for the following varieties: Chippewa, Blue Victor, Irish Cobbler, Eureka, Bliss Triumph, Spaulding Rose, and Warba. The difference in the values for Blue Mercer at the two dates also is slightly greater than the required difference. Russet Burbank and Pride of Multnomah, on the other hand, show a decrease for the December 24 series, rather than an increase.

This behavior of the varieties in the sucrose change at 5° at different starting dates is also interesting in relation to the group classification. On this basis Irish Cobbler and Eureka seem to belong to the same group, although the reducing sugar values for these two varieties showed significant differences. Bliss Triumph and Warba show the same type of response and are in the same group. Russet Burbank and Pride of Multnomah are the only ones to show a downward trend at the late starting date and they belong in the same group. Spaulding Rose and Early Rose respond differently, and also belong to different sections even though in the same group. And, finally, we get a definite difference in the behavior of Blue Victor and the Rural group. In the discussions of variety behavior up to this point, Blue Victor has appeared to be similar to the varieties in the Rural group as a whole, but in the sucrose response to the date for starting cold storage, Blue Victor is differentiated by its capacity to develop a higher amount of sucrose with the late starting date.

GROUPING OF VARIETIES WITH RESPECT TO SUGAR CONTENTS OF JUICE

An attempt was made to classify the varieties on the basis of the sugar content with respect to the two forms of sugar, with the result shown in Table XII. The limits chosen are admittedly empirical, and, since the data for the table are restricted to those obtained in the experiments here reported upon, it is not intended that undue emphasis be placed upon the grouping.

However, one advantage of the present experiment is that the conditions to which tubers of the different varieties were subjected were held

strictly comparable from the time of harvest, which is an important factor for such a comparison as that shown in Table XII.

TABLE XII
APPROXIMATE CLASSIFICATION OF POTATO VARIETIES ON THE BASIS OF SUGAR
CONTENT OF JUICE AFTER STORAGE OF TUBERS AT 5° C.

		Sucrose		
		High, over 110	Medium, 65-110	Low, below 65
Reducing sugar	High, over 60	Eureka Green Mountain Delaware Bliss Triumph Axtell's Bugless	Early Ohio Russet Burbank Spaulding Rose Warba	Pride of Multnomah Blue Mercer
	Medium, 50-60	Irish Cobbler	Early Rose Early Six Weeks	Katahdin
	Low, below 50	Chippewa	Neverblight (see square at right)	Rural group (except possibly Neverblight) Blue Victor

Note: The values 50, 60, 65, and 110 refer to the sums of values in columns 2 and 3, Tables VI and XI, for 5° storage including the October 25 and December 24 starting periods.

Further work would be necessary to show whether this grouping is valid in general, or whether crops from different years or from different localities will show other types of grouping.

EFFECT OF A TEMPERATURE DIFFERENCE OF ONLY 1° C.

The changes brought about in the sugar content of tubers stored at temperatures in a series in which the temperature differences were only 1° C. are shown in Table XIII. The tubers of the varieties Spaulding Rose, Blue Victor, and Carman No. 3 were from a late crop planted in July and harvested October 7 to 9. The start of the test was five to seven days after harvest for these three varieties. The tubers for Green Mountain and Irish Cobbler were small tubers from the main harvest in August.

The analysis of variance for the results in Table XIII is given at the bottom of the table. It is seen that the error variances are 1.60 and 1.11, respectively, for reducing sugar and sucrose. The questions to be decided are whether there are significant differences between the values for 5°, 6°, and 7°, and whether the duration of the storage was an important factor.

Considering first the reducing sugar totals for 5°, 6°, and 7°, Table XIII, line 6, and computing the difference necessary for significance between two sums of five items each from the expression $\sqrt{1.60 \times 5 \times 2 \times 2.120}$ (the factor 2.120 being that corresponding to the 16 degrees of freedom in this test) (3, p. 248), it is found that the necessary difference between totals is 8.5 mg. The difference between the totals for 5° and 6° is greater than

this and therefore is significant for each of the three duration periods, but the difference between 6° and 7° is sufficiently large only at the 40-day period.

TABLE XIII
EFFECT OF A TEMPERATURE DIFFERENCE OF ONLY 1° C.

Type of sugar	Variety	Milligrams of sugar per cc. of juice									Totals	
		At start	Length of storage period									
			10 days			20 days			40 days			
			5°	6°	7°	5°	6°	7°	5°	6°		7°
Reducing	Spaulding Rose	2.1	13.4	9.2	9.1	20.6	12.6	10.5	21.0	12.0	11.9	120.3
	Blue Victor	0.0	6.6	3.8	5.5	12.1	6.4	8.7	11.6	7.8	6.6	69.1
	Carman No. 3	0.0	5.7	4.3	3.8	8.8	5.3	5.0	8.4	9.5	4.8	55.6
	Green Mountain	0.0	4.4	2.9	4.1	11.4	9.0	10.0	21.4	16.6	12.5	92.3
	Irish Cobbler	0.0	1.8	1.6	0.0	5.7	5.7	4.4	11.2	11.3	8.4	50.1
	Totals	2.1	31.9	21.8	22.5	58.6	39.0	38.6	73.6	57.2	44.2	387.4
Sucrose	Spaulding Rose	4.3	11.8	10.4	8.1	10.5	8.8	5.8	8.5	6.0	6.3	76.2
	Blue Victor	1.2	8.7	5.9	6.0	7.0	5.2	4.5	7.2	5.4	3.7	53.6
	Carman No. 3	2.1	6.8	6.8	4.3	5.5	5.9	4.2	5.4	5.1	3.5	47.5
	Green Mountain	5.6	13.5	13.4	10.0	17.2	13.0	11.6	7.7	8.1	8.3	102.8
	Irish Cobbler	1.1	8.9	6.3	5.9	9.3	5.9	5.0	9.8	4.3	4.1	59.5
	Totals	14.3	49.7	42.8	34.3	49.5	38.8	31.1	38.6	28.9	25.9	339.6

Analysis of Variance

Source of variation	Degrees of freedom	Reducing sugar		Sucrose	
		Sums of squares	Variance	Sums of squares	Variance
Total	44	1061.12	—	404.35	—
Between varieties	4	372.43	93.11	219.79	54.95
“ durations	2	330.36	165.18	41.03	20.52
“ temperatures	2	127.65	63.82	72.80	36.40
Durations×temperatures	4	24.20	6.05	2.78	0.695
Varieties×temperatures	8	55.42	6.92	12.63	1.58
“ ×durations	8	125.52	15.69	37.53	4.69
Error	16	25.54	1.60	17.79	1.11

It will be noted that a rapid reducing sugar gain was made in the first ten days of storage by the three varieties with recently-harvested tubers, Spaulding Rose, Blue Victor, and Carman No. 3, while, with the other two varieties, the tubers of which had been harvested about two months previously, the gain was much slower. Allowance must be made, of course, for the final sugar value for each variety in making this comparison. This is in confirmation of the results in the main experiments here reported upon, in which it was shown that storage of tubers at an early date after harvest induces a more rapid gain in reducing sugar than that which occurs if the start of storage is delayed.

Considering next the sucrose totals, Table XIII, line 12, the error variance is 1.11, and the required difference between the sums at the bottom of each column under the headings 5°, 6°, and 7°, is 7.1. The difference between 5° and 6° at the ten-day interval is 6.9, just below the level of significance. But all of the other differences between pairs of temperatures differing by only 1° C. are significant, except the last one, that between 6° and 7° at the 40-day period.

Another interesting item is found in the comparative reducing sugar values of the first three varieties in the list for Table XIII. The values are high for Spaulding Rose and low for Blue Victor and Carman No. 3, a confirmation from tubers of a different harvest of the varietal characteristics shown in Table III.

The results in Table XIII show that a temperature difference of only 1° C. is sufficient to induce differences in both reducing sugar and sucrose, although it may take more than 10 or 20 days for the full effect to become apparent, especially if the temperatures compared are 6° and 7°. Probably with a larger number of varieties or tests within a variety at an early date after harvest, differences over shorter storage intervals and between temperatures differing by less than 1° C. could be established with certainty.

EFFECT OF FERTILIZER FORMULAS ON THE RESPONSE OF TUBERS TO STORAGE CONDITIONS

Tubers for a preliminary test of the fertilizer effect upon reducing sugar and therefore upon potato chip color were obtained through the kind cooperation of Prof. E. V. Hardenburg of Cornell University. The tubers were of the variety White Rural and were from a fertilizer test on silt loam soil at Ithaca, New York. The four fertilizer formulas used for the samples received were: 0-10-5; 5-10-5; 5-0-5; and 5-10-0, the fertilizer being applied at the rate of 1000 lb. per acre.

Upon receipt in Yonkers, New York, the tubers were distributed evenly into lots which were placed in storage at 5° and 7°, and at intervals of 10, 20, and 40 days samples were removed for the sugar analyses.

The results are shown in Table XIV. The analysis of variance shows an error variance value of 0.51 mg. of reducing sugar. The required difference between the totals of two series each with three items for an error variance of 0.51 with 6 degrees of freedom is $\sqrt{0.51 \times 3 \times 2 \times 2.447} = 4.3$. Fertilizer 5-10-0 tends to show higher reducing sugar values, significantly higher than 5-10-5 in the 5° series and higher than any of the others in the 7° series. The fertilizer effect was small, influencing the reducing sugar value by only a little more than 1 mg. per cc. of juice, and this amount, even if the result was consistently obtained, would be important for potato chip color only at a critical range in the sugar content. Besides, it would require more evidence than this to show that a deficiency in potash fertilizer induces a high reducing sugar content in the tubers.

The required difference for totals of sucrose content of potatoes grown with various fertilizer formulas is 2.6, which difference is not reached in any case (see column totals for sucrose in Table XIV).

TABLE XIV
EFFECT OF FERTILIZER FORMULAS ON THE RESPONSE OF TUBERS
(VARIETY WHITE RURAL) TO COLD STORAGE

Type of sugar	Days of storage	Milligrams of sugar per cc. of juice								Totals
		Stored at 5° C.				Stored at 7° C.				
		Fertilizer formulas				Fertilizer formulas				
		0-10-5	5-10-5	5-0-5	5-10-0	0-10-5	5-10-5	5-0-5	5-10-0	
Reducing	10	5.5	4.1	5.6	7.2	1.6	2.0	1.4	2.8	30.2
	20	7.0	6.2	6.5	8.5	1.9	2.2	1.5	3.0	36.8
	40	7.3	7.1	7.2	7.2	2.7	2.1	1.7	4.3	39.6
	Totals	19.8	17.4	19.3	22.9	6.2	6.3	4.6	10.1	106.6
Sucrose	10	3.2	3.3	3.9	3.4	1.7	2.2	2.0	2.5	22.2
	20	3.3	3.8	3.5	4.3	2.0	2.0	1.8	1.9	22.6
	40	3.8	2.8	3.4	3.4	1.7	2.6	1.8	2.1	21.6
	Totals	10.3	9.9	10.8	11.1	5.4	6.8	5.6	6.5	66.4

Analysis of Variance

Source of variation	Degrees of freedom	Reducing sugar		Sucrose	
		Sums of squares	Variance	Sums of squares	Variance
Total	23	135.24	—	15.79	—
Between durations	2	5.83	2.92	0.06	0.03
“ temperatures	1	113.54	113.54	13.20	13.20
“ fertilizers	3	9.50	3.17	0.31	0.10
Temperature × fertilizer	3	1.15	0.38	0.43	0.14
Duration × temperature	2	1.62	0.81	0.30	0.15
“ × fertilizer	6	0.56	0.09	0.34	0.06
Error	6	3.04	0.51	1.15	0.19

EFFECT OF LOCALITY OF GROWTH OF TUBERS UPON THE RESPONSE OF TUBERS TO STORAGE CONDITIONS

Again through the cooperation of Prof. E. V. Hardenburg of Cornell University, tubers of four different varieties each from two different localities in New York State were made available for comparison as to the effect of locality upon the response of tubers to cold storage.

There were three removal dates, at intervals of 9, 21, and 42 days after start of storage. The reducing sugar results are given in Table XV, line 5. The analysis of variance shows an error variance of 0.98 and since there are four items in the sum for each removal interval the required difference for the sums (using the proper factor for 6 degrees of freedom) is 6.9. The dif-

ferences between the Ithaca and the Steuben totals at each removal date are in each case smaller than this value. However, the totals at each removal date are always larger for Steuben than for Ithaca. If the sum of all items for Ithaca and Steuben are obtained, and if the required difference

TABLE XV
EFFECT OF LOCALITY IN WHICH TUBERS WERE GROWN

Type of sugar	Variety	Milligrams of sugar per cc. of juice						Totals
		Source of potatoes						
		Ithaca upland			Steuben Co. upland			
		Days stored at 5° C.			Days stored at 5° C.			
		9	21	42	9	21	42	
Reducing	Irish Cobler	0.0	1.5	7.6	0.0	1.5	7.4	18.0
	Heavyweight	0.1	2.5	9.4	0.0	3.9	7.6	23.5
	Green Mountain	1.1	6.5	16.3	2.7	11.5	21.9	60.0
	Chippewa	0.3	1.2	5.8	0.0	1.1	6.6	15.0
	Totals	1.5	11.7	39.1	2.7	18.0	43.5	116.5
Sucrose	Irish Cobbler	14.0	26.4	29.7	12.1	20.4	22.2	124.8
	Heavyweight	6.6	11.1	9.1	5.7	11.7	7.8	52.0
	Green Mountain	11.5	22.2	20.1	11.4	17.1	10.8	93.1
	Chippewa	17.0	28.0	28.4	10.4	22.9	18.2	124.9
	Totals	49.1	87.7	87.3	39.6	72.1	59.0	394.8

Analysis of Variance

Source of variation	Degrees of freedom	Reducing sugar		Sucrose	
		Sums of squares	Variance	Sums of squares	Variance
Total	23	727.30	—	1227.27	—
Between varieties	3	217.99	72.66	596.66	198.88
“ sources	1	5.86	5.86	118.83	118.83
“ duration intervals	2	399.76	199.88	356.48	178.24
Sources × durations	2	1.70	0.85	23.00	11.50
Varieties × sources	3	19.02	6.34	36.12	12.04
“ × durations	6	77.09	12.85	81.73	13.62
Error	6	5.88	0.98	14.47	2.41

is computed from the expression $\sqrt{0.98 \times 12 \times 2 \times 2.447} = 11.9$, it is seen that the difference between the sums of both localities for all removal intervals is 11.9 which is just sufficient to reach the level of significance. However, it will be noted that the entire amount of the difference is made up of differences furnished by Green Mountain in the two series and that the other varieties show no differences.

The sucrose totals are shown in Table XV, line 10. The required dif-

ference between column totals is $\sqrt{2.41 \times 4 \times 2 \times 2.447} = 10.8$. The Ithaca upland values for sucrose are higher than those for Steuben County upland, the differences at the 42-day and 21-day intervals exceeding the required difference; the difference at the 9-day interval is 9.5, and just fails to reach the required level.

DE-SUGARING TESTS

There was a sufficient quantity of tubers of some of the varieties to permit a test of the rate of de-sugaring when tubers were transferred from 5° to 27°. Again, there was a series starting October 25 and another starting on December 24. The number of days that the lots had been in storage at 5° at the time of removal varied from 63 to 186 days as shown in the headings of Table XVI. The intervals for removal from 27° storage after the start of de-sugaring were such as to give duration periods of 10, 20, and 40 days. The results for reducing sugar are shown in the top portion of Table XVI.

From the standpoint of suitability for the production of potato chips the main question is whether within 20 to 40 days of storage the quantity of reducing sugar was brought down to values such as 5.0 or preferably 3.0 mg. With most of the varieties the reducing sugar was lowered to values of 3.0 or even to 0.0. With some varieties, notably Chippewa, Spaulding Rose, Green Mountain, and Delaware, de-sugaring was incomplete even after 40 days at 27°. Irish Cobbler and Early Ohio were exceptional, in that even though the reducing sugar content was high at the start of storage at 27° the loss of sugar was rapid and the lower limit of 3.0 was reached or passed in from 10 to 20 days.

There was some evidence that de-sugaring was less prompt if the tubers had been in cold storage for a long time before the storage at 27° was started. Compare the values in the 20-day columns for the 63-day, 126-day, and 186-day lots in Table XVI in the October 25 series, and the same intervals in the December 24 series for the 66-day and 126-day groups.

It is interesting that Early Ohio, a variety that would not otherwise be considered promising for potato chip manufacture on account of the accumulation of reducing sugar at low temperature, shows indications of a capacity to de-sugar rapidly at higher temperatures; and that Russet Rural, a variety that tends to maintain a low reducing sugar value in cold storage, may lose its sugar slowly during high temperature storage. Further work would be desirable to determine whether the various varieties of potatoes have characteristic de-sugaring capacities with respect to reducing sugar. The facts are not satisfactorily disclosed by the present tests.

The sucrose values are shown in the bottom half of Table XVI. In contrast to the reducing sugar values, there is much less reduction in sucrose values at various intervals after the start of storage at 27°. Indeed, there

TABLE XVI

DE-SUGARING: EFFECT UPON REDUCING SUGAR AND SUCROSE BY TRANSFERENCE OF TUBERS FROM STORAGE AT 5° C. TO STORAGE AT 27° C.

Variety	Type of sugar	Milligrams of sugar per cc. of juice											
		Storage started Oct. 25, 1940						Storage started Dec. 24, 1940					
		After 63 days at 5°, put at 27° C. for			After 126 days at 5°, put at 27° C. for			After 186 days at 5°, put at 27° C. for			After 66 days at 5°, put at 27° C. for		
		Days			Days			Days			Days		
		0	10	20	40	0	10	20	40	0	10	20	40
Reducing	Russet Rural	7.4	2.4	0.1	0.0	5.8	3.5	1.7	0.0	6.1	3.2	3.0	1.6
	Irish Cobbler	11.6	4.0	1.7	0.0	15.2	3.5	2.1	0.0	12.3	2.8	0.0	0.4
	Chippewa	5.8	3.9	3.3	0.0	11.9	6.8	4.3	2.4	15.6	10.1	7.0	5.0
	Green Mountain	17.4	5.7	2.7	0.0	15.6	5.9	2.9	2.1	13.1	7.2	5.0	2.9
	Carman No. 3	7.8	3.0	0.0	0.0	7.7	3.2	1.5	0.0	7.0	4.1	2.1	0.0
	Katahdin									15.1	7.0	2.8	2.9
	Sir Walter Raleigh									7.6	3.0	0.7	0.0
	Spaulding Rose									17.0	7.1	5.7	4.6
	Delaware									15.1	6.7	6.6	5.2
	Early Ohio									12.2	2.0	0.0	0.0
Sucrose	Rural New Yorker									5.2	3.5	0.0	0.0
	Heavyweight									4.8	4.2	2.0	0.0
	Eureka									17.0	5.7	3.4	1.8
	Russet Rural	5.5	4.6	4.3	2.2	4.7	6.4	4.9	5.8	6.5	7.9	6.4	8.2
	Irish Cobbler	10.7	6.1	3.3	3.0	5.8	4.4	4.1	5.5	11.9	5.5	5.0	8.3
	Chippewa	8.8	6.6	7.0	6.0	4.5	9.0	10.0	11.1	11.6	14.4	14.2	16.2
	Green Mountain	14.4	7.0	6.4	5.7	8.5	6.8	7.0	8.6	9.4	7.5	7.0	11.5
	Carman No. 3	5.4	4.4	3.7	3.0	5.3	4.4	6.2	5.3	7.5	6.7	6.6	6.3
	Katahdin									7.8	6.0	3.4	4.0
	Sir Walter Raleigh									3.2	5.5	4.9	7.6
* Rotted.	Spaulding Rose									6.7	4.5	5.5	8.7
	Delaware									11.3	8.6	8.1	13.1
	Early Ohio									7.5	4.2	4.7	5.2
	Rural New Yorker									3.6	4.1	3.9	6.3
	Heavyweight									5.6	4.3	6.3	7.2
	Eureka									9.4	5.3	5.4	7.9

* Rotted.

were some instances in which the sucrose increased during the storage at 27°, e.g. see the values for Chippewa in the 126-day and 186-day lots in the October 25 series, and for Spaulding Rose, Delaware, and Heavyweight in the 126-day lots in the December 24 series. Only in the lots removed from cold storage at the shortest interval (those for the 63-day interval in the October 25 series), were there consistently decreasing sucrose values at each successive removal interval at 27°, and even in this group it is not certain that there was any reduction of values in the case of Chippewa.

SUMMARY

Potato (*Solanum tuberosum* L.) tubers of 25 varieties with known history from harvest to storage were stored at temperatures of 5°, 7°, and 8.2° C. for varying lengths of time at each temperature, storage starting at two intervals after harvest, i.e. one series on October 25 and the other on December 24.

When the samples of tubers were removed from storage, potato chips were prepared by removing two slices from each tuber and cooking the combined slices in lard at 195° C.; the tissue not used for chips furnished juice which was analyzed for reducing sugar and sucrose.

Confirmation was obtained of the fact reported in the previous paper that the concentration of reducing sugar and not that of sucrose determines the color of chips. Again, the value 5.0 mg. of reducing sugar per cc. of juice was found to be the dividing line, tissue with amounts higher than this furnishing chips too dark brown in color; for a really satisfactory commercial color the value should probably be as low as 3.0.

Of the 25 varieties, 11 were the same as those in experiments of the previous year, and 9 of these showed responses nearly identical with those shown by the corresponding varieties in the previous year, even to the number of milligrams of reducing sugar per cc. of juice under comparable storage conditions. The other two showed differences between the two years.

The reducing sugar values of the lots stored at 7° were only about 35 per cent of the values at 5°, and those at 8.2° were only about 16 per cent of those at 5°.

Delaying the start of storage retarded the rate of increase of reducing sugar at 5° and lowered the value which was reached after, say, 90 days of storage.

The temperature of 7° was a favorable one for storage for potato chip manufacture since many of the varieties were maintained at low reducing sugar values for periods of 108 to 131 days after start of storage; 8.2° was also satisfactory, giving reducing sugar values even lower than at 7° but sprouting was more extensive at 8.2° than at 7°.

The varieties of the Rural group were outstanding in maintaining low

values for reducing sugar. Of the varieties tested, seven (and probably eight) belonged to this group. Of the varieties with low reducing sugar values but outside the Rural group Blue Victor and Chippewa may be mentioned. But the tubers of Chippewa sprout readily and finally become too soft for potato chip manufacture. Irish Cobbler came next in the series listed in order of varieties with regard to reducing sugar values, and the suitability of tubers of this variety for potato chips depended on the time after harvest at which cold storage of tubers started. When the storage was delayed until the late starting date the tubers were held satisfactorily at 7° for at least 108 days without undue accumulation of reducing sugar and furnished chips comparable in color to those of varieties of the Rural group. Varieties, the tubers of which attained high amounts of reducing sugars and which were unsuitable for potato chip manufacture on account of the dark color of chips caused by this high sugar content, were Eureka, Green Mountain, Delaware, Bliss Triumph, Pride of Multnomah, Spaulding Rose, Blue Mercer, Warba, and Axtell's Bugless.

Sucrose values also showed differences among the three temperatures but the change from 5° to 7°, and 7° to 8.2° produced less effect than the changes in reducing sugar at the corresponding changes in temperature.

The order of varieties for increasing amounts of reducing sugar was not at all the order of varieties for increasing amounts of sucrose. A table was prepared showing a classification of the varieties with respect to amounts of each of these two types of sugar.

A temperature difference of only 1° C. was found to be sufficient to show significant changes in the amounts of reducing sugar and sucrose.

Tubers grown under different soil conditions with respect to added fertilizers, and tubers of the same variety grown in two different localities, showed no important differences in the amounts of reducing sugar developed during cold storage.

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EFFECT OF THE VAPOR OF THE METHYL ESTER OF α -NAPHTHALENEACETIC ACID ON THE SPROUTING AND THE SUGAR CONTENT OF POTATO TUBERS

F. E. DENNY, JOHN D. GUTHRIE, AND NORWOOD C. THORNTON

It was shown in a previous report (2) that the vapor of the methyl ester of naphthaleneacetic acid inhibited the sprouting of intact potato tubers (*Solanum tuberosum* L.). The method consisted in placing papers impregnated with the methyl ester in the container in which the tubers were stored. The chemical volatilized slowly and continuously, and furnished enough vapor to bring about marked retardation of sprouting.

The tests were continued with the potato crops of the two following years, 1939 and 1940, with the objects of determining the length of time during which sprouting could be inhibited, the limits of concentration and temperature for favorable results, the proper time after harvest at which to start the treatments, and the effect of the treatment upon the sugar content of the tubers.

The results show that sprouting of tubers can be completely inhibited for a period of at least one year at 10° C. (a temperature at which sprouting occurs freely with untreated tubers), and also even at 15° C., although at this temperature there is some shrinkage (but no sprouting) after a storage period of six to eight months. Sprouting is inhibited for several months at room temperature (20° to 25° C.), even though this temperature is unfavorable on account of loss of moisture from the tubers.

Only very small amounts of the chemical are needed to inhibit the sprouting. For about 1 to 1.3 kg. of tubers in a container, filter papers impregnated with a total of 400 mg. of the methyl ester of α -naphthaleneacetic acid induced complete inhibition for many months; when the amount was 100 mg. small sprouts appeared in many cases after a few months; but an observable effect was obtained with 30 mg., and perhaps with an amount as low as 10 mg. Since the jars holding the treated tubers were not closed tightly but only with a paper cover in which there were small holes for ventilation, not all of the chemical which was applied in the treatment came into contact with or was absorbed by the tubers. Furthermore, it was found that papers that had been impregnated with the chemical and had been used in a test with one lot of tubers over a period of several months, still retained enough chemical to be used successfully with a second lot of tubers. The amounts of chemical actually absorbed by the tissue under these conditions have not been determined.

The chemical treatment had little effect upon the sugar content of the juice of the tubers. In most of the tests no differences in the sugar content

were found between the treated and the control lots. In other tests there were significant differences, but there was no agreement among the tests as to the direction of the change. In any event, the effect was small and the results indicate that tubers can be treated and stored under temperature conditions favorable for maintaining low sugar contents in the tubers, and that sprouting of the tubers can be inhibited under these same conditions.

METHODS

The methyl ester of α -naphthaleneacetic acid ($C_{10}H_7CH_2COOCH_3$) was dissolved in acetone at such dilutions as to give 40, 10, etc. mg. in each cc. Filter papers 15 cm. in diameter were then moistened with these solutions, using 10 cc. to each five filter papers. The acetone was allowed to evaporate and the dry papers, now containing 400, 100, etc. mg. of the methyl ester (but no acetone) in each set of five papers, were used for treating the potatoes. The filter papers for the control lots were prepared by incorporating 10 cc. of acetone in five papers and allowing the acetone to evaporate. After they were thoroughly dry the papers were used in the tests.

The potato tubers were placed in one-gallon glazed earthenware jars, the five filter papers being distributed between layers of tubers from bottom to top. The number of tubers per jar varied from 12 to 18, and the weight from 1 to 1.3 kg., depending upon the variety and the size of tubers.

The jars were covered by cutting off a paper bag at the proper height, inverting it over the jar, fastening it with a rubber band, and poking two holes the size of a lead pencil in the paper cover.

TABLE I

SPROUTING OF POTATO TUBERS STORED AT VARIOUS TEMPERATURES IN THE PRESENCE OF FILTER PAPERS IMPREGNATED WITH THE METHYL ESTER OF α -NAPHTHALENEACETIC ACID

Variety	Weight of sprouts in grams per 10 tubers											
	Continuous treatment						Treatment applied for 10 days only					
	15° C.		18° C.		22° C.		15° C.		18° C.		22° C.	
	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.
Russet Rural	0.8	22.6	0.1	20.4	0.0	17.1	4.2	19.4	3.2	25.3	2.0	11.3
Carman No. 3	3.0	15.5	0.2	21.6	0.0	13.8	3.1	15.2	3.0	17.2	1.1	11.1
Irish Cobbler	1.6	51.4	0.1	76.0	0.1	58.3	12.7	39.4	5.9	51.8	5.0	37.0
Russet Burbank	0.4	15.6	0.1	26.2	0.0	22.3	2.1	11.7	1.1	25.4	1.4	18.2
Bliss Triumph	1.0	35.1	0.1	52.0	0.2	33.3	8.3	29.0	5.3	55.8	4.6	39.1
Chippewa	0.7	83.9	0.8	95.7	6.2	70.6	17.8	65.1	18.4	83.5	18.5	46.3
Early Ohio	0.1	12.0	0.0	21.2	0.0	15.9	1.9	9.8	1.0	15.0	0.6	10.9
Green Mountain	0.7	15.5	0.0	29.0	0.1	20.5	6.8	16.1	2.4	22.0	4.6	11.5
Blue Victor	0.8	18.1	0.0	29.6	0.0	24.9	5.7	16.3	0.8	26.3	2.0	22.8
Katahdin	3.1	29.0	0.6	31.2	0.1	29.2	13.6	24.8	5.2	37.5	3.8	25.8
Spaulding Rose	3.4	18.8	0.2	38.8	0.3	31.3	7.9	18.8	3.8	33.8	5.8	26.1
Totals	15.6	320.2	2.2	441.7	7.0	332.2	84.1	265.6	50.1	393.6	49.4	260.1

The preparation of juice from the tubers and the method of analysis for sugar content of the juice were the same as those previously described (1).

RESULTS

WITH TUBERS OF THE 1939 CROP

Main Experiment

The results of an experiment starting December 1, 1939 and ending March 9, 1940 are shown in Tables I and II. The treatment applied was

TABLE II

SUGAR CONTENT OF THE JUICE OF POTATO TUBERS STORED AT VARIOUS TEMPERATURES IN THE PRESENCE OF FILTER PAPERS IMPREGNATED WITH THE METHYL ESTER OF α -NAPHTHALENEACETIC ACID

Variety	Reducing sugar, mg. per cc.						Sucrose, mg. per cc.					
	15° C.		18° C.		22° C.		15° C.		18° C.		22° C.	
	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.
Russet Rural	0.0	0.0	0.0	0.0	0.0	0.0	3.3	3.4	4.2	4.9	3.7	4.0
Carman No. 3	0.0	0.0	0.0	0.0	0.0	0.0	1.9	1.8	3.4	4.0	2.2	1.6
Irish Cobbler	1.5	1.1	0.8	0.5	0.0	0.0	3.2	3.5	4.8	6.2	3.8	6.8
Russet Burbank	3.5	3.2	2.2	1.9	1.2	0.0	2.5	2.1	3.4	3.0	3.0	3.7
Bliss Triumph	5.1	4.6	4.2	3.2	3.1	2.5	2.9	2.2	4.4	5.5	5.0	6.2
Chippewa	1.9	0.3	0.0	0.0	0.0	0.0	3.6	3.3	4.9	6.1	5.6	5.5
Early Ohio	2.1	2.3	2.2	0.9	0.0	0.9	3.6	3.1	3.4	4.1	5.1	4.9
Green Mountain	4.1	3.3	2.7	2.7	1.7	1.3	3.5	2.3	3.6	3.8	5.2	5.5
Blue Victor	1.9	0.4	0.4	0.0	0.3	0.8	1.6	1.4	2.9	2.5	3.1	2.8
Katahdin	0.3	0.0	0.8	0.0	0.0	0.0	4.0	2.3	3.6	2.9	3.5	2.6
Spaulding Rose	2.3	2.3	1.8	2.4	1.1	1.4	2.2	1.9	3.3	3.8	3.8	4.6
Totals	22.7	17.5	15.1	11.6	7.4	6.9	32.3	27.3	41.9	46.8	44.0	48.2

Analysis of Variance

Source of variation	Degrees of freedom	Variance	
		Reducing sugar	Sucrose
Total	65	—	—
Treatments	1	1.280	0.400
Temperatures	2	7.630	14.405
Varieties	10	9.058	4.354
Treat. $\times$ temp.	2	0.255	1.545
Var. $\times$ treat.	10	0.211	0.622
Var. $\times$ temp.	20	0.507	0.830
Error	20	0.113	0.156

100 mg. of the methyl ester of α -naphthaleneacetic acid per jar containing one dozen tubers. This amount of the chemical was incorporated into five filter papers which were distributed throughout the container. It will be noted that some of the lots were treated continuously at temperatures of 15°, 18°, and 22° C., while other lots were treated in the earthenware con-

tainers for only 10 days and were then removed from the containers and stored at these same temperatures for the rest of the time in paper bags.

Effect on sprouting. The weights of sprouts in grams per 10 tubers at the end of the experiment are shown in Table I. The treatment depressed the sprouting markedly in each comparison of treated and check, at each temperature, and with each variety. The values in columns 8 to 13 in Table I are those obtained when the treated lots were exposed to the chemical for only 10 days and were then removed from contact with the chemical, and stored in paper bags. Removing the treated tubers from the presence of the chemical permitted a certain amount of sprout growth in the subsequent period, but the sprout weights for the treated lots were

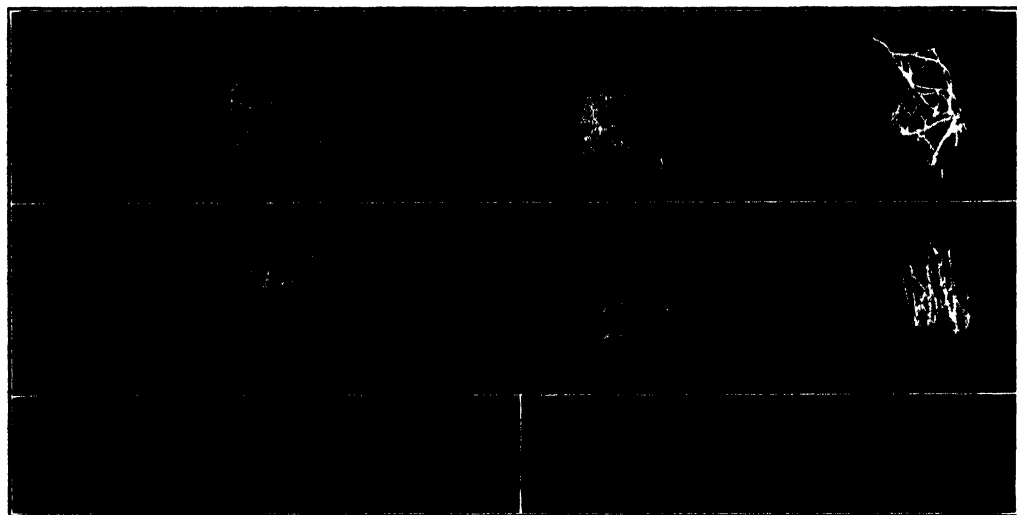


FIGURE 1. Rows A and B, tubers of the 1939 crop after 3 months' storage at 18° C. Row A, left pair, Irish Cobbler; center pair, Russet Burbank; right pair, Bliss Triumph. Row B, left pair, Chippewa; center pair, Early Ohio; right pair, Green Mountain. In each pair: left lot, treated with vapor of methyl ester of α -naphthaleneacetic acid; right lot, not treated. Row C-D, tubers of the 1940 crop after one year from harvest, storage at 10° C. Tubers harvested August 12-13, 1940; this photograph taken August 13, 1941. The C lots are Green Mountain, the D lots are Chippewa. In each set of three lots: left, treated with 400 mg. of the methyl ester of α -naphthaleneacetic acid; center, treated with 100 mg.; right, not treated. The check lot (third from left in C) shows the second crop of sprouts, the first growth of sprouts having been removed on May 21, 1941.

much lower than those for the checks even under these conditions. The retarding effect of even a short period of treatment on the later growth of the sprouts is clearly shown.

The effectiveness of the chemical in retarding growth of sprouts is also shown in Figure 1 A and B. The treated lots in this case were exposed to the chemical for 10 days in early December at 22° C., and then, without removal of the chemically-treated papers, the jars were stored at 18° C.

until March 9 at which time the photograph was taken. After about three months at 18° C., therefore, the treated lots of all six varieties had failed to sprout, while the check lots showed extensive sprouting. There was some shrinkage even of the treated lots at this temperature, 18° C., which is too high to maintain good condition of tubers, but which is not too high for the effectiveness of the chemical in preventing sprouting.

Sugar content. The amounts of sugar per cc. of juice are shown in Table II. The values obtained by the analysis of variance are shown at the bottom of the table.

For the reducing sugar data the treatment variance is many times the error variance and is highly significant. The required difference in the total of 11 items is $\sqrt{0.113 \times 11 \times 2 \times 2.086} = 3.3$ (see 3, p. 49), and on this basis the treatment increased the reducing sugar at storage temperatures of 15° and 18°, but at 22° the difference is not significant. The average increase in reducing sugar, however, even at 15° and 18° is only 0.8 mg. per per cc. of juice.

The variance values for sucrose are shown at the bottom of Table II. The variance for the treatments as a whole, 0.400, does not exceed the error variance, 0.156, by the required amount, and is not significant. However, the variance for treatments at different temperatures, 1.545, is many times the error variance and is significant. The required difference between totals of 11 items is $\sqrt{0.156 \times 11 \times 2 \times 2.086} = 3.9$, and on this basis the treatment increased the sucrose at the temperature of 15°, but decreased it at 18° and 22°. Here, again, the absolute effect was small, the average gain at 15° and the losses at 18° and 22° being less than 0.5 mg. per cc. of juice.

TABLE III

RESULTS OF OTHER TESTS OF THE EFFECT OF TREATMENT WITH METHYL ESTER OF α -NAPHTHALENEACETIC ACID UPON THE SUGAR CONTENT OF POTATO TUBERS

Date	Duration of test, months	No. of varieties included	Temp., ° C.	Amt. of chemical, mg. per kg. of tubers	Treatment effect, increase (+), or decrease (-), mg. per cc.	
					Reducing sugar	Sucrose
Mar., 1939	2.0	1	28°	200	-1.48	-0.10
			22°	200	-0.20	+3.34
			10°	200	-0.03	+0.80
Sept., 1939	2.0	17	22°	100	0.0	+1.6
May, 1940	1.5	1	15°	100	-0.9	+1.5
			Room		-0.3	-0.1
June, 1940	4.5	1	15°	100	+0.5	+0.6
				25	+0.4	0.0
				6.25	0.0	0.0

Other Experiments

There were other miscellaneous experiments on various lots of tubers as they became available during 1939 and early 1940. The results of these tests, so far as they relate to sugar content, are shown in Table III. The numerical values and the signs showing the direction in which the sugar constituent was changed by the treatment are given in columns 6 and 7 in Table III. These do not show a consistent effect on the sugar content, and the differences, except in one or two cases, are very small.

WITH TUBERS OF THE 1940 CROP

Main Experiment

The effect of the methyl ester of α -naphthaleneacetic acid upon the sprout development and sugar content of tubers of the 1940 crop are shown in Table IV. Two storage temperatures were used in this test, 10° and 15° C. Storage was started at two different intervals after harvest, one series starting at once after harvest, and the other series about six weeks later. In addition, with some of the samples, the filter papers containing the chemical were removed from the jars after the treatment had proceeded for one month, the lots remaining, however, for the rest of the experiment in the jars and under the same temperature conditions.

The experiment was started on August 13, 1940, at which time the "at once" lots were treated with the methyl ester. The "six weeks" lots remained at this temperature until approximately six weeks later, when the chemically-impregnated papers were placed in the jars. The sprout data were obtained and sugar analyses made on subsequent dates as follows: March 25, 1941 for Green Mountain; April 7, 1941 for Russet Rural and Bliss Triumph; April 25, 1941 for Irish Cobbler and Chippewa; except that the "10° C.—six weeks" lots of Irish Cobbler, Chippewa, and Green Mountain were sampled on May 4, 1941. Thus, the values in Table IV were obtained after an experimental period of about six to eight months.

Effect on sprouting. The weights of sprouts in grams per 10 tubers after an interval of six to eight months are shown in the upper portion of Table IV, lines 1 to 5. When applied continuously at either 10° C. or 15° C., 400 mg. of the chemical per kg. (approximately) completely inhibited sprouting. The 100 mg. amount was less effective but produced a marked reduction in sprout growth. Applying the treatment for only one month, and then removing the chemical from the containers, reduced the effectiveness of the treatment and permitted considerable growth of sprouts (columns 12 to 14), but, in spite of this, the 400 mg. lots showed a distinctly smaller amount of sprout growth than that found for the control lots. Delaying the application of the chemical until six weeks after harvest resulted in a smaller amount of sprout growth in the 15° lots than when

the chemical was applied at once (compare columns 10 and 16, lines 1 to 5), but possibly not in the 10° lots (compare columns 4 and 7, lines 1 to 5).

Further evidence of the effectiveness of the methyl ester of α -naphthaleneacetic acid in retarding sprout growth is shown in Figure 1 C and D. This photograph was taken one year after the tubers had been harvested. During this interval they had been stored at 10° C. The lots receiving treatment at the 400 mg. rate are seen to be still without sprouts one year later. They were quite firm, and in good condition. No renewal of impregnated papers had been made during the entire interval. The treated tubers are being held further in storage under the same conditions. The 100 mg. lots show some sprouts but the amount is much less than that shown by the check lots. The latter have sprouted freely, have shriveled badly, and cannot be held much longer under these conditions. With one of the varieties (Green Mountain) the sprouts shown in Figure 1 C are the second crop, the first crop amounting to 89.7 grams having been removed on May 21, 1941.

Sugar content. The sugar contents of juices obtained from tubers after an experimental period of six to eight months are shown in Table IV, lines 6 to 15. The values are quite low, especially for reducing sugar at 15°, and for sucrose at 10°. The data are meager for the "six weeks" and "discontinued" lots (see columns 6 to 8, and 12 to 17) but there is a full complement in the "at once" lots for all treatments and all varieties. These 30 values, each for reducing sugar and sucrose, furnished the entries for a new table (not shown here) from which an analysis of variance was made and the mean squares and degrees of freedom so obtained are entered in Table V.

TABLE V

ANALYSIS OF VARIANCE OF SUGAR VALUES SHOWN IN TABLE IV, COLUMNS 3 TO 5 AND 9 TO 11, UNDER THE "AT ONCE" HEADINGS FOR BOTH 10° AND 15° C.

Source of variation	Degrees of freedom	Variance for	
		Reducing sugar	Sucrose
Total	29	—	—
Treatments	2	1.10	18.42
Temperatures	1	2.64	119.21
Varieties	4	20.83	27.06
Treat. $\times$ temp.	2	0.16	10.80
Treat. $\times$ var.	8	0.37	3.46
Temp. $\times$ var.	4	1.04	9.45
Error	8	0.32	0.22

The over-all treatment effect upon the reducing sugar content is not significant, the ratio of 1.10 to 0.32 being 3.44, an amount less than the value 4.46 which is the minimum requirement for significance at the 0.05 level with 2 and 8 degrees of freedom. At both temperatures, however, it is seen that the variety totals (columns 3 to 5, 9 to 11, lines 6 to 10) are

lower for the 400 mg. lots than for the checks, and when these are added the values are 12.8 for the 400 mg. lots and 18.6 for the corresponding control, the difference being 5.8 mg. The required difference between the sums of 10 items is $\sqrt{0.32 \times 10 \times 2 \times 2.306} = 5.8$. It appears that the 400 mg. treatment depressed the reducing sugar by an amount which is barely significant. But even if it is significant, the difference caused by the treatment is small, being only about 0.6 mg. per cc. of juice.

The treatment effect in the case of sucrose is significant, especially with the lots at 15° (columns 9 to 11, lines 11 to 15). The column totals are 22.6, 27.3, and 45.3, respectively, for 400 mg., 100 mg., and check, the differences between treated and check being 22.7 and 18.0 for the 400 mg. and 100 mg. lots. The required difference for sums of five items in this case is $\sqrt{0.22 \times 5 \times 2 \times 2.306} = 3.4$ mg. This shows that both the 400 mg. and 100 mg. treatments reduced the sucrose content at 15°. The 100 mg. value is intermediate between 400 mg. and the check, and the difference between the 400 mg. and 100 mg. sucrose totals, i.e. 4.7, is well above the level of significance. The three totals for sucrose at 10° are 10.6, 11.2, and 13.6, respectively, for the 400 mg., 100 mg., and check lots, but the differences in each comparison do not reach the required value, 3.4, although the difference between the 400 mg. and check values is not far below it.

*Effect of the Methyl Ester Applied at the Rate of
100 Mg. per Kg. of Tubers*

In this experiment the chemical was applied at the rate of 100 mg. per kg. of tubers and all lots were stored at room temperature, which was about 23° C. in this case. There were two starting dates, one soon after harvest, and the other one month after harvest. Samples were removed at intervals after harvest, these varying from December 20 to May 12. The results are shown in Table VI.

Effect on sprouting. The weights of sprouts obtained at the different removal intervals are shown in columns 4 and 5 in Table VI. It should be noted that the samples removed on the different removal dates were separate samples, and the crop of sprouts removed at each removal date represents the total growth from the start of the experiment to the date of removal.

The effectiveness of the treatment in depressing the sprout weight was estimated by comparing the average differences between pairs (treated minus check) at each sampling date with the mean error of these differences computed by Student's method (3, p. 20). For the December 20 date there were four pairs, the average difference was 4.80, the mean error was 2.06, the t value is 2.331, which does not reach the required value, 3.182, for 3 degrees of freedom. At the other sampling dates, however, the differ-

ences between treated and check are significant, with *t* values well above the minimum requirement.

TABLE VI

EFFECT OF METHYL ESTER OF α -NAPHTHALENEACETIC ACID APPLIED AT THE RATE OF 100 MG. PER KG. OF POTATO TUBERS

Variety	Time after harvest at which treatment started	Date of sampling	Wt. of sprouts, g. per 10 tubers		Sugar, mg. per cc. of juice			
					Reducing		Sucrose	
			Tr.	Ck.	Tr.	Ck.	Tr.	Ck.
Russet Rural	At once	Dec. 20	0.0	1.4	0.1	0.1	1.7	1.8
		Mar. 24	7.3	19.0	0.0	0.0	4.5	4.6
		May 12	4.1	28.7	—	—	—	—
		May 12	9.1	30.3	—	—	—	—
	One month	Dec. 20	0.0	1.1	0.2	0.0	2.8	1.6
		Mar. 24	4.3	17.8	0.0	0.0	4.3	4.9
		May 12	8.9	33.1	—	—	—	—
		May 12	6.2	30.8	—	—	—	—
Chippewa	At once	Dec. 20	0.2	8.9	0.1	0.1	2.3	1.8
		Mar. 24	7.0	56.8	0.0	0.0	10.1	16.0
		May 12	11.5	67.3	—	—	—	—
		May 12	5.3	73.6	—	—	—	—
	One month	Dec. 20	1.1	9.1	0.1	0.1	1.8	1.9
		Mar. 24	4.2	46.1	0.0	0.0	15.5	16.9
		May 12	8.1	76.2	—	—	—	—
		May 12	6.1	58.2	—	—	—	—
Green Mountain	At once	Mar. 24	14.2	87.3	0.0	0.0	7.7	10.8
		May 12	13.2*	100.0	—	—	—	—
	One month	Mar. 24	7.7	99.9	0.0	0.0	12.6	12.3
		May 12	10.1	111.0	—	—	—	—
Irish Cobbler	At once	Mar. 24	1.9	44.5	0.0	0.0	6.3	6.7
		May 12	12.9	63.6	—	—	—	—
	One month	Mar. 24	1.7	40.4	0.0	0.0	4.9	5.9
		May 12	10.0	71.0	—	—	—	—

* The true value was lost and this entry was made by estimating the value from the proportionate values between treated and check for the May 12 entries.

The sprout weights are seen to be usually greater for the "at once" lots than for the comparable "one month" lots, but these paired differences when tested by Student's method do not furnish a significantly high *t* value. When the values for the treated lots at the March 24 and May 12 sampling dates are pooled the resulting average difference ("at once" minus "one month") is found to be 2.1 g., with a mean error of 0.965, which gives a *t* value of 2.177, whereas the required *t* value is 2.306.

Sugar content. At this temperature (approx. 23° C.) the reducing sugar was held to very low values (see columns 6 and 7, Table VI), and the chem-

ical treatment showed no effect. The sucrose values are shown in columns 8 and 9, Table VI. These furnish 12 paired items which give differences the significance of which may be tested by Student's method. When the differences (check minus treated) are taken, the mean difference is 0.90 mg., and the error of this difference is 0.55, giving a ratio of 1.636, which fails of significance, since the required ratio for 11 degrees of freedom is 2.201.

Effect upon Sprout Growth of Small Amounts of the Methyl Ester

Table VII shows the effect upon sprout development when smaller amounts of the methyl ester of α -naphthaleneacetic acid were applied. The amounts chosen were 30, 10, and 3.3 mg. of the methyl ester per kg., and these were compared with check lots receiving no chemical. The "at once" lots were placed under the experimental conditions on August 22, 1940, and the other lots were not started until about one month later. The removal dates as shown in column 2 were March 24 and May 12, 1941. The storage temperature was 15° C.

To determine whether the amount of chemical which was applied had reduced the weight of sprouts obtained at the end of the experiment, the differences between the treated lots and the check lots were first computed. For example, the differences between the weights of sprouts in a comparison of the check and 30 mg. lots, columns 4 and 7, Table VII (using first the "at once" lots), are $115.0 - 21.1 = 93.9$; $100.1 - 81.0 = 19.1$; $100.0 - 40.6 = 59.4$; etc. The mean of the five values so obtained is 34.6, which is the entry in column 5, line 1, in Part B of Table VII. The sum of the squares of the deviations, i.e. $(93.9 - 34.6)^2 + (19.1 - 34.6)^2 + (59.4 - 34.6)^2$, etc. was found to be 6730.91, which is the entry in column 4, line 1, in Part B. Since there are five items in each comparison, the number of degrees of freedom is 4 (see column 3, Part B). Similar computations for the check minus 30 mg. differences in the "one month" series, and for both series for the check minus 10 mg. and check minus 3.3 mg. differences were made and the values are shown in columns 3 and 4, Part B. The variance for the pooled values is $16,109.00 \div 24 = 671.21$, the standard deviation is $\sqrt{671.21} = 25.91$, and the error of the mean of five items is $25.91 \div \sqrt{5} = 11.59$.

The t values, i.e. the ratios of 34.6 to 11.59, 48.7 to 11.59, etc., are shown in column 6, Part B. The required t value for odds of 19 to 1 with 24 degrees of freedom is 2.064.

The 30 mg. treatment definitely decreased the sprout weights, and the 3.3 mg. treatment showed no significant differences. The situation with regard to the effect of 10 mg. is doubtful, since the t value for the "one month" lots shows a significant effect, while that for the "at once" lots does not. When the two series for the 10 mg. treatment are combined, the average difference between check and treated is 16.25, the error of this

TABLE VII

EFFECT OF SMALL AMOUNTS OF THE METHYL ESTER OF α -NAPHTHALENEACETIC ACID UPON
THE SPROUTING OF POTATO TUBERS

Part A

Variety	Date of sampling	Time after harvest at which treatment started	Weight of sprouts, g. per 10 tubers				Totals
			Amount of methyl ester applied, mg. per kg. of potato tubers				
			30 mg.	10 mg.	3.3 mg.	Check	
Green Mountain	May 12	At once	21.1	100.6	119.0	115.0	355.7
		One month	40.6	78.5	101.0	98.0	318.1
Irish Cobbler	Mar. 24	At once	81.0	96.2	68.4	100.1	345.7
		One month	30.5	68.0	80.2	118.0	296.7
Chippewa	May 12	At once	40.6	71.1	68.5	100.0	280.2
		One month	37.0	62.4	89.8	98.5	287.7
Russet Rural	Mar. 24	At once	24.4	28.3	27.5	27.3	107.5
		One month	18.2	13.9	36.2	20.0	88.3
	May 12	At once	43.6	50.0	46.0	41.4	181.0
		One month	24.1	46.5	41.6	59.6	171.8
Totals			361.1	615.5	678.2	777.9	

Part B

Significance of Sprout Weight Differences between Treated and Check Lots

Differences taken	Starting dates	Degrees of freedom	Sums of squares	Mean difference, g.	<i>t</i> Value
Ck. minus 30 mg.	At once	4	6730.91	34.6	2.986
	One month	4	4118.81	48.7	4.203
Ck. minus 10 mg.	At once	4	849.99	7.5	0.647
	One month	4	1277.27	25.0	2.157
Ck. minus 3.3 mg.	At once	4	1442.47	10.9	0.941
	One month	4	1689.55	9.1	0.785
Pooled values		24	16109.00	(Required <i>t</i> value = 2.064)	
Variance			671.21		
Standard deviation			25.91		
Error of mean of 5 items			11.59		

mean is $25.91 \div \sqrt{10} = 8.19$, the t value is $16.25 \div 8.19 = 1.983$, which is just below the required value, 2.064.

It appears that, under these conditions, 10 mg. of the methyl ester of α -naphthaleneacetic acid per kg. of tubers is near the lower limit for exerting a pronounced effect in retarding sprouting. But, of course, the variety of potato, the temperature of treatment, and especially the method of applying the chemical may be important factors in determining the lower limit of concentration of chemical for inhibition of sprouts.

Another question is whether there is a difference in the final sprout weights between the lots started at once after harvest and those not put into the experimental conditions until one month later. In this case our interest is only in the 30 mg. and 10 mg. lots, which were the ones giving evidence of having an effect upon the weight of sprouts. The paired differences are now: 21.1–40.6, 81.0–30.5, etc. in the 30 mg. column, and 100.6–78.5, 96.2–68.0, etc. in the 10 mg. column. There are five such differences and 4 degrees of freedom for the data in each column. The pooled sums of squares and degrees of freedom give 19.47 as the standard deviation, 8.71 as the error for the mean of five items, and 6.16 as the error for the mean of 10 items. The differences between the “at once” and “one month” values do not give a t value sufficiently high for significance, either for the 30 mg. or 10 mg. treatments. When the 30 mg. and 10 mg. values are combined, the average sprout weights are higher for the “at once” lots by the amount of 13.73 g., but the error of this difference is 6.16, which gives a t value of 2.224, somewhat less than 2.306 (the t value required for significance). On this point, therefore, the results in Table VII agree with those in Table VI in showing that while the sprout weights in the treated lots were smaller when the chemical was not applied until a delayed period after harvest, the differences are not large enough to be statistically significant.

The amount of chemical used, and possibly the length of the delay period, may be factors in this comparison, as is indicated in Table IV, columns 10 and 16. In this test the amount of chemical was 100 mg., and the delay before treating was six weeks instead of one month.

Effect of Using Impregnated Papers a Second Time Without Renewal of Chemical

Chemically-impregnated papers do not become exhausted after having been used once for inhibiting the sprouting of tubers over a period of several months, but retain enough of the methyl ester to retard the sprouting of a second lot of tubers.

Some of the methyl ester papers that had been used for inhibiting the

sprouting of tubers over a period of about three months in the 1939 tests were stored in a large glass container (culture dishes of the telescoping type), with the top closed but not sealed, from March, 1940 until December, 1940.

Some tubers of the Green Mountain variety, purchased in the local market, were placed in the glazed earthenware jars with a sufficient number of the chemically-impregnated papers to give 400 mg. of the methyl ester (computed on the basis of the amount originally applied to the papers, with no allowance for subsequent loss of chemical) per kg. of tubers. These lots together with controls not receiving chemical were covered with inverted paper bags, and were stored at room temperature from December 19, 1940 until March 24, 1941. The weights of sprouts per 10 tubers obtained from these lots on March 24 were: treated, 20.6 g., 21.0 g.; control, 90.2 g., 82.2 g.

In another test, tubers of the variety Russet Rural of the 1940 harvest were stored in October, 1940 at 15° C. in containers with chemically-impregnated papers from the 1939 series of tests using 400 mg. of the methyl ester (i.e. the amount that was applied to these papers when the 1939 tests were started) per kg. of tubers. On April 28, 1941, these tubers were without visible sprouts, and the condition of untreated tubers can be found by consulting the sprout data for Russet Rural in Table IV, column 8.

POTATO CHIPS

Potato chip samples were made of all of the lots for which sugar analyses were made. No direct effect of the chemical upon the color of potato chips was observed, the color of the chips depending on the quantity of reducing sugar, as was shown in previous papers (1, 4). Since the chemical did not influence the reducing sugar content to any important extent it did not affect the color of chips. The reducing sugar values in Tables II, IV, and VI serve to indicate the chip color which was obtained from the tubers furnishing these sugar values. Values for reducing sugar above 5.0 mg. per cc. of juice were indicative of a chip color entirely too dark. Lots having a reducing sugar of 3.0 mg. furnished chips light brown in color, and chips from those showing very low values were amber-colored or nearly white. The sucrose concentration was not a factor in chip color under the conditions of chip-frying here employed (1, p. 294).

The experiments show that by the use of the methyl ester of α -naphthaleneacetic acid, the intact tubers of many varieties of potatoes can be stored at temperatures which are sufficiently high to maintain the reducing sugar at a low value (such as is needed to produce potato chips of good color), without the occurrence of sprouting.

However, no recommendations are given at present for such a treatment with tubers that are to be used as food, because of the lack of infor-

mation as to the toxicity of potato tissue from tubers treated in this manner with the methyl ester of α -naphthaleneacetic acid.

SUMMARY

The methyl ester of α -naphthaleneacetic acid ($C_{10}H_7CH_2COOCH_3$), when incorporated into filter papers which were then distributed among potato tubers stored in glazed earthenware containers, inhibited the sprouting of the tubers.

When the amount of the chemical was as much as 400 mg. of the methyl ester per kg. of tubers, sprouting was inhibited completely for at least one year from harvest. When the amount applied was reduced to 100 mg. per kg. of tubers, a small amount of sprout development occurred after six to eight months. Sprouting was definitely retarded by 30 mg., and the lower limit, under these conditions, was about 10 mg.

The jars containing the tubers and chemically-impregnated papers were not closed tightly but only with paper covers, and it is not known what amounts of chemical entered the potato tissue. Filter papers containing the methyl ester having once been used to inhibit sprouting of tubers, after storage for several months in a closed (but not entirely sealed) container, were effective in inhibiting or retarding the sprouting of a second lot of potato tubers.

When the treatment was applied for a short period such as 10 days or one month, a definite retardation of sprout development was obtained, but the inhibition was incomplete and the result after three to eight months was much less marked than when the treatment was applied continuously.

At 10° C., tubers were stored under methyl ester treatment for at least one year without the occurrence either of sprouting or shrinkage of tubers. At 15° C., sprouting was inhibited satisfactorily for a period of at least eight months, but there was a moderate amount of shrinkage due to loss of moisture. Sprouting was inhibited for three to six months at 18° C. and at room temperature (approx. 23° C.), but some shriveling occurred.

No definite effect of the treatment upon the sugar content of the juice of potato tubers was established. In some cases both reducing sugar and sucrose were increased, in others they were decreased, in still others there was no effect. When either an increase or a decrease was obtained in any test, the change in sugar content was small, usually less than 1 mg. per cc. of juice.

The chemical treatment permitted tubers to be stored at temperatures sufficiently high to maintain low values for the reducing sugar content, and so to furnish potato chips of satisfactory color. However, no recommendations are given at present for such a treatment with tubers that are to be used as food, because of lack of information as to the toxicity of the chemical.

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SOME CONDITIONS WHICH AFFECT RATE OF DECOMPOSITION OF KITCHEN WASTE

M. M. McCool

Owing to the possibility of aiding in the disposal and conservation of an enormous total aggregate of soil-improving materials, a study of the rate of decomposition of kitchen waste or garbage from cities has been undertaken. This report deals with the pH values, nitrogen, ash, and ether soluble contents of representative samples. In addition, the rate of nitrate formation and the effect of different treatments on the rate of decomposition are considered. The salient results to be noted are that the materials collected varied in composition and availability as measured by the rate of nitrate formation. The speed of decomposition, moreover, was accelerated by placing them in an aerated and heated chamber, by the addition of inoculum from active composts and of steapsin. A rise followed by a decline in the percentage of nitrogen took place as decay proceeded.

MATERIALS AND METHODS

The materials utilized in these studies were derived from different sources. Those designated as Hastings represent the materials collected on each of five successive days from a restaurant in Hastings-on-Hudson, New York. The specimens labeled Dobbs Ferry were taken from 40 household kitchens in an apartment in Dobbs Ferry, New York, on different dates. Each lot of Yonkers waste was that collected from 35 restaurants in Yonkers, New York. The latter were delivered by the Department of Public Works, each delivery consisting of about 12 cubic yards. Upon receipt of the waste, paper, glass, cans, rags, and other materials were removed. The bulk lots from Yonkers were chopped as finely as practicable by means of sharpened shovels, mixed, and about three bushels taken to the laboratory and put through a Hobart food chopper. When the plans called for such, the mass was air-dried by spreading in a thin layer on waxed paper, blowing air over it by means of an electrically driven fan. In the rise-in-temperature studies one-half of a delivery was placed in a heap, the other treated with calcium carbonate and composting horse manure, and placed in a heap. These were covered with boards to prevent rain from falling on them. The covers were so arranged that the circulation of air around them was not impeded. The process tankage was a composite of seven commercials.

The rate of nitrate formation was measured in the usual manner (2). Each culture contained 100 g. of Sassafras soil with which was mixed a sufficient amount of the material in question to provide 30 mg. of nitrogen.

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The ash content of the materials was determined by ignition in an electric furnace heated to 700°C .

The rate of decomposition was determined by placing from 200 to 500 g. of the variously treated materials in Erlenmeyer flasks. The mouths of the flasks were covered by one layer of cheesecloth. The containers were placed in a horizontal position in the culture chamber the temperature of which was controlled by passing heated air through it. The air in the incubator was changed four times hourly. The inoculum which was utilized in the series of cultures held at 27° and 40°C . was obtained by incubating kitchen waste, with which a small amount of horse manure was mixed, four days at 40°C . A portion of this was shaken with distilled water and the extract added to the cultures in question. A like mixture incubated at 55°C . was the source of the inoculum for the cultures which were held at this temperature. Thus, no attempts were made to obtain pure cultures or to select those most active.

The rotation of the cultures was accomplished by fastening a metal disc to a motor-driven shaft which was inserted through the wall into the chamber. The container, a reagent bottle of 3-liter capacity, was attached to the disc in a horizontal position. Two aluminum strips, one inch in width, were placed within the bottle. Adjustments were such that the container could be rotated at the rate of two revolutions per minute. The containers which were not rotated were likewise placed in a horizontal position. Holes one and one-half inches in diameter were made in the plastic covers on the reagent bottles.

LITERATURE

O'Brien and Lindemuth (3) made a comprehensive study of the composition of garbage collected in several sections of Washington, District of Columbia, over a four- to five-month period. They did not find samples to vary widely.

Ashbrook and Wilson (1) give results of analyses of cantonment garbage as reported by the Bureau of Chemistry which show much wider range in composition than those reported by O'Brien and Lindemuth.

Stoller and others (4) arranged a wooden butter churn so that either air or water could be forced into it through holes in an iron pipe which was inserted into the churn. Fresh horse manure decayed rapidly when it was aerated, kept moist, and turned.

According to Tripp (5) the refuse from Royal Borough of Kensington, England, is dumped into large pits, whence it is removed by large grabs, put on belts, conveyed to crushers, screened, sprayed with a bacterial culture, and thence taken to large chambers or cells. He states that aerobic and anaerobic fermentation takes place with rise in temperature to 170° to 175°F . Sixteen days later the product carries about 30 per cent moisture and the nitrogen ranges from 0.97 to 1.42 per cent.

RESULTS

The percentage of nitrogen, ash, ether extractable materials, and the pH values of the various samples of waste are shown in Table I. The moisture content ranged from 67 to 73 per cent, the nitrogen from 1.73 to 3.37, the ether extractable materials from 6.42 to 25.63, the ash from 2.77 to 21.20, and the pH value from 4.38 to 5.95. Thus, aside from the water content, the variations were quite wide.

TABLE I
COMPOSITION OF KITCHEN WASTE. RESULTS EXPRESSED AS PER CENT DRY BASIS

Source and date of sampling			Water	Nitrogen	Ether soluble	Ash	pH
Hastings	April 13, 1941	1	72.3	1.73	13.44	2.77	4.38
	April 14, 1941	2	67.0	3.37	21.93	9.98	4.96
	April 15, 1941	3	70.2	2.11	13.60	6.22	4.61
	April 16, 1941	4	68.1	1.96	12.55	6.69	4.62
	April 18, 1941	5	69.4	1.82	20.37	8.41	4.70
Dobbs Ferry	April 25, 1941	1	67.6	2.38	25.63	7.79	5.73
	April 29, 1941	2	73.2	2.21	13.13	6.59	5.68
Yonkers	July 1939		70.2	2.10	7.4	Not run	5.40
	September 1939		69.4	2.48	10.4	10.4	5.72
	October 1939		68.0	2.56	19.1	20.6	5.41
	April 1940		71.0	2.23	12.0	19.6	5.13
	May 1940		68.4	2.31	7.80	12.4	5.68
	July 1940		67.4	2.40	17.62	14.6	5.58
	September 1940		71.2	3.22	6.42	21.20	5.54
	May 1941		69.4	2.66	18.2	10.95	5.95

One of the first phases of the investigation was that of temperature change in bulk samples of waste collected October 11, 1939, in Yonkers. The temperature of the untreated mass rose to 35° C. one foot inward from the surface three days after it was laid down. The heap with which was mixed 1 part of calcium carbonate to 20 parts of waste, dry basis, and inoculum from an active compost was more active, the temperature at the time being 45° C. One day later the differences were more striking. After five days the temperature of the control or untreated material was 51° and that of the treated was 67° C. The average temperature of the former for the period was 41° and the latter 48° C.

The rate of nitrate formation from the different materials is shown by the data in Table II. The most active of the garbage collections taken from Yonkers were those in September and in May. Those cultures which contained materials from Dobbs Ferry yielded less nitrate-nitrogen than did the controls at the end of the 30-day period, and those from Hastings and Yonkers (July collection) contained only slightly more than did the untreated ones. Thirty days later the differences between the amounts of nitrate extracted from the garbage lots were less striking and after 90 days

all cultures, with the exception of those which carried the Dobbs Ferry materials, contained much more nitrate-nitrogen than did the control cultures. It should be noted that the process tankage was more active at all periods than were the other materials. Judging from these results the nitrogen in kitchen waste or garbage should be looked upon as being rather slowly available to plants when added to the soil.

TABLE II

RATE OF NITRATE FORMATION FROM WASTE. RESULTS EXPRESSED AS P.P.M. NITRATE NITROGEN IN DRY SOIL

Cultural Treatment	Incubation period in days		
	30	60	90
Control	14.6	43.0	54.0
Process tankage	58.1	92.2	102.4
Dobbs Ferry waste (composite)	7.4	58.7	65.5
Hastings waste (composite)	15.1	59.0	98.5
Yonkers, July 1940 waste	16.7	48.0	98.6
Yonkers, Sept. 1940 waste	36.4	64.4	76.1
Yonkers, May 1941 waste	28.5	62.9	81.0

The effect of calcium carbonate and inoculum on the nitrogen and ash contents after different intervals of time is given in Table III. As decomposition proceeded, changes in structure and color of the treated composts took place rapidly, the mass became granular and darker in color. In addition, moisture was released. Moreover, ammonia was liberated a few days after the cultures were placed in the incubator. It should be noted also that the obnoxious volatile compounds for the most part had been dissipated. Owing to the changes and the time factor involved in a practicable method of procedure for processing the waste, the periods of incubation were relatively short.

An examination of the data in Table III reveals that the percentage of nitrogen in the various cultures increased and then declined as the periods of incubation lengthened. It should be noted, however, that the trend with respect to this element during the four-day period was upward, the change being highest in the controls followed in order by those to which lime, lime and inoculum, and inoculum were added. Four days later the percentage of nitrogen in each of the Yonkers composts was less than it was at the termination of the first period of incubation. The percentage of this element in the untreated composite materials and those from Hastings was about the same as it was four days earlier and that in the Dobbs Ferry materials

was greater. The reduction in the percentage of nitrogen in the composts to which lime had been added was slightly greater than it was in the remainder of the composts at the close of this period. After 16 days the cultures to which the inoculum was added contained less of this element than formerly and also less than the control cultures.

TABLE III

EFFECT OF CALCIUM CARBONATE AND INOCULUM ON RATE OF DECOMPOSITION OF WASTE.
RESULTS EXPRESSED AS PER CENT, DRY WEIGHT BASIS

Source of waste and culture number		Time of composting in days							
		0		4		8		16	
		Nitro- gen	Ash	Nitro- gen	Ash	Nitro- gen	Ash	Nitro- gen	Ash
Yonkers, May, 1941	1*	2.66	10.9	2.89	10.9	2.37	12.4	2.12	14.3
	2	2.56	14.7	2.78	15.7	2.53	16.2	—	—
	3	2.56	14.7	2.67	17.3	2.35	17.6	—	—
	4	2.66	10.9	2.70	15.1	2.39	15.0	1.78	16.4
Composite Hastings, Dobbs Ferry and Yonkers, May, 1941	1	2.40	14.6	2.69	14.0	2.66	15.0	2.30	17.4
	2	2.31	18.4	2.47	17.2	2.27	19.0	—	—
	3	2.31	18.4	2.45	18.3	2.36	20.7	—	—
	4	2.40	14.6	2.47	15.9	2.35	18.9	1.98	21.3
Dobbs Ferry composite	1	2.29	7.2	2.70	7.8	3.02	9.4	2.65	11.2
	2	2.20	10.9	2.45	11.4	2.59	14.0	—	—
	3	2.20	10.9	2.56	11.7	—	14.5	—	—
	4	2.29	7.2	2.59	9.2	2.48	10.8	2.23	12.5
Hastings composite	1	2.47	7.0	2.54	6.4	2.54	7.4	2.26	8.7
	2	2.38	10.8	2.43	9.7	2.21	11.5	—	—
	3	2.38	10.8	2.43	12.6	2.15	13.4	—	—
	4	2.47	7.0	2.52	9.3	2.40	12.4	2.09	15.4

* 1 = No treatment; 2 = 132 lbs. CaCO_3 per ton; 3 = 132 lbs. CaCO_3 per ton + inoculum; 4 = inoculum.

As measured by the increase in the ash content of the cultures (brought about by losses in the percentage of the organic portion) the addition of calcium carbonate alone did not alter significantly the rate of decomposition of the waste. The addition of the inoculum along with the carbonate increased somewhat the ash content of the waste from Yonkers and Hastings after four days and at the end of the eight-day period that of these and also the composite samples. The inoculum without the lime produced the greatest change in the materials and thus proved to be the most effective treatment for speeding up decomposition.

The question arose as to the effect of the presence of the fats in the waste on its value as a soil amendment. Accordingly the action of steapsin on the decomposition of the fats in waste from Dobbs Ferry, Hastings, and Yonkers, May 1941, as measured by the change in the percentage of ether

soluble materials, was determined. According to the data in Table IV two days after the cultures were placed in the incubator the ether soluble portion increased in the Dobbs Ferry and Hastings waste. Two days later or after four days of incubation it had decreased strikingly in those cultures to which the enzyme was added. This was also true of the Yonkers cultures during the next or eight-day period. It should be noted also that the percentage of nitrogen increased and then declined in all composts except in those made up of the waste from Dobbs Ferry.

TABLE IV

EFFECT OF STEAPSIN ON DECOMPOSITION OF KITCHEN WASTE. TEMPERATURE OF INCUBATION 40° C. RESULTS EXPRESSED AS PER CENT, DRY BASIS

Source of waste and culture number	Time of composting in days							
	0		2		4		8	
	Ether soluble	Nitrogen	Ether soluble	Nitrogen	Ether soluble	Nitrogen	Ether soluble	Nitrogen
Dobbs Ferry composite 1*	18.64	2.20	23.42	2.30	19.75	2.44	17.22	2.72
2	18.64	2.20	23.95	2.52	13.80	2.47	12.25	2.74
Hastings composite 1	15.67	2.38	20.60	2.36	21.40	2.48	22.97	2.29
2	15.67	2.38	24.20	2.37	12.58	2.58	15.75	2.46
Yonkers, May 1941 1	17.54	2.56	17.10	2.71	16.75	2.78	14.31	2.39
2	17.54	2.56	17.95	2.61	12.75	2.92	7.18	2.51

* 1 = Control + inoculum; 2 = Inoculum + 0.02 per cent steapsin.

It appears likely that the increase in the percentage of the ether soluble portion and nitrogen was due to the more rapid decrease in the amount of such materials as starches and sugars. The decline in the percentage of nitrogen probably was due to an increased rate of decomposition of the nitrogenous materials and to the loss of nitrogen by volatilization.

Whether or not it is advisable to add steapsin and thus decrease the fat content of garbage remains to be ascertained. When the digestive power of the microorganisms of the soil and the cost of the enzyme are taken into consideration, however, it appears to be doubtful.

Two series of cultures were prepared from Hastings 1, 3, 4 and Yonkers, May 1941, collections by adding inoculum in the usual manner. One set was placed in an incubator the temperature of which was 40° C. and the other in one held at 55° C. In addition, compost comprised of the Yonkers waste was incubated at 27° C. The percentage of nitrogen, ether soluble and ash contents after two and four days are given in Table V. It is obvious that the percentage of nitrogen in the cultures held two days at

40° C. invariably was greater than it was at the beginning of the test. Two days later it was greater in each. The percentage of this element in the cultures which were in the 55° C. chamber did not change significantly.

The percentage of ether soluble fraction after two days was less in those cultures taken from the incubator which was held at 40° C. than it was in those composted at 55° C. Two days later the same relationship held with the exception of the Hastings number 1 composts.

TABLE V

EFFECT OF TEMPERATURE ON RATE OF CHANGE IN NITROGEN, ETHER SOLUBLE AND ASH CONTENTS OF GARBAGE COMPOSTS. RESULTS EXPRESSED AS PER CENT, DRY WEIGHT BASIS

Source of waste and sample number	Temp., ° C.	Time of incubation								
		0 days			2 days			4 days		
		Nitro-gen	Ether soluble	Ash	Nitro-gen	Ether soluble	Ash	Nitro-gen	Ether soluble	Ash
Hastings	1 40	1.73	13.44	2.8	2.09	16.20	3.1	2.46	17.50	7.0
	1 55	1.73	13.44	2.8	1.89	19.25	5.2	1.93	17.70	5.8
	3 40	2.11	13.60	6.2	2.36	14.00	7.5	2.65	12.80	9.2
	3 55	2.11	13.60	6.2	2.22	17.20	7.6	2.30	16.20	8.8
	4 40	1.96	12.55	6.7	2.11	9.38	8.8	2.17	12.28	10.2
	4 55	1.96	12.55	6.7	1.97	13.00	9.6	1.95	13.50	9.3
Yonkers	27	2.66	18.20	10.9	2.66	18.52	11.0	2.70	18.02	11.5
May 1941	40	2.66	18.20	10.9	3.05	14.53	12.4	3.21	14.75	15.4
	55				2.72	17.70	11.5	2.69	17.53	12.9

The Yonkers cultures which were placed in a chamber the temperature of which was 27° C. did not change either in appearance or in composition.

Slowly rotating cultures comprised of Yonkers, May 1941, and composts of Hastings and Dobbs Ferry collections did not prove to be successful owing to the formation of the mass into pellets or balls of various sizes. These formed about 24 hours after the tests were inaugurated.

It appears that in the construction of a plant for processing kitchen waste or garbage for use as a soil improver provision should be made for heating the mass quickly to 40° C. by passing heated air through the chambers and by the incorporation of inoculum. The decomposition should be stopped before the percentage of nitrogen has declined. The mass should then be dried, mixed, and ground.

SUMMARY AND CONCLUSIONS

Representative samples of kitchen waste or garbage varied in nitrogen, ether soluble and ash contents.

The temperature of bulk samples when limed and inoculated rose more rapidly than did that of those which were not so treated.

The rate of nitrate formation in soil cultures to which were added

samples of various specimens in general was relatively slow, thus indicating delayed availability to plants when added to the soil.

Inoculum from active composts increased the rate of decomposition as measured by the change in the ash content of the composts. The percentage of nitrogen increased and then declined as the period of incubation lengthened.

The addition of steapsin resulted in a marked decrease in the content of ether soluble materials.

The waste incubated at 40° C. broke down more rapidly than it did when composted at either 27° or 55° C.

Suggestions as to what should be provided in the construction of a garbage processing plant are offered.

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DORMANCY IN SEEDS OF CONVALLARIA MAJALIS L. AND SMILACINA RACEMOSA (L.) DESF.

LELA V. BARTON AND ELTORA M. SCHROEDER

Dormancy in relation to seeds is a general term which has been used to indicate the failure of the embryo to resume growth when placed under conditions of temperature, moisture, and oxygen supply which ordinarily bring about germination. Many temperate-zone plants are known to produce seeds with embryos which continue to develop only after a period of exposure to low temperatures in a moist condition. These embryos after-ripen in a moist medium at temperatures of 1° to 10° C. so that when they are transferred to greenhouse temperatures of 20° to 25° C. germination proceeds immediately. In these cases the shoots emerge soon after the radicle and the plant appears above soil in a relatively short period of time. Among seeds possessing such characteristics are such widely different forms as *Pinus resinosa* Ait., *Celastrus scandens* L., *Gentiana crinita* Froel., *Rosa multiflora* Thunb., and *Typha latifolia* L.

Some other seeds which might be expected to respond favorably to this type of treatment have failed to germinate. It has been found in the cases of *Symphoricarpos racemosus* Michx., *Tilia americana* L., and some others that fully imbibed embryos will after-ripen in the same manner as described above but the impermeable coats characteristic of these seeds prevent the absorption of water without which after-ripening cannot take place. The procedure for bringing about germination in these instances is treating the seeds to render the coats permeable and then following with a period in a moist medium at low temperature to after-ripen the embryo, after which it resumes growth in the manner already described. These hard coats may be broken down by any of the approved methods of mechanical abrasion or by sulphuric acid treatment. They may also be made permeable by planting in a moist medium and leaving for a period of a few months at a temperature of 20° C. or above during which time microorganisms become active and break down the seed coats.

Another class of dormancy has been found in which the initial stages of germination, i.e. protrusion of the radicle and hypocotyl, occur without any pretreatment. Such roots continue to develop until the food material stored in the seed is exhausted. In these, the root systems will eventually die unless the dormancy of the epicotyl or the bud which forms it is broken. This requires a period at low temperature which must be given *after* the seed has germinated and the root started to elongate. The dormancy may be broken at any time between the first appearance of the radicle and the

time of the maximum development of the root system from the stored food. Seeds reported to show epicotyl dormancy are those of tree peony and some species of *Viburnum* and *Lilium*. It becomes evident, then, that it is necessary to distinguish between root and shoot production especially as regards the dormant state.

All of the dormancy types listed above have been more fully described with accompanying citations to detailed experiments in a general article on "Experiments at Boyce Thompson Institute on germination and dormancy in seeds" (4).

The experiments reported below, in which seeds of *Convallaria majalis* L. and *Smilacina racemosa* (L.) Desf. were the test material, show epicotyl dormancy of a slightly different type from any reported to date in that the period at low temperature must be given, not merely after root production, but after the shoot has developed to a certain stage. Low temperature is ineffective in breaking the dormancy if applied before this certain stage of development is reached.

All seeds were either mixed with moist granulated peat moss or planted in soil for these tests.

EXPERIMENTAL RESULTS AND DISCUSSION

CONVALLARIA MAJALIS L.

A preliminary experiment with a limited seed supply was begun in March, 1937, using seeds harvested in 1936. Seeds were mixed with moist granulated peat moss and placed at 5° C. Samples of 100 seeds each were removed after two, three, four, five, and six months at 5° C. and were mixed with moist granulated peat moss at 20° C. or were planted in soil in the greenhouse. Results indicated that roots were obtained with comparative ease. Up to 92 per cent of the seeds showed radicle growth in moist peat at 20° C. within two months after transfer from 5° C. Three months' pretreatment at 5° C. resulted in 92 per cent root development after transfer to 20° C. as compared with 46 per cent after two months' pretreatment. Periods longer than three months at 5° C. preceding the germination temperature of 20° C. showed no advantage over the three-month period.

Samples planted in soil in the greenhouse after various periods failed to produce seedlings. It should be kept in mind that this failure refers to the production of green shoots above soil. Examination revealed the presence of roots underground. This pointed to epicotyl dormancy. This conclusion was further indicated by the fact that green shoots were formed when two of these flats were removed from the greenhouse and placed in a cold frame with a board cover for one winter. Furthermore, seeds with no pretreatment planted in soil in a flat and placed out-of-doors in March, 1937, and allowed to remain in a board-covered frame over winter, produced 82 per cent green shoots by April of the following year. Hence a period at high

temperature during the summer caused germination and the development of roots, and the cold of the following winter broke the dormancy of the epicotyl so that green plants were produced. These results also indicated that a pretreatment period in moist condition at low temperature was not necessary for root production but was necessary for shoot production. However, since the seeds were planted in March, they received a short period of cool weather before summer. Later experiments have shown that this may have had some effect on root production.

A large supply of hybrid seeds collected at the New York Botanical Garden in September, 1939, made possible a more complete experiment. These seeds were cleaned of their pulp and experiments were begun within a month after collection. The results of the experiments with this seed lot will be discussed under three headings: 1. Root production, 2. Production of the first green leaf, and 3. Production of the second green leaf.

Root Production

Samples of 200 seeds each were mixed with moist granulated peat moss and placed at various controlled temperatures. Constant temperatures of 15°, 20°, 25°, and 30° C. were used and resulted in root production of 0, 16, 62, and 20 per cent respectively. Daily alternating temperatures of 10° to 20°, 10° to 30°, 15° to 30°, and 20° to 30° C. produced 0, 10, 4, and 84 per cent germination. These cultures were left at the lower temperature for 16 hours and at the higher temperature for 8 hours daily. At all temperatures where roots were produced, development began within a month and was practically complete within two months. Cultures were left at 15° C. and at 10° to 20° C. for three months, but no seedlings appeared. Other experiments have shown that roots will be produced at these temperatures if they are allowed to remain for a longer time.

Some untreated seeds were also planted in soil in flats on October 18, 1939. These flats were placed in greenhouses with approximate temperatures of 21° and 15° C., and in cold frames provided with mulch and board cover and with board cover only for the winter months. Sample flats from each of these conditions were examined for seedlings in May and September, 1940, and May, 1941. The results showed a root production of 61 and 60 per cent respectively for the greenhouses of 15° and 21° C. by May, 1940, while only 7 and 5 per cent of the seeds had produced roots in the cold frames. By September, 1940, from 74 to 82 per cent of the seeds in the 15° C. greenhouse and in both cold frames had produced roots, while root production in the 21° C. greenhouse remained 60 per cent. These figures show that although *Convallaria* seeds do not require pretreatment at low temperature for root production, such production is increased in soil by previous exposure to low temperature. This fact was further demonstrated by data to be presented below. By May, 1941, the root production under

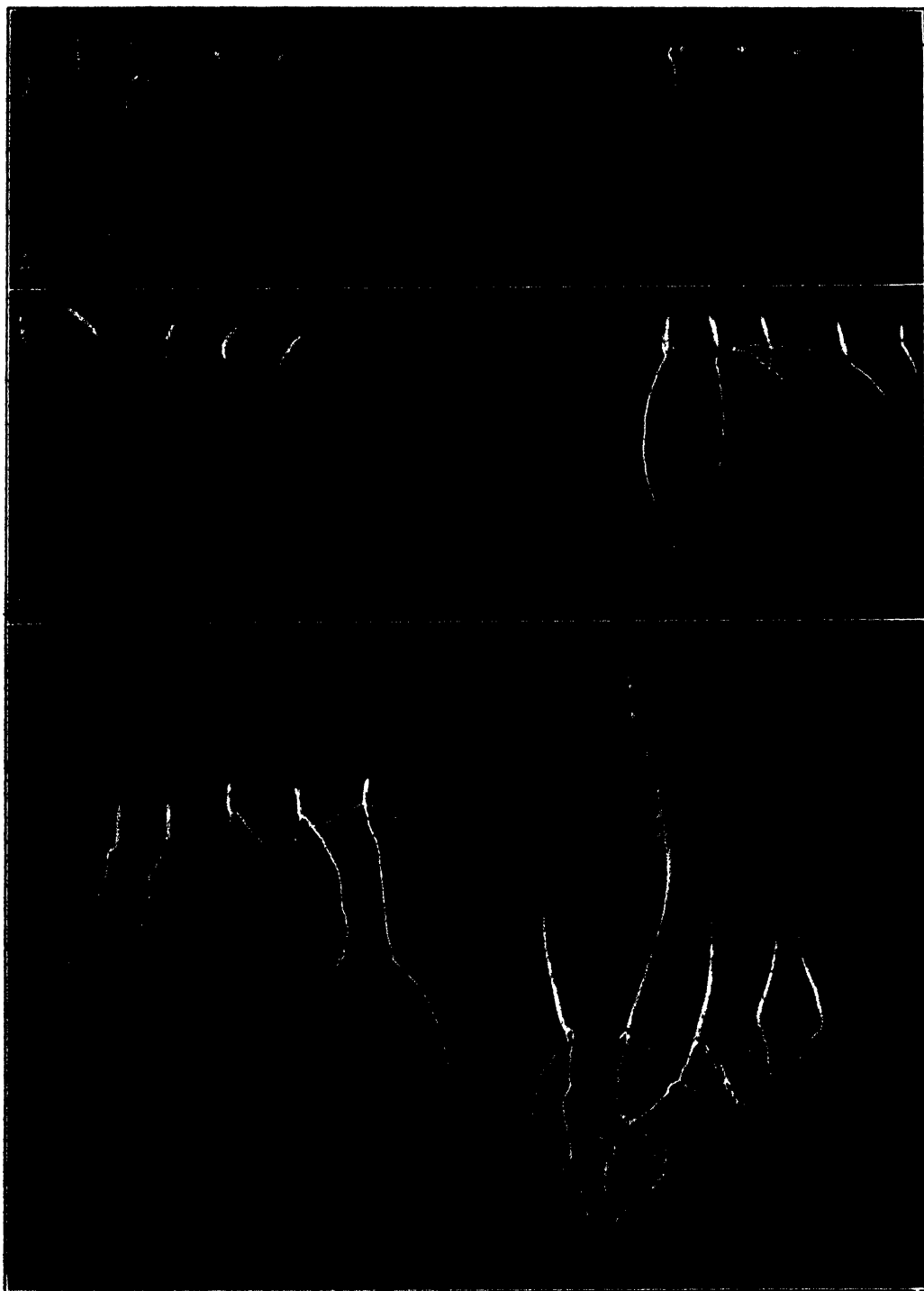


FIGURE 1. Germination of seeds and development of seedlings of *Convallaria majalis* planted October, 1939 and removed from the soil for examination. A. May, 1940, B. October, 1940, and C. May, 1941. Representative lots of five each selected for photograph. Left, kept in greenhouse; right, kept in cold frame with a board cover during the winter months.

these conditions had not increased, but there was a differential production of shoots depending on the temperature. This will be discussed below.

The results of planting seeds on October 18, 1939 in a greenhouse at a temperature of approximately 21° C. or in the board-covered frame is shown in Figure 1. Five representative seedlings were selected in each case for photographs. Here it will be seen that there was a great deal of difference in root development under the two conditions in May, 1940, but this difference had disappeared by September, 1940. In May, 1941, however, those seedlings receiving another winter in the board-covered frame had continued to develop, while those remaining at 21° C. were in the same stage of development as in May of the previous year.

Twelve thousand seeds mixed with moist granulated peat moss were placed at 5° C. at the same time the previous tests were begun. After three months at 5° C. all of these were transferred to 20° C. where 11,814 seedlings were produced. These seedlings were used for epicotyl dormancy tests to be reported below. Germination at 20° C. began in three weeks to one month after transfer from 5° C. and was practically complete in two months. From another seed lot at 5° C., samples were removed to 20° C. after two and four months at the low temperature. Four months offered no advantage over the three-month period while a slightly lower final germination percentage was obtained after two than after three months' low-temperature treatment. The germination period at 20° C. was the same regardless of length of pretreatment at 5° C. Twenty degrees C. was used here for germination because of indications of preliminary tests that this was a favorable temperature. However, since 25° C. and 20° to 30° C. had proved better for this particular crop, some additional samples from low temperature were placed at these temperatures for germination, with the same results as at 20° C. It would seem, then, that *Convallaria* seeds, pretreated at 5° C. for a period, become less specific in their temperature requirement for root production.

It will be noted that no advantage is gained by low-temperature pretreatment in time required for root production. On the contrary, the three months at 5° C. is additional since germination is complete at the higher temperatures in two months, regardless of whether low-temperature pretreatment has been given. However, the percentage root production at temperatures around 20° C. can be increased by low-temperature pretreatment from 16 per cent in peat in an oven at 20° C. to 82 per cent, and from 60 per cent in soil at 21° C. to 92 per cent.

To summarize the requirements for root production in *Convallaria*, it may be said that either a constant temperature of 25° C. or a daily alternating temperature of 20° to 30° C. was favorable. Other temperatures were less effective. Unless controlled temperatures are available, better results will be obtained if the seeds are pretreated in a moist medium for three months at 5° C.

Production of the First Green Leaf

The failure of *Convallaria* seedlings to produce shoots even long after normal roots had developed pointed to the possibility of epicotyl dormancy as has been reported for some other forms (2, 3, 6). Consequently, seeds which had produced roots in moist granulated peat moss at controlled temperatures in the 1937 preliminary experiment described above, were removed and planted in soil in pots. These pots were then placed at 5°, 10°, or 15° C. for various lengths of time. Control lots were placed in the greenhouse. Contrary to expectations, periods at 5° or 10° C. which had been found effective for breaking epicotyl dormancy in other forms could not be depended upon to produce green shoots of *Convallaria*. In some cases



FIGURE 2. Stages in the development of the seedlings of *Convallaria majalis*. Left to right: Stage 1, the protrusion of the radicle and hypocotyl; Stage 2, the first evidence of shoot development; Stage 3, further growth of the shoot to break through the cotyledonary sheath.

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shoots were obtained upon removal of the pots from the cold rooms to the greenhouse, but the results were by no means consistent. Examination of the seedlings of the control lots kept in the greenhouse for two months, as compared with those kept at the lower temperatures for the same period, revealed that those of the former had made much better development than those of the latter, in spite of the fact that no green shoots were produced in the controls whereas low-temperature treatment sometimes brought about such development. Some of the control seedlings in an advanced stage of development were then transferred to 5° C. for a period of two months after which they were replaced in the greenhouse where excellent stands of green leaves were obtained. It appeared then that not

only did the epicotyl need an after-ripening period at low temperature, but this low temperature was effective only if given after a certain stage of development had been reached. It was further indicated that this stage was attained by certain seedlings at low temperatures in three months but that many more seedlings failed to develop rapidly enough at low temperature. On the other hand, the greenhouse temperature was effective in bringing about development but not effective for after-ripening.

The adequate supply of hybrid seeds collected at the New York Botanical Garden in September, 1939, and the large number of seedlings with the roots developing obtained from oven tests, made possible a thorough study of the problem involved. In addition to experiments using seedlings, seeds were also planted in soil and the green shoots resulting from specific treatments noted.

First, it was necessary to know how much development of the shoot took place at various temperatures. For convenience in the ensuing discussion, we shall refer to three stages of seedling development, illustrated in Figure 2. Stage 1 is represented by a very small seedling formed by the protrusion of the radicle and hypocotyl. In Stage 2 the development of the shoot first becomes evident, while in Stage 3 the embryo has grown and broken through the cotyledonary sheath. The seedlings at Stage 3 are still below the surface of the soil.

Very young seedlings (Stage 1) were removed from the peat medium in ovens at controlled temperatures and planted in soil in pots. These pots were placed at various temperatures for 1, 2, 3, 4, and 5 months. Duplicate pots were used in each case and at the end of the allotted period, one pot was transferred to the greenhouse where green shoot production was noted, and the corresponding pot was sieved carefully and the condition of all the seedlings recorded. Photographs of five representative seedlings of each lot are shown in Figure 3. Here it will be seen (Fig. 3 A) that the best seedling development during the first month had taken place in the greenhouse which had an approximate temperature of 21°C . All of these seedlings had reached Stage 3. Seedlings grew more slowly at 15° and 10°C ., but all had reached Stage 2 in one month. At 5°C ., however, the development was very much retarded. Other samples of the same lot of seedlings planted in soil at the same time and allowed to remain in low temperature for four months before transfer and examination appeared as shown in Figure 3 B. No further development had taken place in the greenhouse but the seedlings at 15° and 10°C . had advanced to Stage 3. Five degrees C., on the other hand, not only inhibited development, but actually injured the seedlings. These tests were repeated many times using hundreds of seedlings with essentially the same results. If seedlings were allowed to develop to Stage 2 before being placed at various temperatures the time required to reach Stage 3 in the greenhouse or at 15° or 10°C . was short-

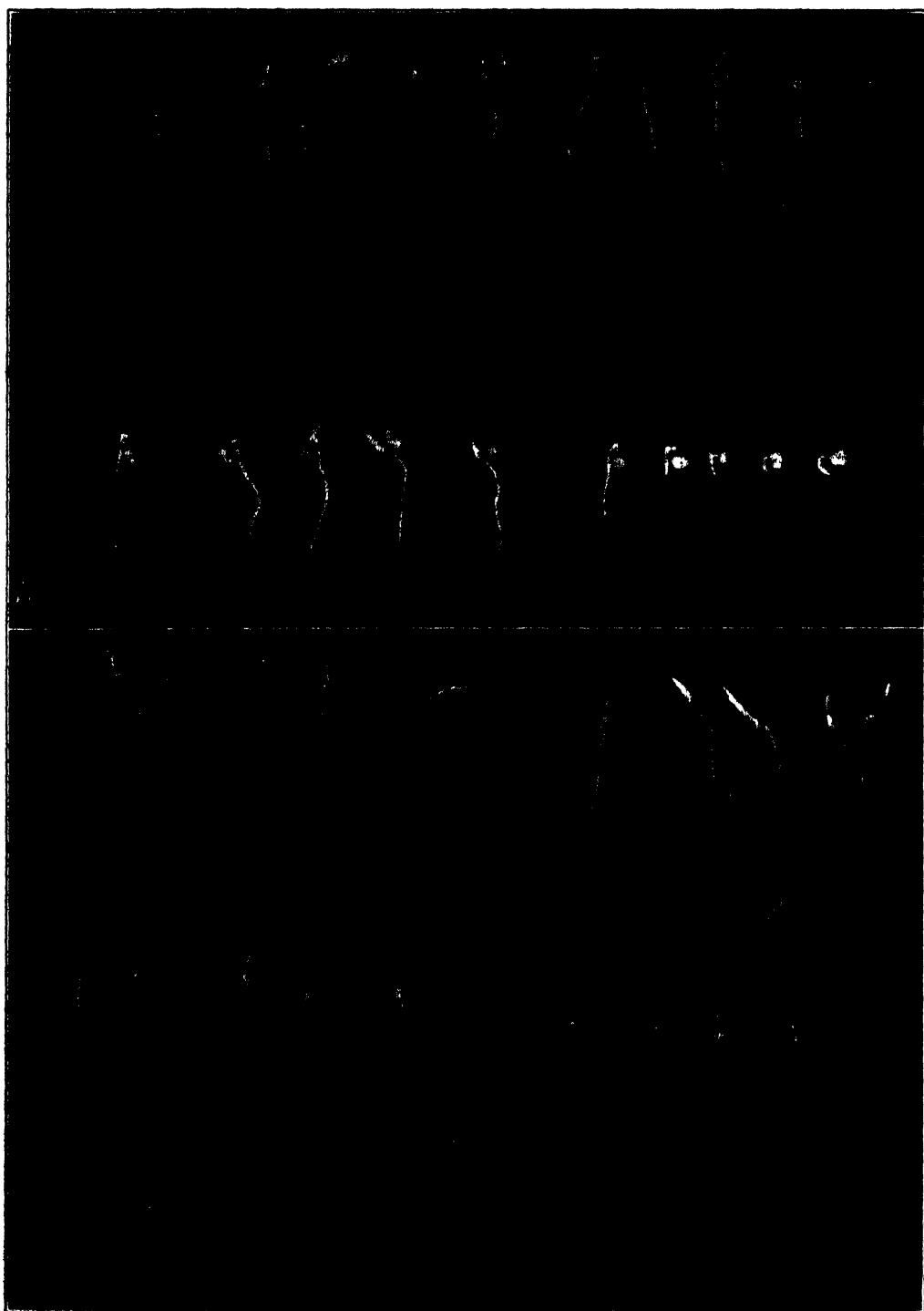


FIGURE 3. Development of seedlings of *Convallaria majalis* planted in soil and placed at different temperatures for A. 1 month; B. 4 months. Five seedlings each. Top row: greenhouse; 15° C.; bottom row: 10° C.; 5° C.

ened while less injury was sustained by the seedlings at 5° C. In fact, in this case, some of the seedlings developed normally, although very slowly at 5° C.

The next question was whether these seedlings would continue to develop and form green leaves. Up to 20 per cent of those left at 10° C. for five months produced green shoots when transferred to the greenhouse. When seedlings were allowed to develop to Stage 2 before they were planted, up to 32 per cent of them continued growth and formed green leaves after five months at 5° C. Smaller seedlings placed at 5° C. did not develop so well. Fifteen degrees C. was not satisfactory for after-ripening although the seedlings grew to Stage 3 at this temperature and appeared healthy. Similarly, the greenhouse, while furnishing a favorable temperature for growth to Stage 3, did not after-ripen the epicotyl, so no green leaves appeared above ground there unless a period at low temperature was provided.

It would seem, then, that *Convallaria* seedlings must develop to the point where the embryo has broken through the cotyledonary sheath, before dormancy of the shoot bud can be broken. Growth to this stage was more rapid at a temperature of approximately 21° C. than at lower temperatures. It is desirable that young seedlings should be allowed to remain in the greenhouse for a time, after which they should be given a period at low temperature and then transferred back to the greenhouse for green shoot production.

In order to test this point further, an experiment was designed whereby the seedlings were planted in pots which were kept for 1, 3, or 5 months at 5°, 10°, or 15° C. after periods of two weeks or 1, 2, 3, 4, or 5 months in the greenhouse. Twenty-five seedlings were planted in each pot. Enough pots were planted to permit the sieving of one pot for the examination of the seedlings every time a transfer was made, so that it was always possible to compare the stages of seedling development with the effectiveness of epicotyl after-ripening, as measured by the appearance of green shoots in replicate pots transferred to the greenhouse. Two control sets were used, one placed directly at 5°, 10°, or 15° C. without a previous period in the greenhouse, and the other set allowed to remain in the greenhouse for the entire period. No green shoots ever resulted from the greenhouse treatment alone. The results of the other tests are shown in Table I. As noted above, better results were obtained when seedlings at Stage 2 rather than smaller ones were planted. In one series, a mixture of Stages 1 and 2 was used, while in the second series, Stage 2 only was planted. The data in this table show clearly the beneficial effect of a period in the greenhouse preceding the low-temperature treatment. Even two weeks in the greenhouse increased the effectiveness of the low temperatures in overcoming epicotyl dormancy, but one month proved better. Examination of the seedlings

after one month in the greenhouse revealed that all except an occasional one had developed to Stage 3. A temperature of 5° C. was better than 10° C. for after-ripening as evidenced by the percentage of green shoots appearing

TABLE I

PRODUCTION FROM GERMINATED SEEDS OF THE FIRST GREEN LEAVES ABOVE SOIL IN THE GREENHOUSE AFTER VARIOUS TEMPERATURE TREATMENTS

Species	Time in greenhouse	Seedling stage at time of planting	% Green leaf production after months at low temp.								
			5° C.			10° C.			15° C.		
			1	3	5	1	3	5	1	3	5
<i>Convallaria majalis</i>	None	1 & 2 2	0 0	0 36	12 32	0 0	0 0	20 8	0 0	0 0	0 4
	2 wks.	1 & 2 2	0 0	44 48	28 56	0 0	12 8	8 24	0 0	0 0	0 0
	1 mo.	1 & 2 2	0 4	48 64	40 84	0 0	8 8	16 16	0 0	0 0	0 0
	2 mos.	1 & 2 2	0 0	32 76	32 72	0 0	8 0	24 48	0 0	0 0	0 0
	3 mos.	1 & 2 2	4 0	4 52	40 60	0 0	0 8	4 28	0 0	0 0	0 0
	4 mos.	1 & 2 2	0 8	20 60	24 60	0 0	8 0	0 12	0 0	0 0	0 0
	5 mos.	1 & 2 2	0 4	0 64	52 56	0 0	4 0	12 12	0 0	0 0	0 0
<i>Smilacina racemosa</i>	None	1 & 2 2	0 0	0 0	0 0	0 0	0 4	8 16	0 0	0 0	0 12
	2 wks.	1 & 2 2	0 0	0 0	0 0	0 0	0 4	68 48	0 0	0 0	4 8
	1 mo.	1 & 2 2	0 0	0 4	36 16	0 0	4 0	64 52	0 0	0 0	8 40
	2 mos.	1 & 2 2	0 0	40 —	68 44	0 0	12 —	56 64	0 0	0 —	32 24
	3 mos.	1 & 2 2	0 0	— —	28 16	0 0	— —	48 52	0 0	— —	40 24
	4 mos.	1 & 2 2	0 5	— —	28 55	0 5	— —	40 57	0 5	— —	12 57
	5 mos.	1 & 2 2	4 41	20 45	38 41	4 18	16 32	28 23	0 5	4 86	20 50

after such treatment, and five months were better than three months. One month at low temperature was without effect. Periods longer than five months (six and seven months) were tried in a limited number of cases but showed no advantage over the five-month period. Indeed, injury was apt

to result from extended treatment, since the cotyledonary sheaths started to elongate in the cold rooms which were dark. These shoots became etiolated and often died upon removal to the greenhouse. A temperature of 15°C ., while effective for growth up to Stage 3, was not favorable for after-ripening. It is of interest to note that while the seedlings may be given low temperature effectively after only two weeks in the greenhouse, this treatment could be given at any time up to five months. Even after five months, however, the development had not exceeded Stage 3 shown in Figure 2. The seedling production after different periods in the greenhouse preceding three months' treatment at 5°C . is shown in Figure 4. The failure to obtain higher percentages of green shoots with favorable conditions was due to the death of some of the seedlings, which may have been injured at the time of removal from the peat moss at controlled temperatures and planting in the soil.



FIGURE 4. Effect of different periods in the greenhouse preceding treatment at 5°C . on subsequent green leaf production of *Convallaria majalis* in the greenhouse from germinated seeds. A. 4 months at 5°C .; B, C, D, E, and F. 3 months at 5°C . preceded by 2 weeks, 1 month, 2 months, 3 months, or 4 months, respectively, in the greenhouse. Twenty-five seedlings planted in each pot.

The appearance of the pots transferred for shoot production after pretreatment of one month in the greenhouse followed by three months at 5° , 10° , or 15°C . is shown in Figure 5 A. The effectiveness of 5°C . as an after-ripening temperature is seen here. Figure 5 B shows the comparative effectiveness of one and three months at 5°C . for after-ripening. In Figure 5 C. is shown the effect of a total of four months' treatment on the production of green leaves. Here again it is demonstrated that the pretreatment with high and low temperatures must be properly distributed. Whereas one month in the greenhouse plus three months at 5°C . resulted in a good stand of seedlings, the reverse, or three months in the greenhouse plus one month at 5°C ., was ineffective. This does not mean that the seedlings were dead or even injured in the latter case, but simply that one month at 5°C . was insufficient for after-ripening the epicotyl and three months in the greenhouse was longer than is necessary for development to the stage where low temperature is effective.

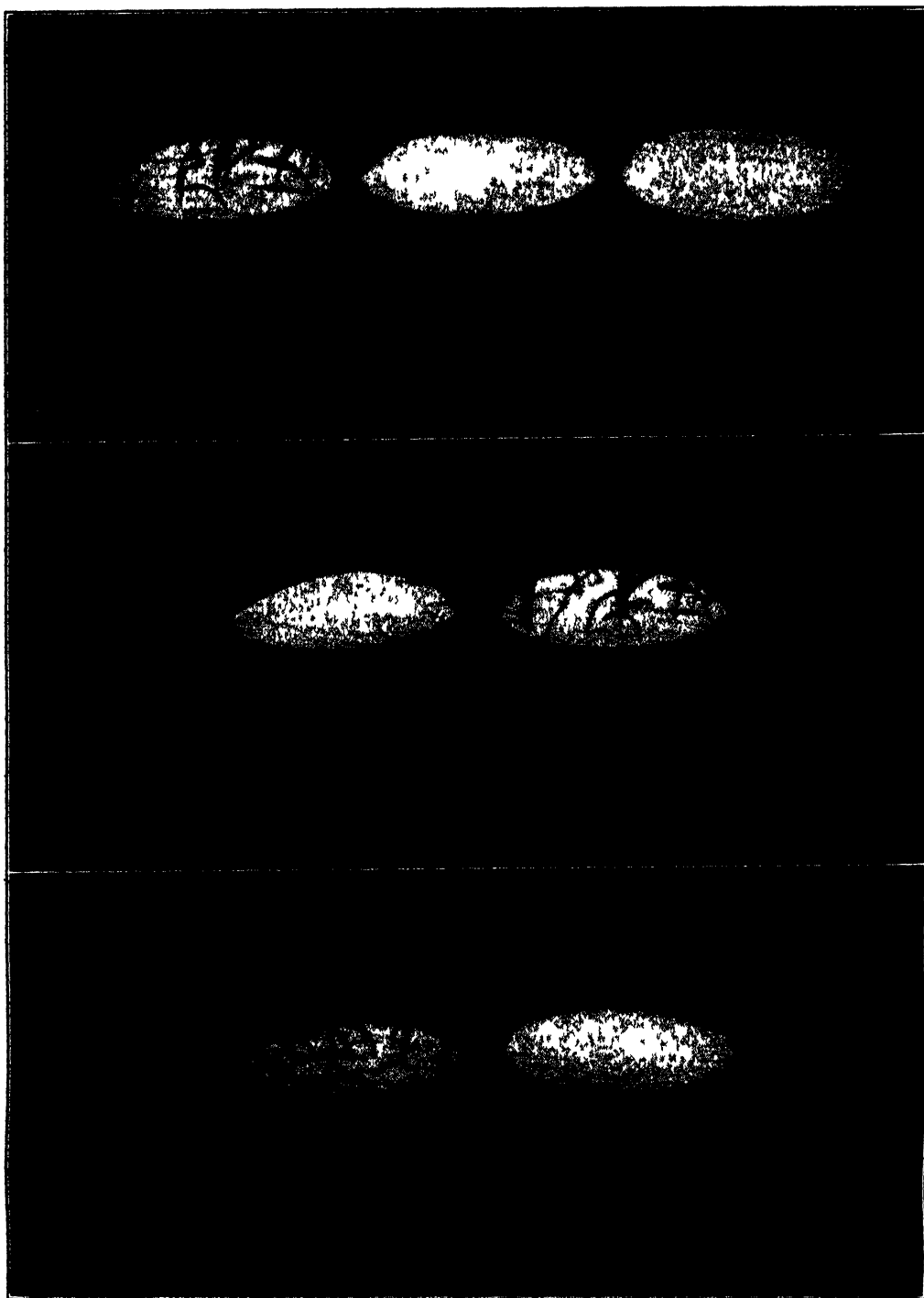


FIGURE 5. Effect on green leaf production of different treatments of seedlings of *Convallaria majalis*. A. One month in the greenhouse followed by 3 months at 5° C., 10° C., and 15° C. B. One month in the greenhouse followed by 1 and 3 months at 5° C. C. Left: 1 month in the greenhouse followed by 3 months at 5° C.; right: 3 months in the greenhouse followed by 1 month at 5° C.

It should be emphasized that seedlings which have developed to Stage 3 in the greenhouse followed by three or more months at lower temperatures appear the same whether that lower temperature has been 5°, 10°, or 15° C. That this appearance is deceiving, however, as far as actual after-ripening is concerned, has been shown in Table I and Figures 4 and 5. This was exhibited in an even more striking manner when the seedlings which were obtained by sieving the treated pots at the time of final transfer to the greenhouse for green shoot production were placed on moist filter paper in light at room temperature. Such seedlings as had received pre-treatment consisting of one month in the greenhouse followed by four months at 15°, 10°, or 5° C. are seen in Figure 6. Again five representative seedlings from each lot were selected for photographs. Figure 6 A shows their appearance after four days on moist filter paper. No apparent differences were to be found in the development of the seedlings from the three temperatures. That there were differences, however, was evidenced by their appearance after two weeks on moist filter paper in light as shown in Figure 6 B. Here it was discovered that the changes necessary to break the dormancy of the epicotyl had not occurred at 15° C. since no further development took place. On the other hand, seedlings which had been exposed to 10° or 5° C. continued to develop, and formed green leaves.

The discussion of shoot production up to this point has been confined to experiments using seeds which had already germinated to form a root.

Experiments were also performed in which 100 seeds each which had been in moist granulated peat moss at 5° C. for two, three, or four months were planted in soil and placed in the greenhouse where they were allowed to remain until a duplicate sample in peat moss, made at the same time and also kept in the greenhouse, had completed root production. This last sample was made in order to permit an estimate of the time required for full germination of the seeds in the flats without disturbance for examination. Here again, as in the cases of seedling plantings in pots, enough flats were sown to permit examination of the seedlings of a flat by sieving every time a transfer was made, so that the stage of development of the seedlings was noted at every step. After 62 to 98 days in the greenhouse, at which time sieving revealed 89 to 93 per cent root production and practically all of the seedlings developed to Stage 3, the flats were transferred to cold rooms at 5°, 10°, or 15° C. for periods of three, four, or five months. They were then replaced in the greenhouse for green shoot production. Control lots were kept in the greenhouse where no green leaves were ever formed. The results are shown in Table II.

Again 5° C. proved the most satisfactory temperature of those tried for breaking epicotyl dormancy of *Convallaria*, while 15° C. was totally ineffective. Three to five months was the best time. Sieving the soil of all of these flats, when no more green shoots appeared, revealed, as reported

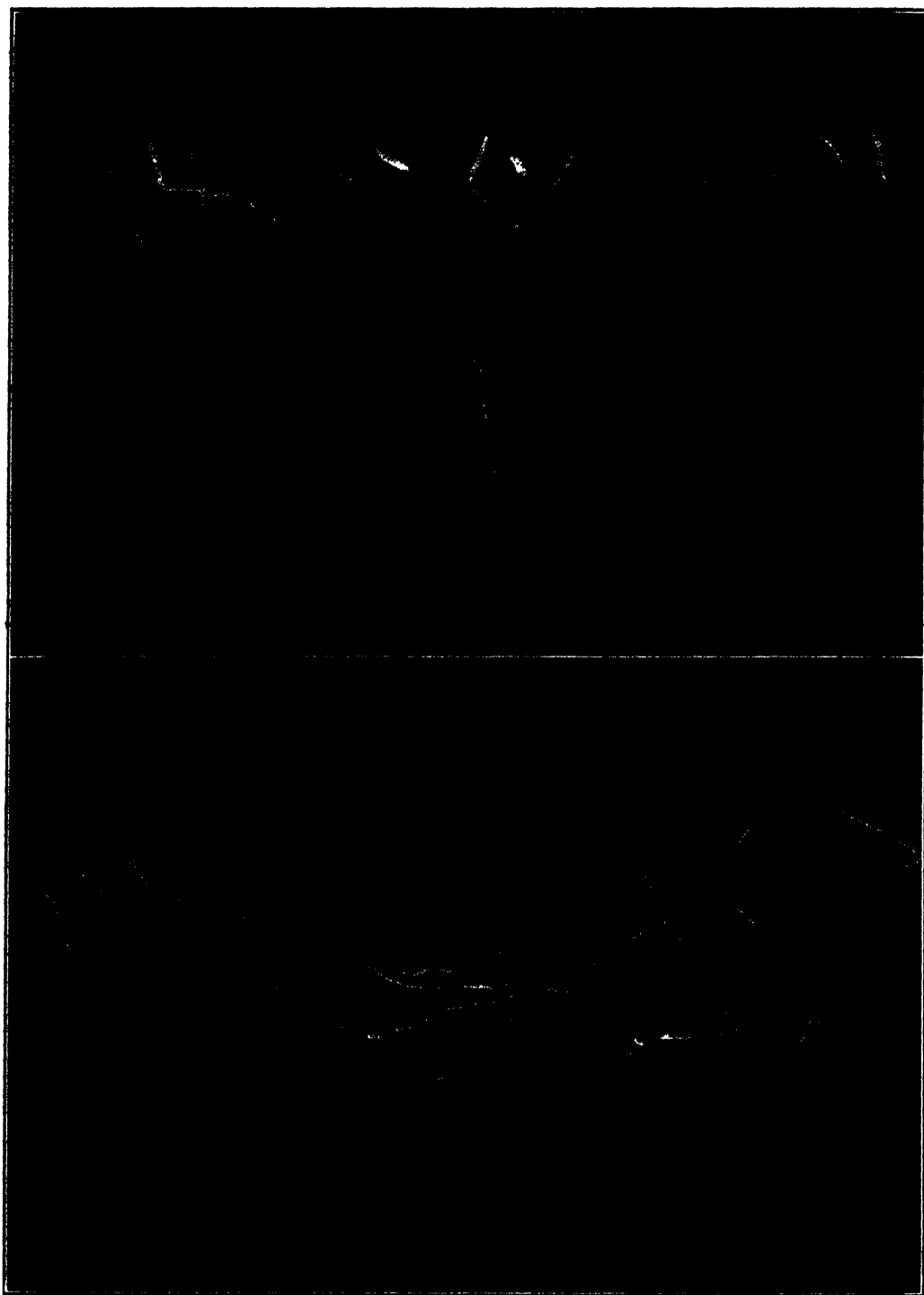


FIGURE 6. Seedlings of *Convallaria majalis* on moist filter paper in light at room temperature A. 4 days, and B. 2 weeks after removal from cold rooms where they had been in moist soil for 4 months. Five seedlings each. Left to right: 15° C., 10° C., and 5° C.

above, that the seedlings which were still below the ground were, for the most part, in very good condition but not further developed than Stage 3.

It will be recalled from the discussion of "Root Production" above, that the initial treatment or stratification of seeds at low temperature was not absolutely necessary since 60 per cent root production occurred when seeds

TABLE II

PRODUCTION OF THE FIRST GREEN LEAVES ABOVE SOIL IN THE GREENHOUSE. SEEDS PRE-TREATED FOR VARIOUS LENGTHS OF TIME IN MOIST GRANULATED PEAT MOSS AT 5° C. PLANTED IN SOIL FOR FURTHER TREATMENT

Species	Months in peat at 5° C.	Days in soil in greenhouse	% Root production	Months in soil at low temperature	Green leaf production after period at		
					5° C.	10° C.	15° C.
<i>Convallaria majalis</i>	2	98	92	3	69	12	0
				4	57	12	0
				5	84	21	0
	3	76	93	3	55	7	0
				4	71	13	0
				5	40	28	0
	4	62	89	3	12	0	0
				4	44	10	0
				5	58	21	0
<i>Smilacina racemosa</i>	3	99	40	4	—	21	0
				5	—	36	1
	5	94	54	4	—	18	1
				5	—	41	3
	6	85	80	4	—	23	1
				5	—	84	4
	7	89	90	4	—	41	8
				5	—	95	14
				6	—	98	33
				7	—	79	53
	8	78	74	4	—	22	3
				5	—	57	45
				6	—	54	49
				7	—	60	44

were planted directly in soil in the greenhouse. However, after low-temperature pretreatment, up to 93 per cent root production was obtained. This advantage of initial low-temperature treatment of the seeds or of a fairly low germination temperature (cf. various temperature effects on germination as discussed above) was further shown by plantings made at different times of year and wintered in a board-covered cold frame. Planting the seeds in October, 1939 or in March, 1940 resulted in good seedling production by April, 1941. In the former case the seeds received cold for one winter while in the latter, fairly low temperatures prevailed during the

germination period. In both cases, root systems were formed during the spring and summer of 1940 and the cold of the following winter broke the epicotyl dormancy so that green shoots were formed in the spring of 1941. Planting the seeds as late as May exposed them to higher temperatures, less favorable for germination, so that few roots were produced. Planting as late as July allowed the germination of very few seeds.

The aspect of seedlings kept in the greenhouse, as compared with those kept outside in a cold frame covered with boards during the winter, has been shown in Figure 1. Attention should be called once more to the fact that seedlings in the two places appeared similar in May, 1940, after planting in October, 1939. However, by May, 1941, those seedlings which had been exposed to low temperatures after the roots were formed and the seedlings had developed to Stage 3, continued to grow and formed green shoots. In addition to a board-covered frame, a frame with both mulch and board cover was used for winter conditions. The two cold frames proved equally adequate for bringing about shoot production.

In conclusion, it may be stated that *Convallaria* exhibits an unreported type of epicotyl dormancy. This dormancy exists in the leaf primordium *after* it has started to develop and has broken through the cotyledonary sheath. Low temperature, preferably 5° C., is effective in bringing about further growth forming a green leaf above the soil surface, only if the seedling is exposed to low temperature after it has developed to this stage. Previous exposure is ineffective but the leaf primordia will remain dormant and in good condition for several months if no low temperature is given.

Production of the Second Green Leaf

About four weeks after the full development of the first green leaf of *Convallaria*, the bud for the second green leaf appears below the surface of the soil. As this bud completes its formation, the first leaf begins to turn brown but does not die back completely for several months.

Some preliminary tests have been made to determine the conditions which will bring about resumption of growth of this second bud and the formation of the second green leaf.

Seedlings with the second bud well developed and with the first leaves dying back, were placed at 5° C. for two weeks, and one, two, and three months, after which the pots were transferred back to the greenhouse. One pot of seedlings was left in the greenhouse for a control. Two weeks were not sufficient to break the dormancy of the buds, but one month at 5° C. induced the development of the second green leaf in six out of ten plants while two or three months induced second-leaf development of all plants.

These facts, together with those reported above, offer a possible means of hastening the growth and maturity of *Convallaria* plants used in breeding or other special tests.

SMILACINA RACEMOSA (L.) DESF.

Seeds of *Smilacina racemosa* show the same type of dormancy as that exhibited by *Convallaria majalis* but to a greater degree. Only occasional roots appear in soil in the greenhouse from seeds which have not received low-temperature pretreatment, and a longer period of cold is necessary to after-ripen the epicotyl so that the first green leaf develops above the surface of the soil.

Root Production

The percentages which appear in the germination results of *Smilacina racemosa* are based on the number of seeds planted. However, an examination of 100 of these seeds revealed 191 embryos distributed as follows: 30 seeds with one embryo each; 39 with two embryos; 12 with three embryos; 10 with four embryos; three with one large and one small embryo; one with one embryo which had two cotyledons; and five without embryos or with embryos disintegrated.

As in the case of *Convallaria majalis*, many preliminary experiments were performed over a period of several years using seeds collected in the vicinity of Yonkers, New York. The production of roots at various temperatures without any kind of pretreatment was noted for three different seed collections. Constant temperatures of 1°, 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. were used in addition to daily alternating temperatures of 10° to 20° C., 10° to 30° C., 15° to 30° C., and 20° to 30° C. In the various tests, the largest root production ever obtained was 25 to 27 per cent at a constant temperature of 25° C. or a daily alternating temperature of 20° to 30° C. No seedlings ever developed at constant temperatures of 30° C. or 35° C. and only occasional ones at the other temperatures tried in spite of the fact that many of these cultures were kept for seven months or longer. It will be recalled that some of these conditions afforded higher percentages of roots from *Convallaria majalis* seeds. When *Smilacina* seeds were planted in soil and kept in a greenhouse of approximately 21° C. during the winter months, no roots appeared within a period of 21 months. An effective method for root production consisted of treatment in a moist medium for seven months at 1° or 5° C. followed by a transfer to the greenhouse or to controlled temperatures of approximately 20° to 25° C. After treatment in this manner, up to 90 per cent of the seeds germinated to form roots. Low-temperature pretreatment permitted germination over a range of temperatures greater than that tolerated by untreated seeds, but no roots were ever produced at constant temperatures as high as 30° C. regardless of manner of exposure. Periods shorter than seven months at low temperature resulted in less germination upon removal to higher temperature and 8, 9, 10, or 11 months failed to give any improvement over the seven-month period. Four months at low temperature were of some advantage to root

production, resulting in an increased germination over the untreated lots.

In contrast to the complete failure to germinate of seeds planted in soil in the greenhouse without pretreatment, 40, 54, 80, 90, and 81 per cent germination was obtained in 78 to 99 days after pretreatment for three, five six, seven, and eight months in moist peat at 5° C. These counts were secured by recovery of the seeds and seedlings from the soil by sieving.

While it cannot be said that low-temperature pretreatment is absolutely necessary to the resumption of growth by the embryos of *Smilacina*, it can be said that it is essential to success if the seeds are placed in soil in the greenhouse. Here good root systems can be obtained with certainty in nine months after planting if the first six months are at 5° C., whereas with no low temperature period the seeds remain ungerminated for at least 21 months.

Production of the First Green Leaf

As in the case of *Convallaria*, epicotyl dormancy tests were conducted along two lines. First, the young seedlings with the roots beginning to grow were removed from moist granulated peat moss, planted in soil in pots, and exposed to various temperatures to determine the requirements for after-ripening. Second, seeds which had been pretreated at low temperature were planted in the soil in flats and allowed to remain in the greenhouse until root production was complete, after which the flats were transferred to various temperatures for the epicotyl-after-ripening period. The young seedlings with the roots beginning to grow were removed from moist granulated peat moss at 25° C. where they were procured after pretreatment at 5° C., and planted in lots of 25 each in soil in six-inch pots. These pots were sown in replicate to permit examination of the developmental stages reached at the time of each transfer to a new condition leaving some pots undisturbed at the same time so that green shoot development might be studied. Thus, the correlation between extent of growth of the seedlings and their continued development into green plants could be noted. For these tests, 1589 seedlings from the 1937 seed crop, and 6888 from the 1939 seed crop were used.

It was discovered here as in *Convallaria* that a certain stage of development must be reached before low temperature can be applied successfully to break epicotyl dormancy. Furthermore, the stages are strictly comparable to those of *Convallaria*, as shown in Figure 2. When the seedlings have reached Stage 3, or the point at which the leaf primordium has developed sufficiently to break through the first enclosing sheath, the after-ripening process can proceed at low temperatures. However, seedlings of *Smilacina* develop more slowly than those of *Convallaria*, and if placed at low temperatures at Stages 1 or 2, they became established with difficulty at a temperature as low as 10° C. At 5° C. the growth of such seedlings was

arrested and death finally resulted if the period was prolonged. At 15° C. and in the greenhouse, however, satisfactory development of mixed lots of seedlings of Stages 1 and 2 took place. This does not signify that after-ripening of the epicotyl will not take place at 5° C., but simply that the seedlings must be in Stage 3 before exposure to 5° C., since development from the younger stages to Stage 3 does not take place at this low temperature. This is in contrast to the behavior of *Convallaria* seedlings which will develop slowly at 5° C. Development of *Smilacina* seedlings to Stage 3 from Stages 1 and 2 proceeded at 15° and at 10° C. but required a long period of time. Therefore, the best procedure was to keep the seedlings in the greenhouse for at least a month after planting in Stages 1 or 2 to permit development to Stage 3 before transfer to low temperature. In general, *Smilacina* seedlings responded to higher temperatures for after-ripening the epicotyl than those of *Convallaria*. This is shown in Table I. It will be seen that 5°, 10°, and 15° C. were all favorable for after-ripening the epicotyl, but 10° C. appeared best. This was corroborated in results of sample plantings of seeds to be reported below. A longer time at low temperatures was required for *Smilacina* than for *Convallaria* to enable the shoots to continue their growth beyond Stage 3 to form green leaves upon final transfer to the greenhouse. An extension of the time beyond five months was tried in a number of cases with no advantage gained. The ineffectiveness of even the best treatment will be seen in Table I since none of the percentages were high. This may have been due to the greater sensitivity and difficulty of establishment in the soil combined with the slow development of the young seedlings.

In the second method for testing epicotyl dormancy, seeds pretreated for three to eight months at 5° C. were planted in soil in flats and placed in the greenhouse where they were allowed to remain until root production was complete (78 to 99 days). All plantings were made in replicates of nine flats with 100 seeds in each flat. In order not to disturb the seedlings in the flat before completion of germination, a replicate sample of each lot of seeds was mixed with moist granulated peat moss and kept in the greenhouse along with the flats. This culture was examined weekly when water was added, and seedlings removed and counted. When no more seedlings appeared in the peat, four flats each were transferred to rooms with constant temperatures of 10° and 15° C. and one flat each was sieved to ascertain the condition of the seedlings, most of which had reached Stage 3. After four and five months at 10° or 15° C. two flats each were removed. Again, one was sieved and the seedlings or seeds removed and examined while the other was transferred to the greenhouse for green shoot production. Table II gives the percentage of green leaves appearing above ground after each treatment. Five months at 10° C. was satisfactory for breaking dormancy of the epicotyl in a large percentage of the seedlings, regardless

of the original period in peat moss at low temperatures. That the length of the original period in peat moss affected root production is evident, however, from the percentage root production in soil in the greenhouse. For example, there was only 40 per cent root production in the greenhouse after three months at 5° C. but there was 80 per cent after six months, and 90 per cent after seven months. All of these seedlings produced green shoots after further treatment of five months at 10° C.

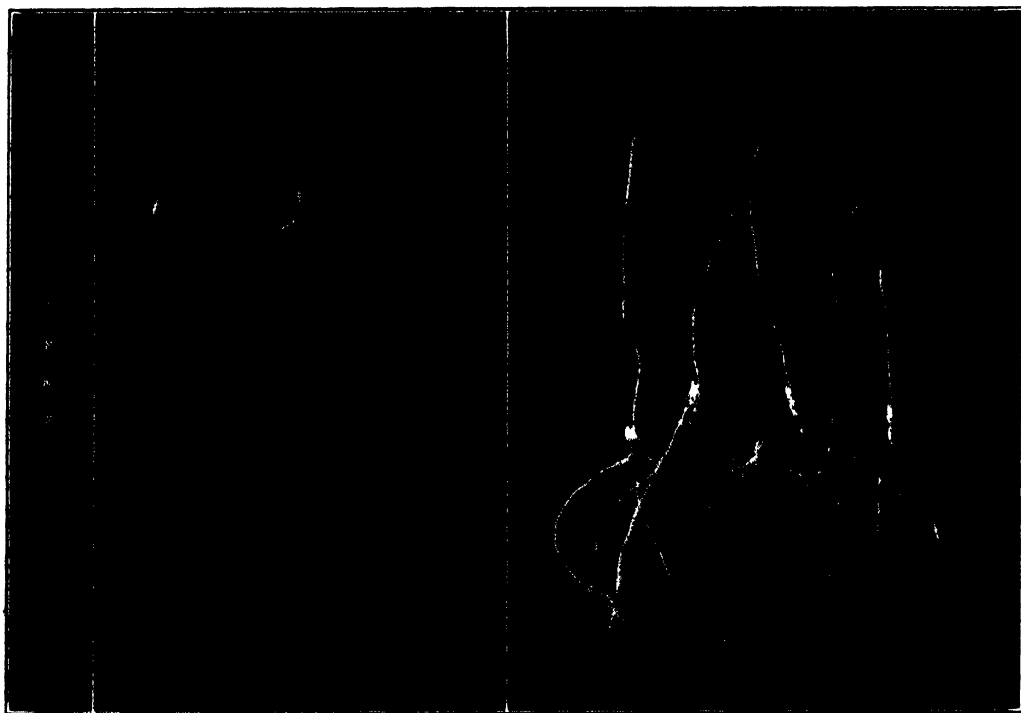


FIGURE 7. The result of planting *Smilacina racemosa* seeds in September, 1939 in soil and keeping in a board-covered cold frame. Seeds or seedlings (5 from each treatment) removed from the soil and photographed A. May, 1940; B. September, 1940; C. May, 1941.

Over a period of several years, many outside plantings of *Smilacina* seeds have been made. Green seedlings have appeared above the ground after the second winter but in no case have they appeared after one winter (Fig. 7). No additional seedlings have ever appeared after the third winter out-of-doors. These plantings were made in flats with 200 seeds each. Some of the flats were kept in cold frames with a protection of mulch and board cover, or board cover only, while others were unprotected. Seeds planted in flats and left in the greenhouse were used as controls. No seedlings have ever appeared in the control flats. A board-covered frame provided a better condition than a mulched and board-covered frame for after-ripening the epicotyl. Both of these were superior to the open or unprotected condition.

The time of year in which outside plantings were made was of the utmost importance for success. Figure 8 illustrates such effects. These seeds were of the 1936 crop and were planted in 1936 and 1937. Clean seeds were stored dry in the laboratory until the time of planting. It is evident that the best seedling stand was obtained from a September, 1936 planting. November and December plantings resulted in decreased seedling production. The yield from a planting made the following January was very much reduced and June and August plantings were ineffective. It was to



FIGURE 8. Production of second green leaves from *Smilacina racemosa* seeds planted at different times of the year. Photographed June, 1939. Duplicates of 150 seeds each planted in each flat

be expected that these latest plantings would have produced seedlings by the time this photograph was made (June, 1939) since by that time they had had the required two periods of cold with an intervening period of warm. The explanation for this failure is to be found in the fact that these seeds rotted in the soil before the beginning of the first winter. This may be attributed to either one or both of two factors. It has been shown above that *Smilacina* seeds will not germinate at 30° C., but that the seeds rot at this temperature before the advent of the first necessary cold period. Again, it is possible that seeds of *Smilacina racemosa* are short-lived and lose their vitality in the interim between harvest and the following summer. If the latter hypothesis proves true, it would also explain the progressive decline in seedling production of plantings made in November, December, and January. However, this latter condition may exist because of the decreasing period of cold weather following the planting and the long period

of cold shown above to be a necessary prerequisite for root production. Giersbach and Barton (7) reported rapid deterioration of *Smilacina* seeds in dry storage at room temperature. The question of the life span of these seeds is now being more fully investigated using seeds of the 1940 crop.

These detailed studies have given an explanation to the germination behavior of *Convallaria* and *Smilacina* seeds as reported by other authors. Adams (1) sowed clean seeds of *Convallaria majalis* and *Smilacina racemosa* (a) on September 20, as soon as possible after collection, in shallow boxes placed out-of-doors and left there during the winter; (b) on September 20, in pots in the greenhouse; (c) on March 5, after being stored dry in a tin box out-of-doors over the winter; and (d) on March 5, after being stored in a heated room over-winter. *Convallaria* plantings (a) and (b) yielded 64 and 0 per cent in 632 days, while (c) and (d) gave 5 and 1 per cent in 466 days. He reported similar results for *Smilacina* plantings. Since these plantings were made in the soil, it is assumed that seeds were counted as germinated only if green shoots appeared above the soil. Nichols (10) listed *Smilacina racemosa* as seed with germination markedly higher (ratio 5:1 or more) after exposure to low temperature. Out of 100 seeds, 30 germinated in 264 days after refrigeration for 112 days. Two seeds germinated in 376 days without low temperature. Fleischer (5) stated that lily-of-the-valley seeds germinated only in the spring, regardless of the time of sowing. If sown right after maturing, they germinated the next spring. If sown at the beginning of winter, they germinated the second or third spring. Giersbach and Barton (7) reported that 25° C. was favorable for the germination of seeds of *Convallaria majalis* and that 95 per cent seedling production was obtained in the greenhouse after five months at 0° C. They also stated that germination of *Smilacina racemosa* seeds was obtained at 25° and 30° C. These data applied to root production only and not to the appearance of green shoots.

Kinzel (8) found that *Convallaria majalis* seeds germinated at 18° to 20° C. up to 89 per cent in a period of one year and three months if kept in the dark. Germination was greatly reduced in light. Mitchell (9) reported a similar favorable effect of dark on the germination of seeds of *Smilacina racemosa*. She obtained 16 per cent in the dark in 72 to 147 days for one seed crop and 5 per cent in 38 to 63 days for another. No germinations were obtained in the light. Moist filter paper at room temperature was used for germination so that results pertain to root production only. A preliminary test by the present authors on the effect of light and dark failed to confirm these results. Germination depended on temperature rather than light. Hence, in the large number of experiments reported here, the seeds were handled in the normal manner with exposure to light during intervals of examination or planting.

The experimental findings reported in this paper serve to establish

further the necessity of a distinction between root and shoot dormancy of seed embryos. In a majority of dormant seeds which respond to an after-ripening period in a moist medium at low temperatures, both root and shoot acquire ability to resume growth following one period of treatment. It has been assumed that the entire embryo has become after-ripened at the same time. Then followed the discovery that though the roots may not be dormant, there were cases where the shoot or the bud which forms it was unable to grow without low-temperature pretreatment.

Such "epicotyl dormancy" has been known for some time, but seeds of *Convallaria* and *Smilacina* show a specialized type of the dormancy since the shoots must be developed to a certain stage before low temperature is effective in after-ripening. This has not been reported for any other seeds. Again, as in many other cases, it may be said that the roots have a partial dormancy. Whereas some root production is obtained without previous exposure to low temperature, especially in *Convallaria*, full root production does not occur without such pretreatment. In *Smilacina* no roots appeared in soil in the greenhouse without low temperature. Hence it might be said that seeds of these two species exhibit a double dormancy. They require an initial period at low temperature to after-ripen the root followed by a period at high temperature during which the root system grows and the shoot develops until it has broken through the cotyledonary sheath. A second period at low temperature is then necessary to after-ripen the epicotyl, followed by a second period at high temperature during which the first green leaf appears above soil.

Seeds of *Smilacina* are more dormant than those of *Convallaria*, requiring longer periods at low temperature for both root and shoot after-ripening.

SUMMARY

Seeds of *Convallaria majalis* and *Smilacina racemosa* were found to show a type of epicotyl dormancy not previously reported. Low-temperature pretreatment was required for forcing shoot growth after the root had started to grow, but was effective only if given after the shoot had started to grow and had broken through the cotyledonary sheath. Exposure to low temperatures at earlier developmental stages was without effect in breaking epicotyl dormancy. Development of the growing embryo to the proper stage for the exposure to low temperature took place more rapidly at greenhouse temperatures than at lower temperatures. At a temperature as low as 5° C. *Convallaria* seedlings developed very slowly and *Smilacina* seedlings failed to continue growth and died with prolonged exposure.

Low-temperature pretreatment increased root production from seeds of *Convallaria* and was essential to root formation in *Smilacina* when plantings were made in soil in the greenhouse.

The best treatments for the production of green leaves above soil in these forms were found to be as follows: for *Convallaria majalis* seeds—three months at 5° C. to after-ripen the partially-dormant roots, two months at greenhouse temperature to grow the root system and to develop the first leaves to the stage where they break through the enclosing cotyledonary sheath, three to five months at 5° C. to after-ripen the shoot bud, then a final transfer to the greenhouse to produce green leaves; for *Smilacina racemosa* seeds—six months at 5° C., three months at greenhouse temperature, five months at 10° C., then a transfer to the greenhouse were required to effect complete after-ripening and produce green leaves.

In commercial practice, seeds should be cleaned and planted soon after harvest in the fall. Seedlings will appear above ground the second spring after planting. Some green leaves of *Convallaria* may be produced after one winter provided the planting is early enough to afford several weeks of warm weather, during which a small percentage of roots may grow.

Good seedling stands may be had within nine months after harvest for *Convallaria*, and 14 months for *Smilacina* if controlled temperatures are available.

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GERMINATION STUDIES OF SEEDS OF SYMPHORICARPOS ORBICULATUS

FLORENCE FLEMION AND ELINOR PARKER

Seeds of *Symphoricarpos orbiculatus* Moench., commonly known as coral-berry or Indian currant, are very resistant to germinative conditions. The delayed germination in these seeds is due to a hard impermeable seed coat and to dormancy of the embryo. This condition is similar to that found in snowberry seeds (1).

The effect of the seed coat can be overcome by a treatment with concentrated sulphuric acid or by keeping the seeds in a moist medium at 25° C. for 3 to 4 months. The dormancy of the embryo can be subsequently broken by an after-ripening period of approximately five months at 10° C. The recommended procedure for germinating the seed in the laboratory is to treat the seeds for 30 to 40 minutes with concentrated H₂SO₄ followed by 2 to 4 weeks at 25° C., then 5 to 6 months at 10° C.

MATERIALS AND METHODS

The berries were collected when mature in early November in the vicinity of the Institute at Yonkers, New York. The seeds were immediately freed of the pulp with the aid of a Hobart mixer, washed well, spread out for drying, and subsequently stored at room temperature in containers covered with cheesecloth.

When the seeds were treated with concentrated sulphuric acid, a quantity of acid equal to about five times the volume of the seeds was used. The mixture was stirred about every ten minutes. At the end of the treatment period, the acid was removed, the seeds were thoroughly washed in running tap water, and then kept in a large volume of water for at least an hour to remove any remaining acid. Freshly-harvested seeds were never treated with acid until after 4 to 6 weeks of drying at room temperature because with fresh seeds the acid penetrated and destroyed the embryos due to the higher moisture content of the seeds.

For experiments in the laboratory moist granulated peat moss was used as medium. For the plantings made in flats a mixture of one-third sand, one-third peat moss, and one-third composted sod soil was used. The seeds which were planted in flats were kept at various controlled temperatures or placed out-of-doors in cold frames, either open, covered with a board cover, or mulched and covered with a board cover. Seeds, when fully after-ripened, germinated readily at low temperature or within several weeks after being transferred to the greenhouse (temperature 21° C., except

during warm, sunny days when the temperature rose above this figure). Seedlings in the cold frames appeared early in spring.

The germination percentages given in the tables and figure represent averages of duplicate or triplicate lots of 200 or 300 seeds except when otherwise stated.

RESULTS

Germination tests. In the first experiment intact seeds of the 1934 crop, without pretreatment to remove the retarding effect of the seed coat, were mixed in moist peat moss and kept at various constant temperatures. As seen in Table I, 10° C. is the most favorable temperature for the after-ripening of these seeds. Several similar experiments, but of shorter duration, were repeated with subsequent crops and invariably the highest percentage germination was obtained at 10° C. However, the total percentage germination varied with the different crops. Various alternating temperatures were also tested but best results were obtained at a constant temperature of 10° C.

TABLE I

PERCENTAGE GERMINATION OF SYMPHORICARPOS ORBICULATUS SEEDS WHEN MIXED IN MOIST PEAT MOSS AND KEPT AT VARIOUS TEMPERATURES*

Temperature	Percentage germination					
	0.5 year	1 year	2 years	3 years	4 years	5 years
1° C.	0	0	0	1	2	3
5° C.	1	2	3	3	5	10
10° C.	5	43	62	66	70	74
15° C.	0	0	0	1	2	5
20° C.	0	0	0	0	0	0

* Duplicate lots of 200 seeds of 1934 crop; experiment started March 6, 1935.

Since germination of intact seeds at 10° C. was found to be very slow, various pretreatments of the seeds were undertaken to overcome the effect of the impermeable seed coat before the low temperature after-ripening period. Intact seeds were kept in moist peat moss for various periods of time at 25° C. and were subsequently transferred to several constant low temperatures. In addition, intact seeds were treated with acid, then mixed with moist peat moss and after being kept at 25° C. for 0 and 2 weeks they were transferred to low temperature. The total germination percentages obtained after a six months' period at the various low temperatures are recorded in Table II. Here, also, 10° C. is better than either 1°, 5°, or 15° C. as the after-ripening temperature.

The effect of subjecting the seeds to 25° C. while in a moist medium for various periods preceding the low temperature is shown in Table II. Approximately 70 per cent germination was obtained when intact seeds were mixed in moist peat and kept at 25° C. for 12 weeks followed by the

low temperature. When the seeds were treated with concentrated sulphuric acid for 30 minutes, then mixed in moist peat and held at 25° C. for two weeks before being transferred to 10° C., approximately 60 per cent germinated. Similar experiments were conducted with subsequent crops. It was found that an effective method of overcoming the effect of the seed coat, therefore, was either a period of about 16 weeks at 25° C. or a combination of acid treatment (30 to 40 minutes) and several weeks at 25° C.

TABLE II

EFFECT OF SOAKING SEEDS IN CONCENTRATED SULPHURIC ACID AND OF SUBJECTING SEEDS WHILE IN A MOIST MEDIUM TO 25° C. ON THE GERMINATION OF SEEDS OF SYMPHORICARPOS ORBICULATUS*

Time in H ₂ SO ₄ , minutes	Time at 25° C., weeks	Percentage germination**			
		1° C.	5° C.	10° C.	15° C.
0	0	0	0	0	0
0	2	0	0	1	—
0	4	0	5	11	—
0	8	10	28	56	—
0	12	9	59	72	—
0	16	24	72	70	—
15	0	—	—	3	0
15	2	—	—	12	—
30	0	—	2	14	0
30	2	—	28	58	—
45	0	0	11	—	—
45	2	4	55	—	—

* 1935 crop. Experiment started January 15, 1936.

** Averages of duplicate lots of 200 seeds after 6 months at low temperature.

Seedling production. Seeds were pretreated as described above and after being kept at various low temperatures, lots of 100 seeds each were planted in soil and placed in the greenhouse (21° C.). These sample plantings were not very successful because seeds not fully after-ripened failed to germinate while those after-ripened for some time prior to the planting time had germinated at the low temperature. However, a good seedling stand was obtained by planting the seeds in soil in flats prior to being subjected to the various temperatures. Many of the seedlings which appeared in the soil while at the low temperature survived when transferred to the greenhouse. These, together with those which subsequently germinated, produced a satisfactory seedling stand.

Either a period of about four months in soil in the greenhouse (21° C.), followed by approximately five months at 10° C. (controlled room), or an acid treatment of 30 to 45 minutes followed by a short period of several weeks in the soil in the greenhouse prior to the low temperature treatment, was equally effective in producing seedlings. The results of one of the experiments are illustrated in Figure 1. Approximately 50 per cent ger-

mination was obtained when the seeds were held in the greenhouse for 16 weeks followed by four and one-half months at 10°C . or when treated with acid for 30 minutes and kept in the greenhouse for four weeks, then placed at 10°C . for five months. The other seedlings shown in Figure 1 were obtained by varying the acid treatment or the period at high temperature. The period at 10°C . was determined by the number of seedlings which appeared in the flats while at the low temperature, for when the seedlings remained too long at this temperature they became spindly and etiolated. The maximum number of seedlings was not always obtained because not all of the seeds were fully after-ripened at the time of transfer. However, approximately 50 per cent germination can be obtained by this procedure.

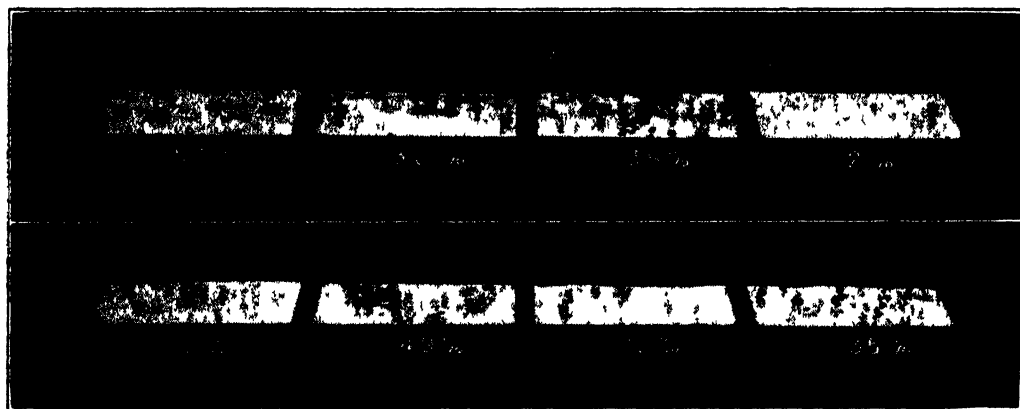


FIGURE 1. Seedling production of coral-berry seeds. Lots of 200 seeds each of the 1939 crop were planted December 18, 1939. Photographed September, 1940. A. Intact seed series: (1, 2, and 3) 16, 12, and 8 weeks, respectively, at 21°C . +4.5 months at 10°C . (4) 0 weeks at 21°C . +6 months at 10°C . B. Concentrated sulphuric acid series: (1 and 3) 30 and 45 minutes acid, respectively, +6 months at 10°C . (2) 30 minutes acid +4 weeks at 21°C . +5 months at 10°C . (4) 45 minutes acid +2 weeks at 21°C . +5 months at 10°C .

In other tests the seeds, after various treatments to overcome the seed coat effect, were planted in flats and placed out-of-doors in cold frames for the low temperature treatment. The seeds mature late and after thorough drying prior to the acid treatment and subsequent subjection to high temperature, it was usually late December or early January when they were finally placed in the cold frames. Some years a very high percentage of seedlings was obtained the first spring but in other years it was not so successful due in all probability to the seeds not having sufficiently after-ripened by spring. These seeds require from four to five months at low temperature. However, for more consistent results, seeds which have been treated with acid for 30 minutes, then planted, and kept at room temperature for four weeks, should be transferred to a cool cellar (about 10°C .) instead of out-of-doors. In the spring, when seedlings appear, the flats can be transferred to the cold frame.

Intact seeds were planted in flats at various intervals and placed in cold frames out-of-doors (Table III). Seedlings were obtained the first spring following plantings made in June and August but with September or November plantings none germinated until the second spring after planting. In the experiments described earlier a period of 12 to 16 weeks at a temperature of 20° to 25° C. is required for overcoming the effect of the coat of intact seeds; therefore, if seeds are not pretreated to overcome the seed coat effect, plantings should be made in the early summer so that the seeds are subjected to the warm temperature for approximately this length of time. The embryos subsequently after-ripen during the winter. Seedlings which appear under the mulch are frequently injured when the mulch is removed in the early spring. The open frame was satisfactory some years but the best condition was the cold frame covered with a broad cover.

TABLE III

SEEDLING PRODUCTION OF SYMPHORICARPOS ORBICULATUS SEEDS WHEN PLANTED IN FLATS AND HELD OUT-OF-DOORS IN COLD FRAMES

Crop	Date planted	Percentage germination							
		Spring 1935				Spring 1936			
		Mulch	Board cover	Open	Control (21° C. greenhouse)	Mulch	Board cover	Open	Control (21° C. greenhouse)
1933	June 25, 1934	1	31	43	0	1	33	50	0
1933	Aug. 6, 1934	12	22	33	0	14	26	40	0
1933	Sept. 25, 1934	0	0	0	0	19	45	38	0
1933	Nov. 19, 1934	0	0	0	0	9	45	24	0
1934	Nov. 11, 1934	0	0	0	0	45	90	35	0
1934	June 28, 1935	—	—	—	—	16	51	34	0

Experiments with excised embryos. The results from preliminary experiments indicate that the viability of coral-berry seeds can be determined in a relatively short period of time by observing the behavior of excised embryos using a technique similar to that described for snowberry seeds (2). Prior to excising, the seeds were treated with acid for 30 minutes, then held in moist peat moss for two weeks at 25° C. Considerable care is necessary when excising since the embryos are quite small. By observing the behavior of the embryos which were placed on moist filter paper in Petri dishes at room temperature, the final readings could be taken on the fourteenth day. Viable embryos either remain intact or show some type of development while nonviable embryos deteriorate. Thus, the viability was determined within four weeks instead of the five to six months required for germination. The method was also found useful for ascertaining the optimum length of time for the acid treatment of a given lot of viable seeds which could be tolerated without injuring the embryos.

DISCUSSION

Intact snowberry seeds rarely germinate (1) while intact seeds of coral-berry germinate slowly, requiring several years to give 50 per cent germination. However, with both species, a speedier germination is obtained when the seed coats are modified prior to the period at low temperature. Either a period of three to four months at 20° to 25° C. or an acid treatment followed by a short period at this temperature is equally effective.

When treating the coats of coral-berry seeds with acid, a given period of time did not prove optimal for seeds of different crops nor for seeds of different ages. Therefore, it is advisable to use a suboptimal period (30 or 40 minutes) followed by a short period in moist peat at a warm temperature. In the case of similarly treated snowberry seeds, Pfeiffer (4) found that during this short period the remaining part of the coats not destroyed by the acid was infected with fungi. She also studied the activity of fungi in the decomposition of the coats of intact snowberry seeds held in moist peat for longer periods as well as when planted in soil and held out-of-doors during the summer months. Such a study has not been made with coral-berry seeds but in all likelihood fungi play a rôle in disintegrating the coats of these seeds because the coats, as with snowberry, become soft and progressively darker as the period at the warm temperature increases.

Regardless of the method by which the coats are modified, the seeds of coral-berry and snowberry must be subsequently subjected to a period at low temperature in order for germination to occur. The optimum after-ripening temperature for coral-berry seeds is 10° C. while for snowberry seeds 5° C. is a much more effective temperature for overcoming the embryo dormancy. This difference in temperature response is not surprising since in the natural distribution (3) of the two species the range of coral-berry is more southern.

SUMMARY

In order to induce germination in seeds of *Symphoricarpos orbiculatus* it is necessary to disintegrate the seed coat as well as to after-ripen the embryo. This can be accomplished by a period of three to four months in moist peat moss at 25° C. followed by approximately five months at 10° C. The period at 25° C. can be reduced to two to four weeks if the seeds are treated with concentrated sulphuric acid for 30 to 40 minutes prior to being mixed in the moist peat moss.

For the production of seedlings on a large scale the best method is to plant the seeds in soil in flats in the spring and place out-of-doors in a cold frame which is covered with a board cover during the subsequent winter. Germination will occur the following spring. By combining the acid treatment with a short period at high temperature seedlings can be obtained the first spring providing the seeds are after-ripened in a cellar maintained at approximately 10° C. (50° F.).

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INACTIVATION OF THE BROWNING SYSTEM IN FROZEN-STORED FRUIT TISSUE

F. E. DENNY

The effectiveness of thiourea, NH_2CSNH_2 (also called thiocarbamide), in preventing the browning of the tissue and juice of various fruits and vegetables was pointed out in a previous article (1). However, in these early experiments, tissue slices which had been dipped into dilute solutions of thiourea and had been allowed to dry in air to a white color, when placed in water promptly turned brown within a few minutes, indicating that "thiourea had to be present to prevent browning, and that it had not destroyed or rendered permanently inactive any constituent that was essential for browning" (1, p. 58).

Subsequent experiments, not with dried tissue but with tissue dipped into dilute thiourea solutions, then frozen and stored in the frozen condition, show that this process brings about an inactivation of the browning system, at least of peaches, apples, and pears, so that browning of the treated tissue does not occur after thawing of the frozen material, or even after leaching it in an excess of water over a period of 20 to 24 hours to remove the thiourea and after grinding the leached tissue in a mortar to bring the fragments into good contact with air.

Peach and pear tissues in cubes or slices were held in the frozen condition for more than a year, and apple tissue for more than 10 months at least, without development of discoloration on thawing or leaching.

Such long periods of storage in the frozen condition, however, were not found to be necessary for the inactivation of the browning system; later experiments showed that this result could be accomplished within a day with peach fruit, two or three days with pear, and a week or two with apple. Indeed, with apple, freezing, although it hastened the process of destruction, was not essential, since merely storing the treated tissue in tubes immersed in chopped ice (which permitted several days' storage without undue action of organisms) resulted in the prevention of browning of the tissue after thawing or leaching in water.

The important factor seems to be the penetration of the tissue by the chemical, and the rate is influenced by the concentration of chemical, duration of contact, and the porosity of the tissue. Possibly freezing disrupts the membranes and allows better or more rapid penetration by the chemical.

METHODS

The fruits were peeled, cores or pits removed, and the tissue was cut into appropriate sizes and shapes (slices or dices), usually into eighths or

sixteenths. These were dropped at once into the thiourea solutions or into water, and after the tissue of two or three fruits had been accumulated, the dipped tissue was removed, allowed to drain for a few seconds, and then was placed in wide-mouthed fruit jars. The tissue remained in the solution for only a few seconds or at most for two minutes. A few tests were made of the effect of soaking for periods of 15 minutes to one hour, and these showed an additional favorable effect of soaking as compared with merely dipping but the experiments here reported upon relate to the results with the method of dipping. The glass jars containing the dipped fruit were placed in a freezing room, the temperature of which varied between -6° and -10° C. Except in the very earliest experiments the dipped tissue was allowed to stand two to six hours at room temperature before freezing was started.

When the fruit was removed from the freezing room it was allowed to thaw and was placed on watch glasses with good exposure to the air for at least a day. After finding that treatments with thiourea in which the dip solutions were of adequate concentration allowed the tissues to remain frozen for long periods, to thaw without browning, and to remain so at room temperature until the condition of the tissue was affected by organisms, the test of the thiourea effect was made more rigid by soaking the tissues after thawing. This was done to remove the absorbed thiourea. The tissue was covered with an excess of water, which was decanted and replaced with a fresh supply of water; this was repeated twice more and finally the tissue was allowed to stand in an excess of water overnight and was placed on a watch glass for four hours before decision was made as to whether browning of tissue had occurred or not. In some tests the tissue after having been soaked was ground in a mortar and the paste was spread thinly on a watch glass. This method was found not entirely desirable since the presence of local brownish tints observable in the intact pieces was obscured when the tissue was ground.

When jars were removed from the freezing room there was found in the earliest tests a tendency for the occurrence of a small amount of brown discoloration of the pieces of tissue at the top of the jar, with retention of the white color in the lower portions. It was believed that this was not due to a greater oxygen concentration at the top of the jar, since at least with apple tissue the pieces retain their form and furnish a continuous air connection among them. It seemed more probable that the failure to obtain full protection of the top layers was due to drainage of the liquid soon after the preparation of the sample, the thiourea solution flowing downward from the top layer to lower layers, so that the uppermost pieces retained a smaller amount of the chemical on the fruit surface. In order to overcome this difficulty, in the more recent experiments (those relating to short periods of storage in the frozen condition), the jars after the dipping and

sealing, and before being placed in the freezing room, were inverted for two to six hours at room temperature, then were placed in the upright position when they were put in the freezing room. This allows a penetration of the chemical into the tissue of the top layers during the period in which the jar is inverted. Drainage to the bottom layers during the period before freezing begins provides for the supply of chemical to the lower layers.

RESULTS

Long Periods of Storage of Tissue in the Frozen Condition

Peach (*Prunus persica* Sieb. & Zucc.). Fruits of the variety Hiley were peeled after immersion in boiling water for one minute, were pitted, and were cut lengthwise into one-eighth slices; these were dipped into 0.1 and 0.05 per cent solutions of thiourea and into water as a control. All lots were put into glass fruit jars, which were sealed, and put in a freezing room at approximately -6° to -10° C. on July 22, 1940. On October 28, 1941, i.e. after 1 year and 98 days, the samples were removed from the freezing room and allowed to thaw at room temperature. The lots treated with thiourea were of good color when removed from the cold room, and samples removed from these lots thawed and remained exposed to air for one day without discoloration. Other samples were placed in an excess of water which was decanted occasionally for a few hours and then soaked in water overnight to remove the thiourea which had been used originally to treat the tissue; in these also there was no subsequent browning. The lots originally dipped in water only, browned during the period of freezing and remained brown on thawing, on standing in air, and on being soaked in water overnight.

A similar result with Hiley peaches was obtained with a lot dipped and frozen on July 27, 1940 and removed on October 28, 1941. Sugar was added to the lots in this experiment. After the slices were dipped in the thiourea solutions or in water, and before they were placed in the glass jars for freezing, crystallized cane sugar was added in an amount corresponding to one-fifth of the weight of the fruit. Figure 1 A shows the results at the conclusion of the test when the frozen fruit was thawed, removed from the jars, and samples were leached with an excess of water over a period of 20 to 24 hours before being placed on watch glasses and exposed to air. It is seen that the lots treated with 0.1 per cent and 0.05 per cent thiourea failed to discolor in air after soaking in water. A slight amount of browning occurred with the lot treated with 0.025 per cent thiourea, and the control tissue gave a dark brown color.

The control in this test at the time of removal of the jar from the freezing room showed a dark brown color only in the top two inches of the tissue in the jar, the lower layers showing very little browning. It seemed that the liquid in the sample produced a viscous syrup with the sugar and this by

interference with the diffusion of the air downward in the jar protected the lower layers from contact with oxygen. However, when the relatively light-colored tissue from the bottom of the jar was removed and exposed to the air or soaked in water, browning took place promptly. This indicated that the conditions within the jar during the long cold storage period were not

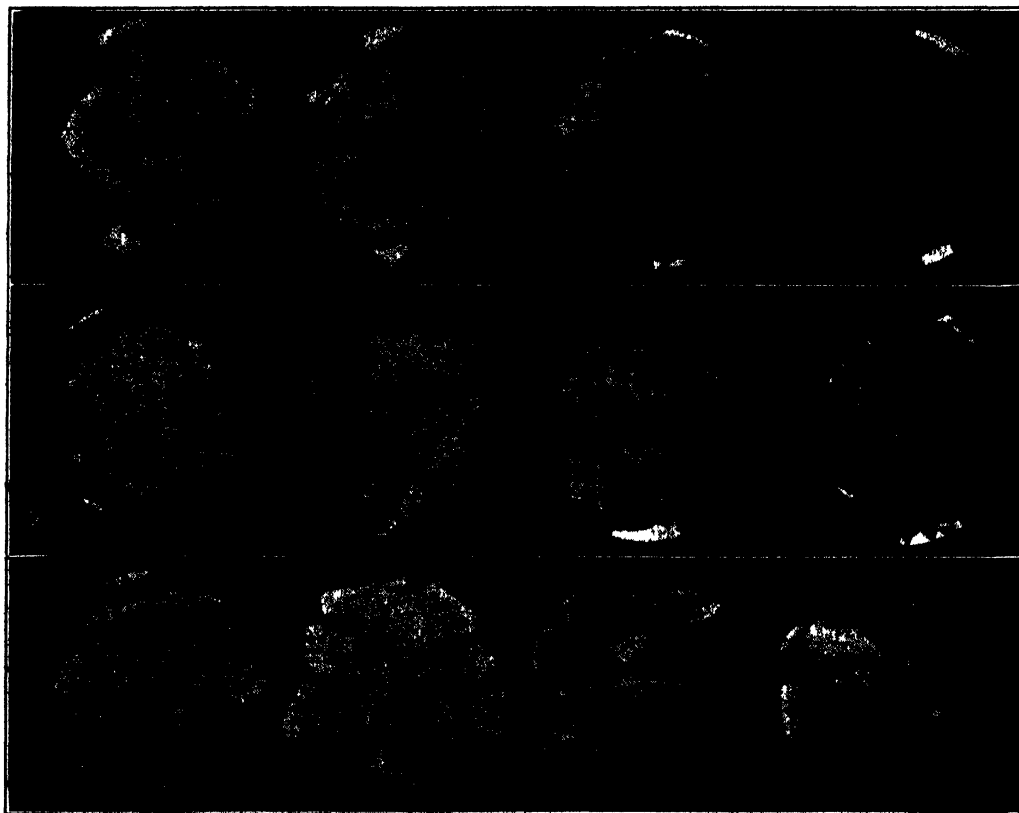


FIGURE 1. Retention of color of fruit tissue after long periods of storage in the frozen condition as a result of treatment with thiourea prior to freezing. Maintenance of white color after leaching the thawed tissue with an excess of water is taken to indicate an inactivation of the browning system of the tissue. A. Peach: left to right, tissue dipped into 0.1, 0.05, and 0.025 per cent thiourea solution, and into water before freezing. Stored in the frozen condition for 1 year and 93 days; then thawed and leached in an excess of water to remove the absorbed thiourea. B. Pear: left to right, tissue dipped into 0.1, 0.05, and 0.025 per cent thiourea and into water before freezing. Frozen during 1 year and 48 days. This photograph after thawing and thoroughly leaching the tissue with water. C. Apple: left pair, dipped in 0.2 per cent thiourea before being frozen; right pair, dipped in water. Within each pair: left sample merely exposed to air after removal from freezing-room and thawing; right sample soaked in an excess of water and exposed to air. Duration of frozen period 10 months and 19 days.

such as to bring about inactivation of the browning system in the absence of thiourea, and showed that the thiourea was the essential factor in the inactivation.

Pear (Pyrus communis L.). Pear fruits of the variety Clapp were peeled, cored, and diced, first cutting lengthwise into quarters, then crosswise, into eighths. The dipping liquids were: 0.1, 0.05, and 0.025 per cent thiourea, with water as a control. These were put in glass jars, and frozen-storage at -6° to -10° C. was started September 9, 1940. These lots were removed from the freezing room on October 28, 1941, i.e. after 1 year and 49 days. Upon thawing, samples were soaked in water until the next day and placed on watch glasses. The pieces of pear tissue failed to maintain their shapes after the long period of freezing and upon thawing disintegrated into a pulp. The result of this test is shown in Figure 1 B. Here, again, 0.1 per cent and 0.05 per cent thiourea completely inactivated the browning system and 0.025 per cent thiourea permitted only slight discoloration. The control lot became brown in the usual manner of pear tissue exposed to air.

In another experiment fruits of the variety Bartlett were treated and frozen starting on September 11, 1940. On October 28, 1941 the samples were removed and thawed. Browning did not occur in the lots treated with 0.1 per cent or 0.05 per cent thiourea, whether the tissue was merely exposed to air after thawing or thoroughly leached in water. Some discoloration was observed in the lots treated with 0.025 per cent thiourea. The control lots became brown. In this test one series was frozen without the addition of sugar and to the other series crystalline cane sugar was added in an amount equal to one-fifth of the weight of the fruit. No important differences were noted between the lots receiving and not receiving sugar. This is not to be taken as a general statement, however, since in a later paragraph it is shown that with apples the addition of sugar decreased the effectiveness of thiourea.

Apple (Pyrus malus L.). On May 17, 1940 fruits of the variety Newtown Pippin were peeled, cored, cut into one-eighth slices and dipped into 0.1, 0.05, and 0.025 per cent thiourea solutions in a series without addition of sugar, and into 0.2, 0.1, 0.05, and 0.025 per cent solutions of thiourea incorporated into sucrose solutions of 35-degree Balling density. These were placed in glass jars and put in the freezing room at -6° to -10° C. On September 9, 1940 these lots were removed and allowed to thaw. The lots receiving the 0.1 per cent dip without sugar and 0.2 per cent dip in the presence of sugar syrup were white and remained so when exposed to air. The soaking or leaching tests had not been started at that time and so were not applied to these lots. There was considerable browning in the other treated lots especially at the top layers in the jar. The control lots dipped in water or sugar solution became dark brown in color. The presence of sugar made a difference in the effectiveness of the thiourea in this test with apples, more thiourea being needed in the presence than in the absence of sugar.

Another experiment with apples was started December 9, 1940 with

fruits of the McIntosh variety. The concentrations of dipping solutions were 0.1 and 0.05 per cent thiourea in a series without sugar syrup, and 0.2, 0.1, and 0.05 per cent thiourea incorporated into a 35-degree Balling sucrose syrup. Control lots were dipped into water. These lots were removed from the freezing room on October 28, 1941, after 10 months and 19 days. The tissue in the lot receiving 0.2 per cent thiourea in sucrose syrup was white and remained so upon standing in air and after thorough leaching in water. The appearance of these lots is shown in Figure 1 C. The lot receiving 0.1 per cent thiourea with or without sugar (not shown in Fig. 1 C) showed some browning especially at the top of the jar, although in most cases only small brown portions were observable either at the core or in the center of the pieces of tissue. In all there were 83 white pieces and 35 pieces with at least some brown areas in the 0.1 per cent thiourea lots. Removing the white pieces and exposing them to the air after thawing or soaking in an excess of water showed no brown development and indicated that the browning system had been inactivated in the portion of the sample that remained white. A similar result was obtained with the 0.1 per cent lot receiving the sucrose syrup except that the proportion of white pieces to those showing some brown areas was 79 to 60. A comparison of the number of brown and white pieces in the two lots of apples treated with 0.1 per cent thiourea, one receiving and one not receiving sugar, shows that the deviation of the observed from the expected number is 8.61 pieces in each of the four groups; this gives a χ^2 value of 4.98, which furnishes odds of about 50 to 1 that the presence of sugar decreased the effectiveness of the thiourea in preventing browning.

Another test with McIntosh fruits (without added sugar) starting January 14, 1941, and ending October 29, 1941, showed complete inactivation with a 0.2 per cent thiourea dip, but some browning of pieces in the top part of the jar with 0.1 per cent thiourea. The white pieces in a jar in which some of the pieces showed brown areas failed to brown when exposed to air or soaked in water. The control (water dip) showed the usual brown discoloration.

The experiments with apple tissue indicate that it is more difficult to inactivate the browning system of apples than that of peaches or pears. The 0.1 per cent dipping solution which is sufficient for peaches and pears appears to be at the lower margin for successful protection of the color of apple tissue, and it may be that a higher concentration may be needed for apples; certainly not higher than 0.2 per cent and perhaps not higher than 0.15 per cent. Soaking for a short period rather than merely dipping the fruit may be needed.

Short Periods of Storage of Tissue in the Frozen Condition

In the preceding section it was shown that tissue dipped into thiourea solutions prior to freezing remained in the frozen condition for many

months without browning, and that when such tissue was thawed, or even soaked in water in order to remove the thiourea by leaching, discoloration did not occur, indicating that the browning system had been inactivated during the storage period.

The experiments now to be described show that a long storage period is not necessary for bringing about this inactivation of the browning system, but that this effect is brought about within a few days at most, after dipping and freezing.

Peach. Slices (one-eighths) of Elberta peaches were dipped into 0.2, 0.1, and 0.05 per cent thiourea solutions and into water. When these were at once soaked in water, or when they were at once put in the freezing-room for two hours and then soaked in water, all lots turned brown subsequently whether treated with thiourea or not. But when the dipped slices were allowed to stand in a container for two hours (to allow time for penetration of the tissue) and then were frozen overnight at -6° to -10°C. , the lots originally dipped into 0.2 and 0.1 per cent thiourea did not turn brown even when soaked in water and exposed to the air. The 0.05 per cent thiourea lot showed some browning and the control lot showed the usual dark brown color of fresh peach tissue after exposure to air. This short-time treatment had sufficed to inactivate the browning system of peach tissue in the presence of suitable amounts of thiourea.

Pear. Pear fruits, variety Bartlett, were peeled, cored, sliced into sixteenths, and dipped into 0.2, 0.1, and 0.05 per cent thiourea solutions and into water. One lot of each was frozen at once for two hours and then the tissue was soaked in water; all of these turned brown whether treated with thiourea or not (Fig. 2 D). Another series was allowed to stand for six hours after dipping before it was put in the freezing-room; after it had been in the frozen condition for one day it was removed and soaked in water. The result is shown in Figure 2 E. The lot dipped in 0.2 per cent thiourea failed to brown on soaking in water but some coloration occurred in the 0.1 and 0.05 per cent lots, although the browning was less than that shown by the control lot. Another lot was allowed to stay in the freezing-room for two days before it was removed and soaked in water. This result is shown in Figure 2 F. Inactivation of the browning system was obtained with the 0.2 and 0.1 per cent thiourea dipping solutions but the 0.05 per cent thiourea lot, although white when removed from the cold-room, subsequently showed a weak development of brown color when soaked in water. The control lot was brown when removed from the freezing-room and it remained brown through the soaking period.

Apple. A similar test was carried out with apple tissue but since previous experience had shown greater difficulty in inactivating the browning system of apple than that of peaches or pears the concentration of thiourea was not decreased below 0.1 per cent and the first removal from the freezing-room was not made until four days after the start of freezing. The

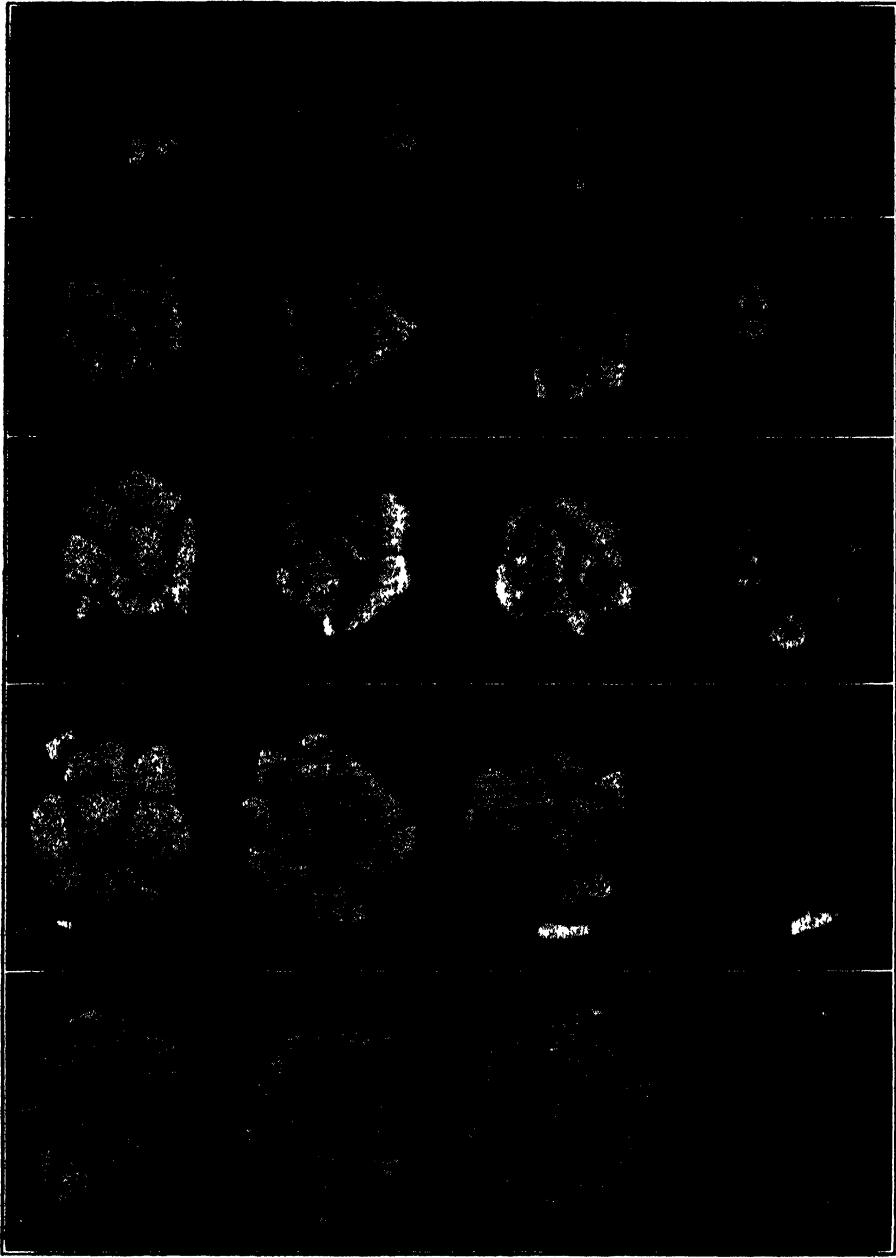


FIGURE 2. Inactivation of the browning system of fruit tissue during short periods of freezing after dipping into solutions of thiourea. D, E, F. Pear tissue; G, H. Apple tissue. D. Dipped into 0.2, 0.1, and 0.05 per cent thiourea solutions and into water (in the order left to right), frozen at once for two hours and then soaked overnight in an excess of water. E. Same but stored for six hours in air after dipping before freezing and allowed to remain frozen for two days before soaking in water. F. Same but frozen for four days. G. Peeled, cored, diced apples dipped into 0.2, 0.15, and 0.1 per cent thiourea and into water (in the order left to right), allowed to stand in air for six hours after dipping, put in freezing room for four days, removed, thawed, and soaked overnight in an excess of water. H. Same but allowed to stand in the freezing room for 10 days before soaking in water.

result is shown in Figure 2 G. There was some browning even in the 0.2 per cent thiourea lot after soaking in water, and considerable browning in the 0.15 and 0.1 per cent thiourea lots (although the discoloration was too slight to show well in the picture). A second series was removed ten days after the start of freezing and leached with water; the result is shown in Figure 2 H. The browning system had become completely inactivated in the lot receiving the 0.2 per cent dip treatment, and nearly so but not entirely in the 0.1 per cent lot. Only a very slight discoloration was obtained in the 0.15 per cent thiourea lot.

The effectiveness of a 10-day period of storage in the frozen condition was greater than that of a 4-day period, which indicates that there was occurring a distribution of thiourea within the tissue even when it was frozen.

The photograph in Figure 2 H fails to show what was clear in the tissue sample after thawing and soaking, that there was a brown development especially in the center of some of the pieces which were originally dipped in 0.1 per cent thiourea. This dipping solution which is adequate to protect peach and pear tissue during freezing, thawing, and soaking usually gave incomplete protection for apples under the same conditions.

Separation of the Freezing and Thiourea Effects

The results that have just been described show that dipping fruit tissues into thiourea solutions and freezing them brings about an inactivation of the browning system; but they do not prove that all of the effect is due to the presence of the thiourea and that the freezing process and the conditions during storage of tissue in the frozen state do not of themselves inactivate the browning system. This would not become apparent in an examination of the control tissue because it discolors during the time of preparation for freezing and in the early stages of freezing.

If tissue could be frozen without the occurrence of browning during the process of freezing and without a treatment with thiourea, it could then be determined whether the freezing process itself had partly or completely inactivated the browning system. It was found possible to make this test in the following way: a sugar solution containing 200 g. of cane sugar dissolved in 500 cc. of water was poured into 100 cc. test tubes which were then placed in the freezing-room along with an intact apple. The intact apple froze without turning brown internally. After 6 to 16 hours in the freezing-room the apple was peeled and dices of the solidly frozen white tissue were put into the tubes and were pushed downward into the sugar solution in the test tube. They were held in the lower part of the tube by a smaller test tube (see Fig. 3 J). Under these conditions even untreated apple tissue did not turn brown (except possibly very slightly at the core line) and was held for many days in the frozen condition without browning.

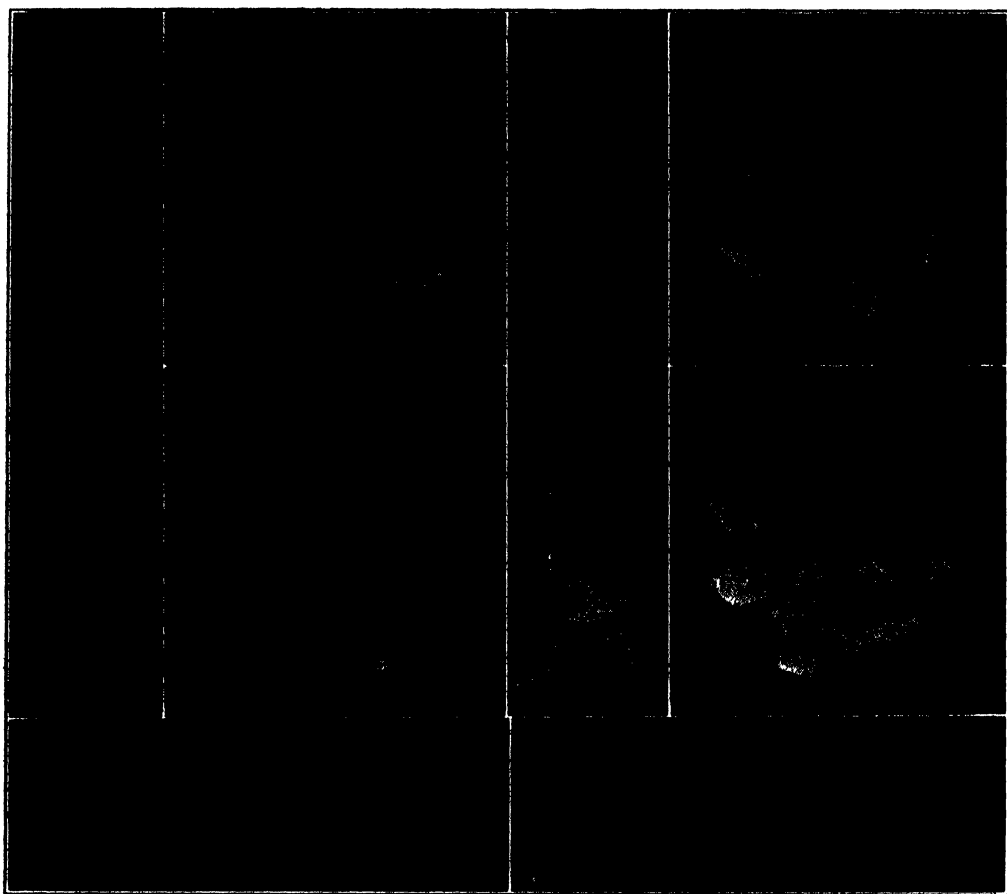


FIGURE 3. Separation of the freezing and thiourea effects in the inactivation of the browning system of apples. J. Obtaining apple tissue in the frozen condition without treatment with thiourea; the large test tube contained a sugar solution brought to the temperature of the freezing room before the tissue was put into it; the apple was first frozen in the intact condition and the frozen apple was peeled, cored, diced, and tissue immersed in the sugar solution, held in the lower layers by the inner test-tube; under such conditions apple tissue was frozen and held in the frozen condition without browning. K. Shows the color of such tissue when first removed from the tube and rinsed with water. L. Shows the color of such tissue when soaked overnight in an excess of water; freezing in the absence of thiourea did not inactivate the browning system. M, N, O. Shows a similar test when thiourea was added to the sugar solution in M to give 0.2 per cent thiourea. N. Tissue when removed and rinsed with water. O. Tissue after soaking in water overnight; presence of thiourea was the principal factor in the inactivation of the browning system. P, Q. Partial or nearly complete inactivation of the browning system of apple tissue with thiourea and without freezing. These lots not frozen but after dipping in 0.2 and 0.1 per cent thiourea solutions and in water, the dipped tissue was stored in tubes immersed in cracked ice. P. Removed after one day, soaked overnight in water, pulped in a mortar, and exposed to air for four hours, left to right in the order 0.2, 0.1 per cent thiourea, H_2O . Q. Same but stored in cracked ice for four days before soaking and pulping. Thiourea caused nearly complete inactivation of the browning system merely by dipping and storing at a low but not freezing temperature.

When, however, after remaining in the freezing-room for eight days, the tubes were thawed, and the tissue was exposed to air (see Fig. 3 K), browning occurred promptly (see Fig. 3 L). This showed that the conditions during freezing and thawing were not of themselves capable of inactivating the browning system of the fruit. On the other hand, tissue treated with thiourea by dissolving thiourea in the sugar solution to give a concentration of 0.2 per cent when carried through the same procedure (see Fig. 3 M) as that just described for the control tissue, did not turn brown during freezing (see Fig. 3 N), after thawing, nor after soaking in water to remove the excess of thiourea (see Fig. 3 O). This shows that the presence of the thiourea was the important factor in inactivating the browning system and not the conditions during freezing, storage in the frozen condition, thawing, or soaking. These were ineffective in the absence of thiourea.

Actual freezing was not found to be essential for the gradual inactivation of the browning system, at least of apples. Peeled and cored apples were cut into sixteenths; the pieces, after having been dipped into 0.2 and 0.1 per cent thiourea solutions and into water, were placed in large test tubes which were stoppered; these tubes were then stored in cracked ice (in order to permit extended storage without undue decomposition of the tissue). At intervals of one, two, and four days, samples were removed, the tissue was soaked in water for one day thereafter, and the tissue was ground in a mortar and exposed to air in a thin layer for four hours. The results for the one-day and four-day samples are shown in Figure 3 P and Q. Nearly complete inactivation of the browning system had occurred by the fourth day. There were some pieces of tissue in the 0.1 per cent lot which showed brown centers but their presence is obscured in Figure 3 Q because of the grinding of the tissue and because of the preponderance in amount of white over brown tissue. Inactivation of the browning system was accomplished more effectively in frozen tissue than in tissue packed in ice, presumably because the freezing increased the permeability of the tissue so that penetration of the thiourea into the tissue was facilitated.

SUMMARY

Tissue of the fruits of peaches, pears, and apples, after having been peeled, cored, or pitted, was dipped in dilute solutions of thiourea, NH_2CSNH_2 (also called thiocarbamide), previous to freezing in a cold room at -6° to -10° C. The tissue so frozen was stored for many months or even for more than a year without the occurrence of browning.

A dipping solution of 0.1 per cent thiourea sufficed for peaches and pears and one of 0.2 per cent for apples.

Fruit tissue so dipped and frozen did not turn brown when thawed and exposed to air, nor after it had been thoroughly leached with water during a period of 20 to 24 hours (to remove the absorbed thiourea). The

treatment had rendered the tissue incapable of turning brown on exposure to air.

Only a short period of storage of the treated tissue in the frozen condition was needed to inactivate the browning system. For peach tissue dipped into 0.1 per cent thiourea the time required for inactivation was one to two days, for pear tissue it was two to four days, and for apple tissue dipped into 0.2 per cent thiourea it was about ten days.

A method was developed for obtaining and holding apple tissue in the frozen condition without the occurrence of browning, and without treating it with thiourea. Such tissue browned rapidly upon thawing and exposure to air, while tissue handled in the same manner except that it was treated with thiourea did not turn brown even when leached with water. This showed that the conditions during the freezing period were not such as to cause inactivation of the browning system in the absence of thiourea, and indicates that thiourea by its presence brings about the loss of the capacity of the tissue to turn brown.

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SUBSTITUTED PHENOXY AND BENZOIC ACID GROWTH SUBSTANCES AND THE RELATION OF STRUCTURE TO PHYSIOLOGICAL ACTIVITY¹

P. W. ZIMMERMAN AND A. E. HITCHCOCK

Molecular configuration of physiologically active molecules has been repeatedly discussed in the literature and some authors have listed minimum structural requirements. Koepfli, Thimann, and Went (7, p. 779) are quoted as follows: "The minimum structural requirements for cell elongation activity in higher plants as indicated by the experimental evidence reported are (a) a ring system as nucleus, (b) a double bond in this ring, (c) a side chain, (d) a carboxyl group (or a structure readily converted to a carboxyl) on this side chain at least 1 carbon atom removed from the ring, and (e) a particular space relationship between the ring and the carboxyl group."

These specifications would eliminate benzoic acid as an active compound since the carboxyl group is not one carbon atom removed from the ring. In fact these authors cite it as an example of an inactive compound.

Recent results in our laboratory show that benzoic compounds are active when bromine and nitrogen groups are substituted in the proper positions in the ring. These substitutions do not change the space relationship between the carboxyl and the ring.

Phenoxyacetamide is an inactive compound but *p*-chlorophenoxyacetamide is active. Phenoxyacetic acid is only slightly active but *o*- and *p*-chlorophenoxyacetic acids are very active substances. The activity here seems to be associated with the substituted groups rather than the side chain alone.

The purpose of this report is to describe the various responses induced with substituted phenoxyacetic acid and benzoic acid, to compare and contrast the effects with those induced with other growth substances, and to consider briefly molecular configuration of physiologically active compounds.

MATERIALS AND METHODS

The plants used as test objects for determining the effects and activity of the various substances were as follows: African marigold (*Tagetes erecta* L.), buckwheat (*Fagopyrum esculentum* Moench.), cucumber (*Cucumis sativus* L.), garden pea (*Pisum sativum* L.), potato (*Solanum tuberosum* L.), *Rosa* var. Briarcliff, sensitive plant (*Mimosa pudica* L.), snapbean

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(*Phaseolus vulgaris* L.), sweet pea (*Lathyrus odoratus* L.), tobacco (*Nicotiana tabacum* L. var. Turkish), tomato (*Lycopersicon esculentum* Mill.), and tropical grape (*Cissus sicyoides* L. var. *jaquini* Planch.).

The chemicals were applied to the plants in water solution, as emulsions, as lanolin preparations, and as vapors. Materials such as Carbowax, Aerosol, and sodium alginate were used as spreaders when the water solutions were sprayed on the plants. The Carbowax was supplied by Carbide and Carbon Chemicals Corporation, New York City. This served as an excellent spreader for the sprays and increased the solubility of the substance. Sodium alginate was supplied by Kelco Company, New York City. Sodium alginate is also a good spreader and increases the solubility of the chemical. Lanolin emulsions were prepared as directed in a booklet on emulsions, published by Carbide and Carbon Chemicals Corporation. One of the constituents of the emulsion, triethanolamine, is a very effective solvent for hormone-like chemicals. Lanolin preparations were made up with a series of concentrations of the chemical, ranging from 0.007 to 20.0 mg./g. of lanolin. These preparations were applied to local parts of the plant with a glass rod. The liquids were usually applied with a nasal atomizer, though various kinds of sprayers were also tested. Plants were exposed to the vapors of the various compounds under bell jars. Usually a small amount of a chemical was placed in a watch glass which was set under the bell jar on an inverted warm or hot crucible. The amount of heat applied varied with the chemical. For example, ethyl chlorophenoxyacetate is very volatile and could be used with little or no heat applied. To produce vapors from the acids medium heat was used.

The following chemicals used in the experiments were purchased from Eastman Kodak Company, Rochester, New York: phenoxyacetic acid, phenoxyacetamide, *o*-chlorophenoxyacetic acid, *p*-chlorophenoxyacetic acid, benzoic acid, 2-bromo-3-nitrobenzoic acid, and 2-chloro-5-nitrobenzoic acid. The following compounds tested in our laboratory were synthesized by Edward K. Harvill of the Boyce Thompson Institute: 2,4-dichlorophenoxyacetic acid, *p*-bromophenoxyacetic acid, 2,4,6-tribromophenoxyacetic acid, *p*-nitrophenoxyacetic acid, *o*-phenylphenoxyacetic acid, *o*-allylphenoxyacetic acid, iso-eugenoxycetic acid, eugenoxycetic acid, *p*-phenylphenoxyacetic acid, ethyl benzoyl malonate, and *o*-chloromandelic acid. Of the latter group, only 2,4-dichlorophenoxyacetic acid, *p*-bromophenoxyacetic acid, and *o*-chloromandelic acid were found active for inducing cell elongation.

EXPERIMENTAL RESULTS

Chlorophenoxyacetic acid and bromonitrobenzoic acid have the capacity to induce many hormone-like responses in plants as described for some 50 other active compounds (11). They differ from the other sub-

stances, except for the β -naphthoxy compounds, in their capacity to cause formative effects on new organs or parts of organs which grow under the influence of the chemical. 2-Chloro-5-nitrobenzoic acid also has this unique capacity but it does not cause unusual cell elongation. The acids, methyl and ethyl esters, salts, and amides of chlorophenoxy compounds were of approximately equal activity.

Cell elongation involving epinasty and bending of stems and leaves. The test for physiological activity was the capacity of a substance to cause cell elongation beyond normal length. Unilateral application of the active compounds to a leaf or stem caused unequal elongation of cells and curvatures resulted in one to four hours. Preliminary tests for activity were usually made with lanolin preparations containing from 0.005 to 2.0 per cent of the chemical. The preparation was applied with a glass rod on the upper side of a leaf and one side of the adjacent stem. A region of the stem approximately an inch below the tip was considered the most responsive. The amount of bending and the degree of widening of the angle between the leaf and the stem were a measure of the activity of the substance. If the concentration of the chemical was too great the growth of one side of the stem and leaf was inhibited and the organs bent toward the treated side. This is a positive response; bending away from the treated side is called negative.

Table I lists 17 substances, 10 of which have growth activity (cell elongation) and 11 the capacity to modify organs. All but one of those having growth activity also modified organs. Two substances which induced formative effects did not cause cell elongation.

Figure 1 A illustrates degrees of responses induced with five different concentrations of 2,4-dichlorophenoxyacetic acid. If observed early, perhaps three hours after the preparations were applied, the stem treated with 0.1 per cent first showed negative curvature but later reversed its direction of growth. This phenomenon is thought to be influenced by the rate of penetration of the chemical.

The high degree of activity of dichlorophenoxyacetic acid for inducing cell elongation with low concentrations places this substance, for scientific consideration, on a par with α -naphthalenaecetic, indoleacetic, indolebutyric, β -naphthoxyacetic, and β -naphthoxypropionic acids, and above phenylacetic acid and cinnamic acid. In fact the responses induced with low concentrations of dichlorophenoxyacetic acid were much more lasting than those of the other hormone-like compounds. This will be brought out more clearly under the heading of formative effects (Table I).

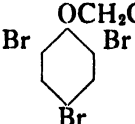
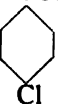
Ethyl *p*-chlorophenoxyacetate was applied as a vapor under bell jars to marigolds, garden peas, sweet peas, and tomato plants. This ester is very volatile and caused pronounced epinasty of leaves of tomato and marigold plants. The etiolated peas made pronounced swelling near the

TABLE I

STRUCTURAL FORMULAE OF GROWTH SUBSTANCES USED IN EXPERIMENTS AND CONCENTRATION RANGE FOR INDUCING NEGATIVE CURVATURE OF STEMS AND LEAVES AND FOR MODIFICATION OF ORGANS.* CONCENTRATION EXPRESSED IN PER CENT IN LANOLIN

Chemical substances	Concn. range inducing negative curvature	Concn. range inducing modification of organs
 Benzoic acid	Inactive	Inactive
 <i>p</i>-Aminobenzoic acid	Inactive	Inactive
 2-Chloro-5-nitrobenzoic acid	Inactive	0.05-5
 2-Bromo-3-nitrobenzoic acid	0.05-2	0.05-5
 2,3,5-Triiodobenzoic acid	Inactive	0.1-2
 Phenoxyacetic acid	1-2	Inactive
 <i>o</i>-Chlorophenoxyacetic acid	0.05-1	0.025-2
 <i>p</i>-Chlorophenoxyacetic acid	0.0125-0.5	0.006-1
 2,4-Dichlorophenoxyacetic acid	0.0015-0.05	0.0003-2

TABLE I (Cont'd.)

Chemical substances	Concn. range inducing negative curvature	Concn. range inducing modification of organs
OCH_2COOH  <i>p</i> -Bromophenoxyacetic acid	0.05-1	0.05-2
OCH_2COOH  2,4,6-Tribromophenoxyacetic acid	Inactive	Inactive
OCH_2COOH  <i>p</i> -Nitrophenoxyacetic acid	Inactive	Inactive
$\text{OCH}_2\text{CONH}_2$  Phenoxyacetamide	Inactive	Inactive
$\text{OCH}_2\text{CONH}_2$  <i>p</i> -Chlorophenoxyacetamide	0.025-5	0.006-1
$\text{OCH}_2\text{CONH}_2$  2,4-Dichlorophenoxyacetamide	0.0015-0.05	0.0003-2
CHOHCOOH  Mandelic acid	2-3	1-3
CHOHCOOH  <i>o</i> -Chloromandelic acid	0.1-2	0.1-2

* Substances inducing negative curvatures of stems and leaves are considered as exhibiting "growth activity." In contrast, substances which cause modification of organs are considered as exhibiting "morphogenetic activity" which involves responses other than curvatures due to cell elongation. A substance may be active for both or for either one of these two responses.

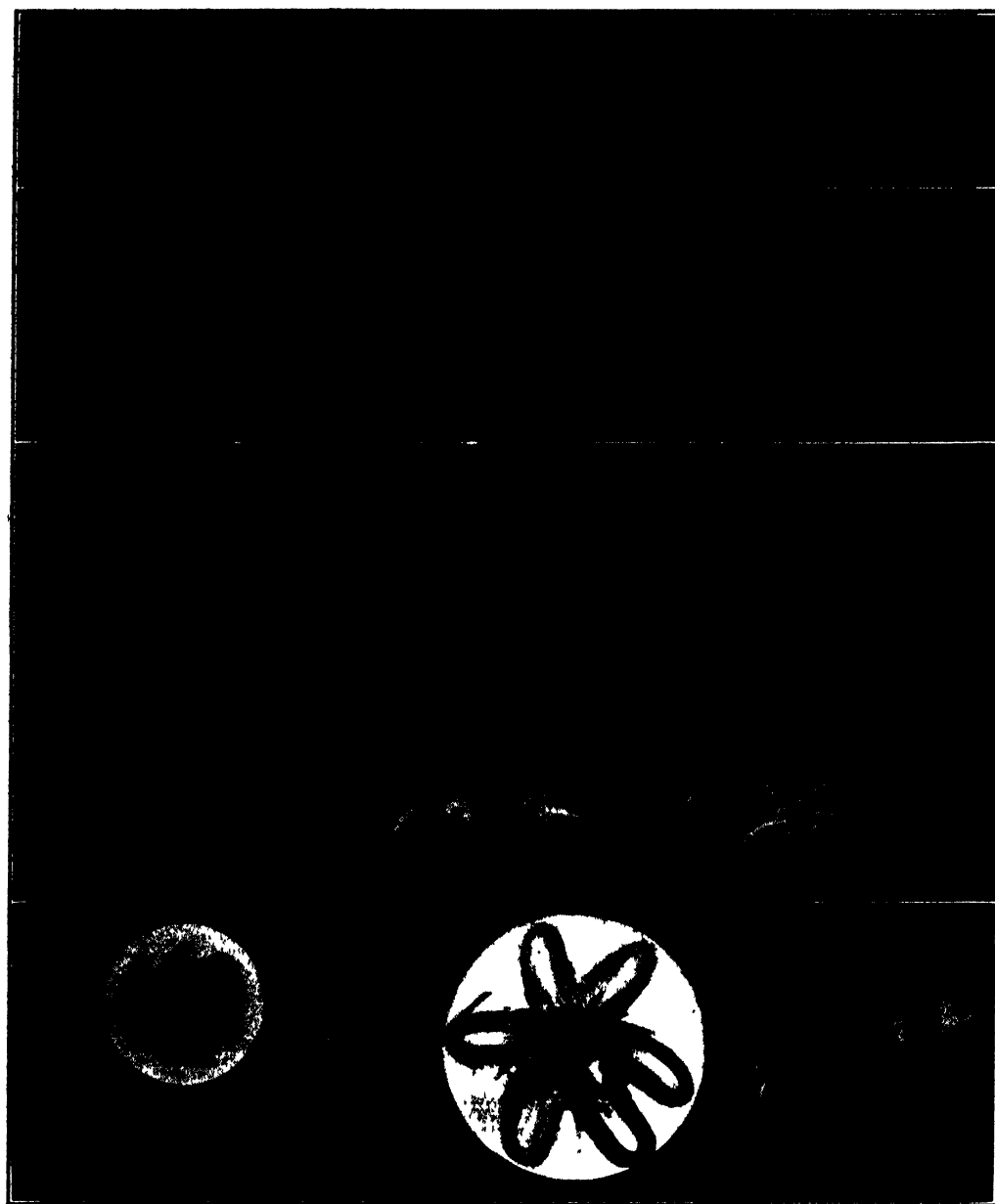


FIGURE 1. Response of tomato and *Cissus*. A. Curvatures induced with a series of concentrations of 2,4-dichlorophenoxyacetic acid. Left to right: (1) Control; (2) 0.03 mg./g.; (3) 0.06 mg./g.; (4) 0.125 mg./g.; (5) 0.25 mg./g.; (6) 10 mg./g. Photo after 24 hours. B. Plants sprayed with 2,4-dichlorophenoxyacetic acid. Left to right: (1) Control; treated with (2) 25 mg./l.; (3) 50 mg./l.; (4) 100 mg./l. Photo after 24 hours. C. Tomato leaf cuttings 7 days after treatment. Left to right: (1) Control; (2) *p*-chlorophenoxyacetic acid 1 mg./l. for 24 hours; (3) indolebutyric acid 1 mg./l. for 24 hours. D. *Cissus* roots (left to right): (1) Control; (2) section of root 2 inches back of modified tip; (3) modified tip after treatment with *p*-chlorophenoxyacetic acid 100 mg./l.; (4) free hand cross-section through modified portion of root; (5) treated with β -naphthoxyacetic acid 300 mg./l.; (6) treated with β -naphthoxypropionic acid 300 mg./l.

tip as described for vapors of several other substances (14). When heat was applied to the ester under bell jars with plants the leaves were injured. When lightly warmed pronounced responses resulted. Once the ester was heated under the bell jar enough remained on the wall so that plants enclosed thereafter were affected as if the chemical had been applied directly to the leaves.

Also the active acids were sufficiently volatile when warmed under the bell jar to cause the responses described for the ester. To expose the plant to the vapors the equivalent of about one drop was placed in a small watch glass, which in turn was placed under the bell jar on a warm inverted crucible.

Dichlorophenoxyacetic acid was dissolved in water in concentrations from 5 mg./l. to 300 mg./l. and sprayed on plants with an atomizer. When so treated the plants showed stem bending and pronounced epinasty. The following plants were used for these experiments: tomato, snapbean, peas, and marigold. The effects of the sprays were more pronounced and lasting when Carbowax or sodium alginate were used as spreaders. Figure 1 B shows the effect of three concentrations sprayed on plants.

One milligram of *p*-chlorophenoxyacetic acid in 50 cc. of water applied to the soil of a four-inch pot was sufficient to cause systemic response of a six-inch tomato plant. When used for soil treatments 0.5 mg. of *p*-chlorophenoxyacetic acid was as effective as 10 mg. of *o*-chlorophenoxyacetic acid.

Proliferations. The chlorophenoxy compounds, like substances previously reported, first caused unusual cell elongation which was followed by increased cell division and finally proliferations with many adventitious roots. Increased cell division became evident within 48 hours by swelling and whitening of treated tissue. In case of treatments with lanolin preparation containing 0.1 per cent or more of the *p*-chlorophenoxyacetic acid the substance spread in all directions from the locally treated tissue. The proliferations were numerous near the treated region but patchy at distant points as shown in Figure 2. The morphological structures induced with chlorophenoxy compounds in general resembled those brought about by treatment with β -naphthoxyacetic (1, 12) and β -naphthoxypropionic acids.

Cross sections examined with the microscope showed that cell division of the cambium and various parts of the cortex was accelerated within 24 hours. Bausor *et al.* (2) described similar responses in tissue treated with β -naphthoxyacetic acid. The vapor treatments caused more uniform swelling than chemicals applied by other methods. The photomicrograph shown in Figure 2 shows irregular growth which occurred after *p*-chlorophenoxyacetic acid had been applied to one side of the stem about two inches above the place where the section was made. This irregularity in growth may be due to the variation in the rate of transport of the chemical



FIGURE 2. Tomato plant showing response to *p*-chlorophenoxyacetic acid 20 mg./g. lanolin preparation applied near the tip of the plant. A. Cross-section approximately 1 inch below the tip showing adventitious roots in the cortex. B. Appearance of the plant 18 days after treatment.

to different regions. Figure 4 shows root primordia originating inside of the cambium and growing into the pith. This condition was induced within one-half inch of the tip of tomato plants which had been treated with lanolin preparations five or six inches below the tip. The tip showed inhibition of growth, swelling, and finally a white color. The older portions of the stem, one-half inch back of the tip, showed many root primordia in the cortex but the region nearest the tip produced root primordia in the pith.

Cuttings and adventitious roots. The use of chlorophenoxyacetic acid for inducing roots on cuttings has not been fully tested. However, its activity is indicated by its effects on tomato leaf cuttings in comparison with indolebutyric acid. Figure 1 C shows the comparative activity of indolebutyric acid and *p*-chlorophenoxyacetic acid seven days after treatment. The basal ends of the cuttings were placed in the chemical solutions (1 mg./l.) for 24 hours and then in tap water for six days. The roots induced with indolebutyric acid are much like those which grow without chemical treatment. The chlorophenoxyacetic acid caused heavy and sometimes fasciated roots, resembling those induced with β -naphthoxy and α -naphthalene substances. In lower concentrations, however, the chlorophenoxy compounds induced more nearly the normal type of roots.

Aerial roots of *Cissus* were particularly sensitive to chlorophenoxyacetic acid. Figure 1 D shows monstrosities induced with phenoxy and naphthoxy substances. Both groups of compounds cause unusual swelling followed by fasciated rows of roots. It appears that several root primordia fail to separate from one another and grow as a kind of wing instead of a round structure. The vascular structure, however, of each primordium was maintained and a microscopic examination disclosed that six or more roots were involved in one fasciated wing. The photomicrograph insert of a cross section in Figure 1 D may help one to understand these structures. Pfeiffer (8) made an extensive study of the anatomical responses of *Cissus* roots to treatment with indole and naphthalene substances. She stated that abnormalities such as the production of vertical extensions of tissue with several rows of vascular cylinders resulted from a second application of the substance. She used from 0.1 mg. to 10 mg. per gram of lanolin.

Para-chlorophenoxyacetic acid and dichlorophenoxyacetic acid were comparatively more effective than other growth substances when applied to the *Cissus* roots. For example, to induce swelling and monstrosities 25 mg./l. of dichlorophenoxyacetic acid was approximately equal to 100 mg./l. of β -naphthoxyacetic and β -naphthoxypropionic acids, 300 mg./l. of naphthaleneacetic acid, and 500 mg./l. of indolebutyric and indoleacetic acids. High activity for this kind of response usually indicates also high activity for inducing adventitious roots and other hormone-like responses. However, the chlorophenoxy compounds have not been fully tested for

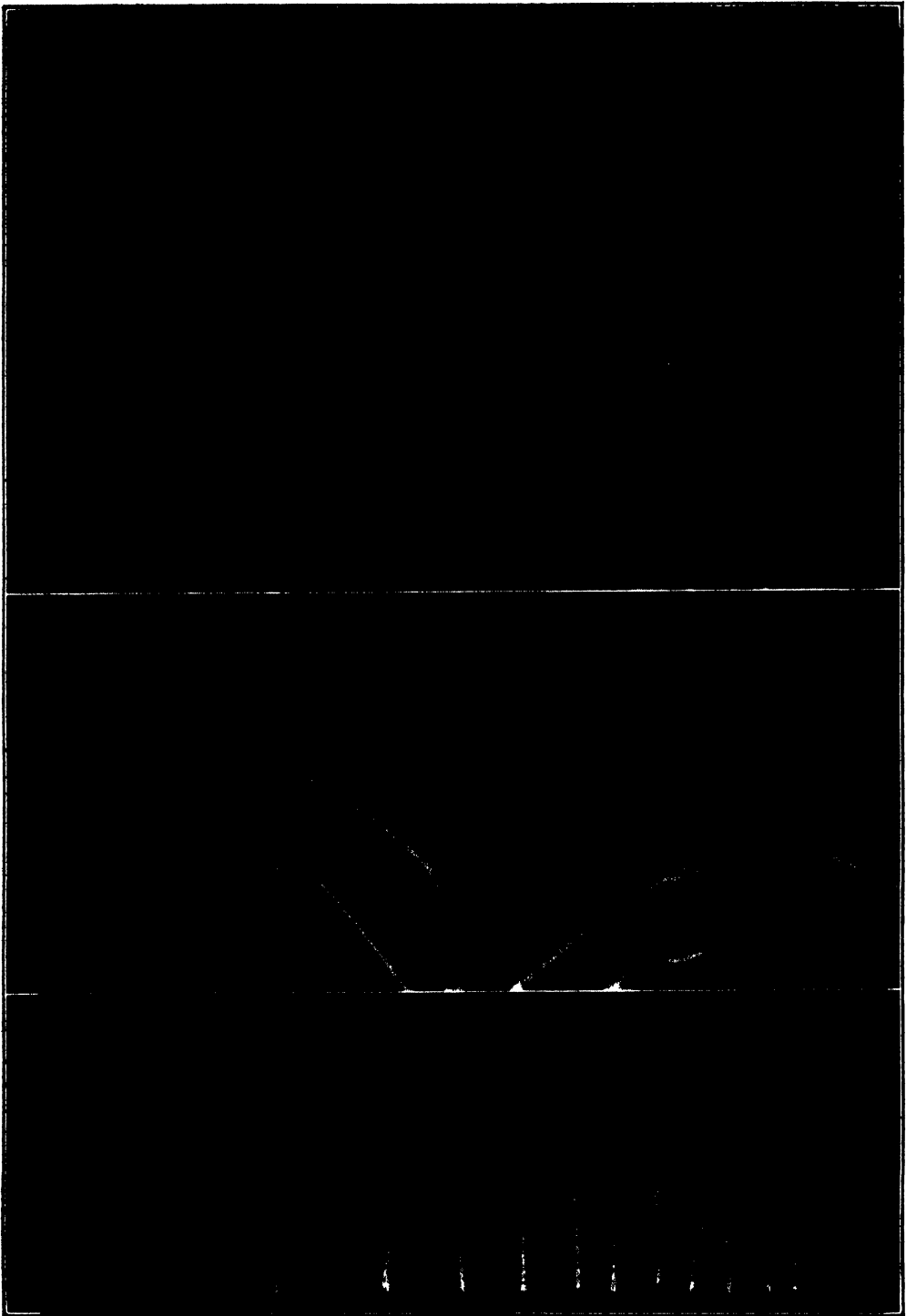


FIGURE 3. A. Tobacco plants to show formative effects induced with *p*-chlorophenoxyacetic acid 200 mg./l. applied at the tip when the plant was approximately 10 inches in height. Control on left. B. Enlargement of plant in (A). C. Series of leaves taken from base to tip of treated plant.

practical applications. When applied to tomato leaf cuttings at 0.5 mg./l. the following comparative activity was indicated by the total number of roots induced on three cuttings:

Control, 5	β -Naphthoxyacetic acid, 33
β -Naphthoxypropionic acid, 137	<i>o</i> -Chlorophenoxyacetic acid, 6
Indolebutyric acid, 116	Naphthaleneacetamide, 20
<i>p</i> -Chlorophenoxyacetic acid, 103	Phenoxyacetic acid, 0
Naphthaleneacetic acid, 86	

Inhibition of buds. As with other growth substances chlorophenoxyacetic acid inhibited axillary buds when the chemical was applied in sufficiently high dosages. One per cent of *p*-chlorophenoxyacetic acid in lanolin, applied directly to axillary buds of tomato plants, caused inhibition for 30 days after the terminal bud had been removed. Under similar conditions control buds normally grow within five days.

Tomato plants exposed to vapors of *o*- and *p*-chlorophenoxyacetic acid and ethyl *p*-chlorophenoxyacetate for 24 hours under a bell jar showed no growth of axillary buds for 30 days. These plants, however, showed pronounced curvatures, swelling, and proliferations on stem and leaf tissues. Recovery occurred and axillary buds grew when the exposure to vapors lasted for only 30 minutes if very little heat was applied to the chemicals. With exposure for 10 minutes to heavy vapors the plants did not recover in 30 days.

When one or two per cent of chlorophenoxyacetic acid was applied near the top of a plant eight inches in height the buds near the treated region remained dormant while the distant buds showed considerable growth. In fact they appeared to have been stimulated into activity since all the axillary buds grew out. When the upper end of the stumps of ten-inch plants was treated with a one per cent preparation of *p*-chlorophenoxyacetic acid in lanolin, all (8 to 10 in number) the axillary buds grew in contrast to controls which averaged only three. Tests to determine the possible stimulating effect of chlorophenoxyacetic acid on axillary buds are still in progress.

Parthenocarpy and seedless fruit. Howlett (6) and Strong (9) conducted extensive experiments using several growth substances to induce seedless fruit of tomatoes and other species. The concentrations used ranged from 1,000 to 10,000 p.p.m. in lanolin, water, and emulsions. Howlett obtained 90 to 100 per cent fruit with lanolin preparations and emulsions containing approximately 3,000 p.p.m. of indolebutyric acid. Strong obtained 80 to 89 per cent set with indolebutyric acid at 5,000 p.p.m. These concentrations are 20 to 50 times greater than those previously reported for naphthoxy compounds (10) and 300 times greater than the dichlorophenoxy compounds reported in the present paper (Table I).

The effective range for the naphthoxy compounds was 50 to 300 p.p.m. in water or emulsions. When the two naphthoxy compounds were mixed together a total of 150 p.p.m. was considered most satisfactory. With this concentration nearly 100 per cent of flowers treated when fully open set fruit.

2,4-Dichlorophenoxyacetic acid induces seedless tomatoes over a range of concentrations from 5 to 100 p.p.m. in water or emulsions. Ten to 25 p.p.m. with Carbowax as a spreader give best results. More than 100

TABLE II

COMPARATIVE EFFECTIVENESS OF THREE DIFFERENT CHLOROPHENOXY COMPOUNDS FOR INDUCING FRUIT SET OF TOMATOES. THE SUBSTANCES WERE DISSOLVED IN WATER WITH 2 TO 10 PER CENT CARBOWAX AS A SPREADER. THE SOLUTION WAS APPLIED WITH A NASAL ATOMIZER, THE ENTIRE CLUSTER BEING TREATED ONCE ONLY

Compounds	Concn., mg./l.	No. and stage of development of flowers and buds when treated				No. of fruit set each stage				Per cent set of each group			
		Old fls.	Newly opened fls.	Buds with color	Green buds	Old fls.	Newly opened fls.	Buds with color	Green buds	Old fls.	Newly opened fls.	Buds with color	Green buds
2,4-Dichloro- phenoxyacetic acid	5	3	16	0	18	3	15	—	5	100	93	—	27
	10	0	21	6	44	—	19	6	22	—	90	100	50
	25	2	21	5	22	2	20	4	5	100	96	75	22
	100	3	14	4	14	3	10	2	2	100	71	50	14
p-Chloro- phenoxyacetic acid	50	0	9	3	13	—	9	1	2	—	100	33	15
	100	3	10	4	10	2	7	2	1	66	70	50	10
	200	0	4	1	4	—	2	0	0	—	50	0	0
	300	3	26	3	26	1	19	1	3	33	73	33	11
o-Chlorophenoxy- acetic acid	50	0	7	0	8	—	7	—	1	—	100	—	12
	100	0	6	1	8	—	6	1	0	—	100	100	0

p.p.m. were toxic to small buds. Five to ten per cent Carbowax provided a very effective spreader. Table II shows some of the results obtained with chlorophenoxy compounds used in water solution with Carbowax as the spreader. Emphasis has been placed upon the effective concentration range rather than upon the influence of concentration for a given substance. Only one treatment is necessary when dichlorophenoxyacetic acid is used. Howlett (6) reported that two or more applications of indolebutyric acid in emulsion were necessary for best results.

The results in Table II show that buds also set fruit when sprayed with dichlorophenoxyacetic acid. When the entire cluster of flowers was sprayed with 10 to 25 p.p.m. the smallest buds showed parthenocarpic development. It is not known whether these should be retreated for best results.

Another method of treatment for seedless fruit is application of lanolin preparations around the peduncle. Concentrations of 0.25 mg. to 0.5 mg. of dichlorophenoxyacetic acid per gram of lanolin were very effective for tomatoes. Higher concentrations induced parthenocarp when applied to the peduncle but they also inhibited growth of the younger buds. Under

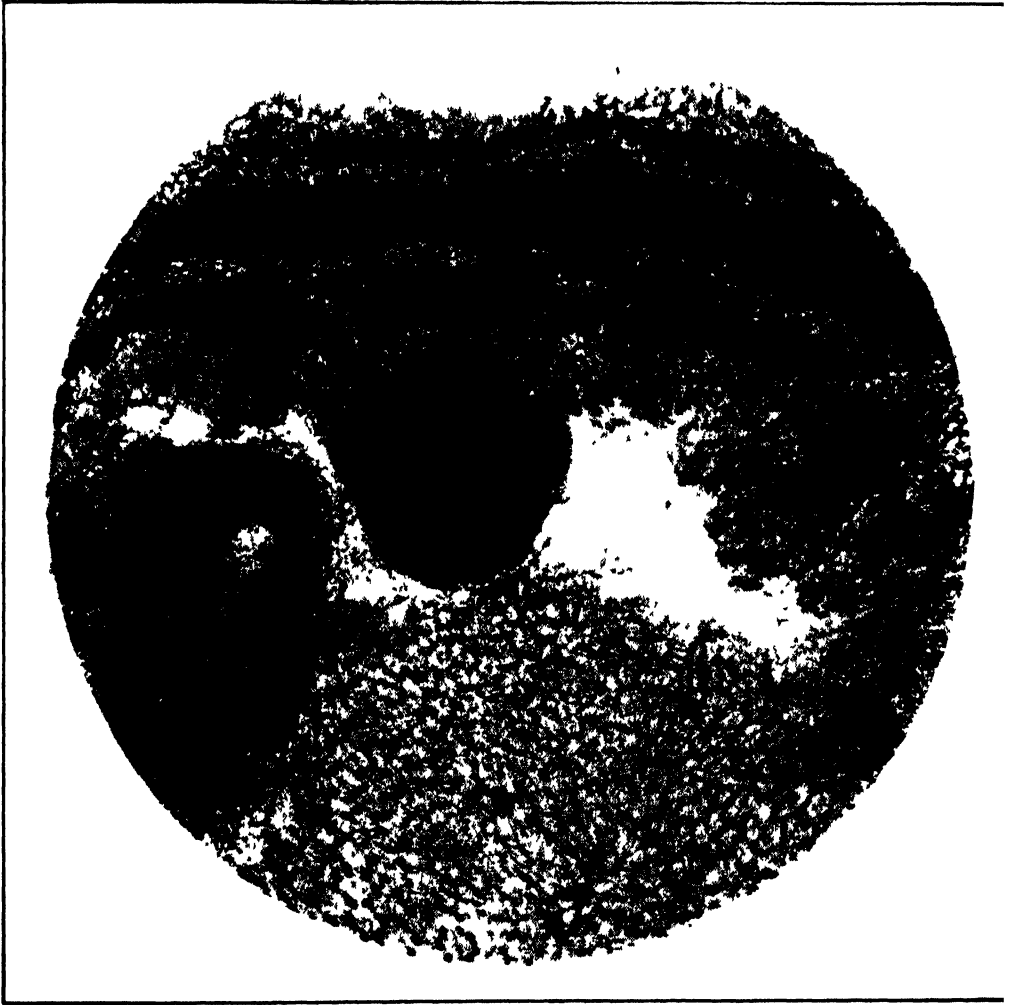


FIGURE 4. Cross-section of stem of tomato plant shown in Figure 3. Section taken approximately $\frac{1}{4}$ inch back of tip. Note root arising from internal phloem and growing into pith.

best conditions the open flowers and buds produced seedless fruit from one application of the preparation around the peduncle. Not enough tests have been made with this method to recommend it for extensive practice but the early results look promising.

Cucumber ovaries developed parthenocarpically when the lanolin preparations of chlorophenoxyacetic acid were applied to the peduncle, to the stigma, and to the cut surface after the stigma was removed. Here

again not enough tests have been made to justify recommendation for practice. Early results appear to favor approximately 5.0 mg. of 2,4-dichlorophenoxyacetic acid per gram of lanolin.

Persistence of floral parts. A few days after pollination of tomato flowers the corolla and stamen wither and become wrapped around the style. As the fruit develops these parts cling for a short time to the blossom end of the ovary and then disappear.

Under the influence of the various chlorophenoxy compounds the corolla and stamens of tomato flowers persist in good condition for 10 to 20 days. There is some variation with the condition of the flowers at the time they are treated. If the flower is just beginning to open when treated, the floral parts last longer than those of old flowers. When young buds are treated the corolla often remains in good condition for 30 days or more. When the corolla persists for some time it does not absciss from the receptacle as described for naturally pollinated flowers. Instead, the corolla and stamen become squeezed between the calyx and the developing ovary (12).

For tomatoes grown under glass the persistence of floral parts throughout the early stages of fruit development, and in some cases up to the fully ripe stage, may be regarded as an approximate index for seedlessness.

Formative influence. One of the most striking effects of chlorophenoxyacetic acid is its capacity to induce modifications in size, shape, pattern, and venation of leaves. A good illustration is shown in Figure 3. In this case a tobacco plant, approximately ten inches in height was sprayed at the tip with a water solution of *p*-chlorophenoxyacetic acid (200 mg./l.) on January 31. The photograph was taken 17 days later. Only one treatment was necessary to cause pronounced modifications. The effects were more lasting than those of β -naphthoxy compounds (12). Also the modifications were different. As shown in Figure 3 some leaves became lanceolate and some developed lobes, though normal tobacco leaves are simple with a smooth edge. The color of leaves on treated plants was darker green than those of controls, suggesting some interference with translocation of synthesized food. Even the stipules of modified leaves were modified, becoming greatly elongated in some cases but nearly absent in others. The lobes on the modified leaves resembled stipules moved out of their normal location.

At the tip of the plant shown in the illustration (Fig. 3 A) the leaves actually fused, forming a tube which enclosed the terminal bud. The upper end of the tube showed three lobes, indicating that three leaves had grown together. On February 25 the bud broke through the tubular leaves and another set of fasciated leaves appeared. This condition is illustrated by the enlargement in Figure 3 B. This is similar to the effect of β -naphthoxyacetic acid on coleus, gynura, and sunflower (10).

The veins of tobacco leaves modified with chlorophenoxyacetic acid changed their direction of growth but they were not like the leaves modified by β -naphthoxyacetic acid. In the latter case the veins affected by high concentrations failed to separate from the midrib making a kind of fasciation for veins. In a similar way leaflets in the primordia of compound leaves were often unable to separate, causing compound leaves to appear simple. Often the leaves were simple on one side while compound on the opposite side. The clearest illustration of failure of primordial organs under the influence of the chemicals to separate from one another is found in induced adventitious roots of *Cissus* and tomato cuttings (Fig. 1 C and D). The insert in Figure 1 D illustrates the appearance of fasciated roots in cross section.

In addition to the naphthoxy compounds which were previously reported (13) the following substances had a formative influence when applied to tomato plants: 2,4-dichlorophenoxyacetic acid, *p*-chlorophenoxyacetic acid, *o*-chlorophenoxyacetic acid, *p*-bromophenoxyacetic acid, 2-bromo-3-nitrobenzoic acid, 2-chloro-5-nitrobenzoic acid.

Chlorophenoxyacetic acid also induced modified leaves of cucumber, buckwheat, marigold, pea, potato, rose, sensitive plant, bush bean, two varieties of tobacco, cotton, and tropical grape. It will not be possible in this paper to describe all of these results in detail. The results with tomato plants will serve as a good illustration of the chemical effects.

Young tomato plants four to six inches in height were treated in four different ways to bring about results of a formative nature. The plants were sprayed with 5 to 500 mg./l. of water solution or emulsion, treated with lanolin preparations containing 0.0007 mg. to 2 mg./g., exposed to vapors of the chemicals, and treated through the roots by applying 0.5 mg. to 20 mg. of the chemicals to the soil of a four-inch pot. All four methods were effective for modifying the size, shape, venation, and pattern of the new organs which formed under the influence of the chemicals. Low concentrations applied only once caused only slight variation from the normal type of leaf while high concentrations caused pronounced modifications and peculiar monstrosities. Difficult to describe, the details are illustrated in Figure 5. The pattern of tomato leaves varied from frenched or fern-leaf types to simple leaves. In many respects the leaves resembled those of virus-diseased or mite-infested plants. Axillary buds of treated plants often produced shoots with leaves rolled into a ball (Fig. 5 C and 6 C). Proliferations sometimes grew along the veins, resembling adventitious buds.

Though there was considerable variation in the new growth of treated plants so much depended on the concentration of the chemical that qualitative differences between the chlorophenoxy compounds could not be detected. It was possible, however, under certain conditions to distinguish

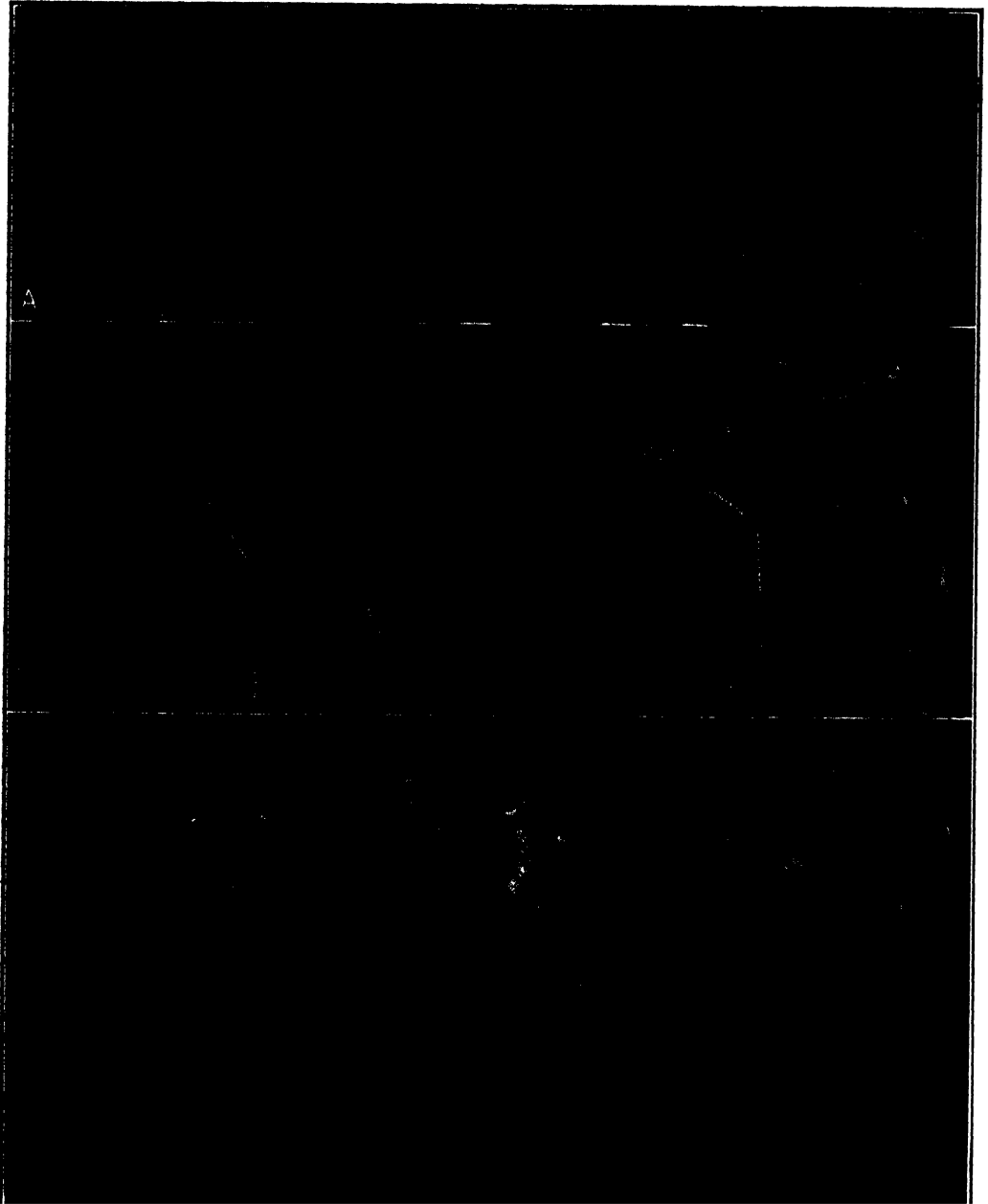


FIGURE 5. Tomato plants showing morphogenetic effects induced with chlorophenoxy-acetic compounds. A. (Left to right) Control; plants sprayed 19 days previously with *p*-chlorophenoxyacetic acid 100 mg./l.; plant treated 15 days previously with *o*-chlorophenoxyacetic acid 10 mg./g. B. (Left) Treated with lanolin preparation of 2-bromo-3-nitrobenzoic acid in lanolin. (Right) Treated with 2-chloro-5-nitrobenzoic acid in lanolin. Photo 16 days after treatment. C. Three plants showing response to *p*-chlorophenoxyacetic acid in lanolin preparation. (L. to R.) Treated near tip with 10 mg./g.; plant with excised tip treated on cut surface and three upper axillary buds; plant with excised tip treated on cut surface only. Photo taken approximately 25 days after treatment. Note formative effects on new shoots.

between the formative influence of naphthoxy and chlorophenoxy compounds. The latter had a tendency to cause the more upward curling (hyponasty) of leaf blades, more slender leaves, and the effects were more lasting. *Mimosa* leaves curled up and became twisted from the effect of chlorophenoxyacetic acid, whereas they grew straight under the influence of β -naphthoxyacetic acid. Both chemicals, however, caused peculiar projections along one side of the leaflets.

The modifications induced with 2-bromo-3-nitrobenzoic acid and 2-chloro-5-nitrobenzoic acid were similar when proper concentrations were used though the former caused cell elongation and the latter did not. The results with both resembled those induced with dichlorophenoxyacetic acid, though the former required approximately 100 times the concentration of the latter.

Phenoxyacetic acid and benzoic acid showed no formative influence on tomato plants. Their failure in this respect indicates the importance of chloro and nitro groups substituted in the proper positions on the ring as in 2,4-dichlorophenoxyacetic acid and 2-bromo-3-nitrobenzoic acid.

Translocation of substances. The fact that chlorophenoxy compounds moved up or down the stem was demonstrated in a number of ways.

Fifty cc. of water containing 0.1 to 1 mg. of 2,4-dichlorophenoxyacetic acid added to a four-inch pot of soil holding a five-inch tomato plant caused pronounced epinasty of leaves up to the top of the plant. The soil around the roots of the plants used in the experiments was wet before the chemical was applied and the solution was considerably diluted. When the roots of the tomato plant were washed free from soil and set into a solution containing 0.1 mg. of dichlorophenoxyacetic acid per liter of water the leaves all showed epinasty within three hours, showing that a highly diluted solution traveled upward in the stem.

Tomato plants ten inches in height were treated with a band of lanolin preparation, containing 5 mg./g. of dichlorophenoxyacetic acid, around the base, around the middle, and around the stem near the tip. All three treatments caused pronounced systemic responses, as shown by epinasty of leaves and curvatures and proliferations of the stems and leaves. Ten days later cross sections of the stem disclosed that adventitious root primordia had formed within an eighth of an inch of the tip. At the extreme tip the root primordia originated inside the cambium ring and grew into the pith (Fig. 4). One-half inch or more back of the tip the primordia arose outside the cambium and grew into the cortex (4). This type of response is illustrated in Figure 2 A. The leaves of plants treated around the stem with lanolin preparations showed swelling and proliferations on the upper side of the petiole, indicating the direction of transport of the active substances.

There were indications that concentrations of dichlorophenoxyacetic

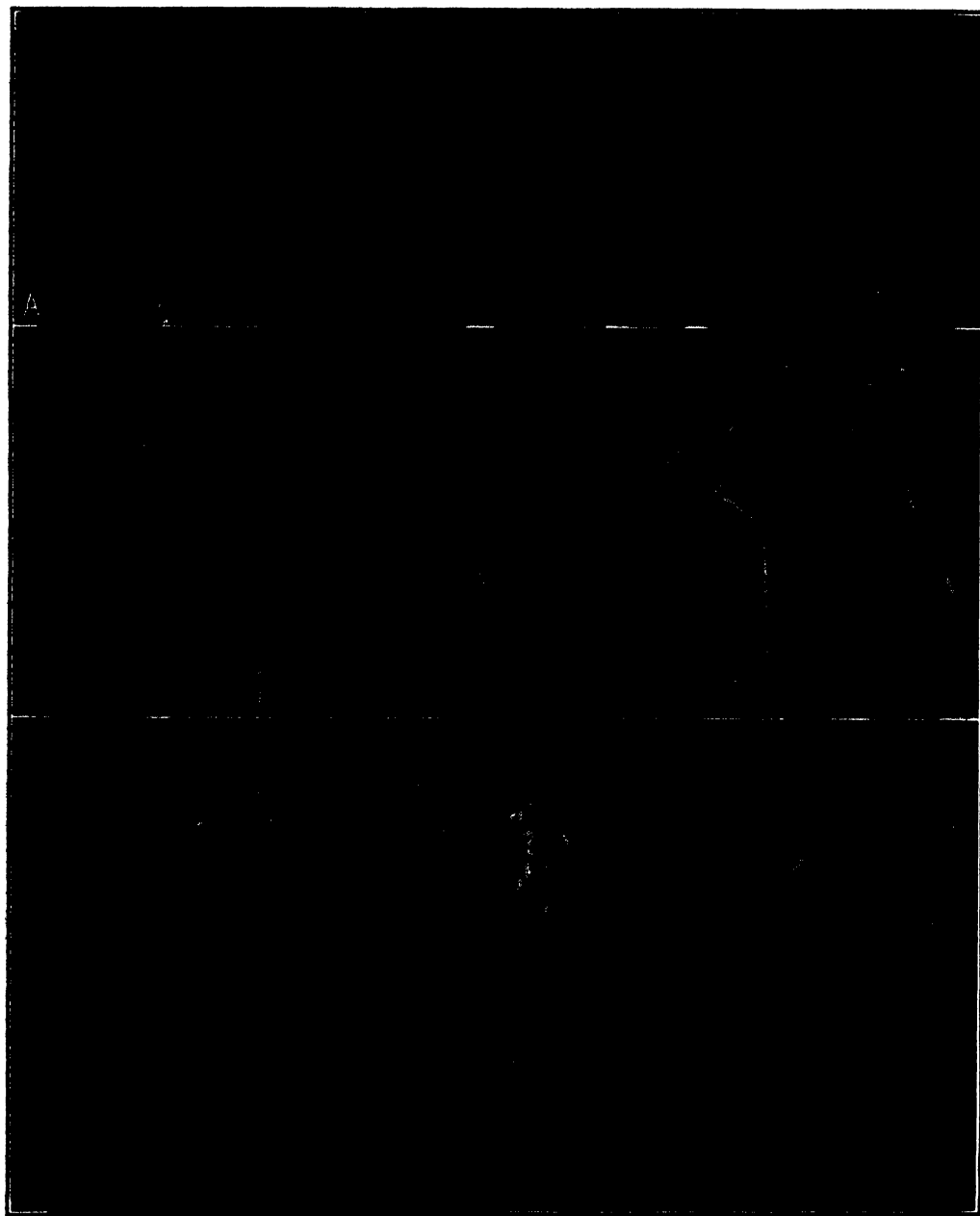


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Translocation of substances. The fact that chlorophenoxy compounds moved up or down the stem was demonstrated in a number of ways.

Fifty cc. of water containing 0.1 to 1 mg. of 2,4-dichlorophenoxyacetic acid added to a four-inch pot of soil holding a five-inch tomato plant caused pronounced epinasty of leaves up to the top of the plant. The soil around the roots of the plants used in the experiments was wet before the chemical was applied and the solution was considerably diluted. When the roots of the tomato plant were washed free from soil and set into a solution containing 0.1 mg. of dichlorophenoxyacetic acid per liter of water the leaves all showed epinasty within three hours, showing that a highly diluted solution traveled upward in the stem.

Tomato plants ten inches in height were treated with a band of lanolin preparation, containing 5 mg./g. of dichlorophenoxyacetic acid, around the base, around the middle, and around the stem near the tip. All three treatments caused pronounced systemic responses, as shown by epinasty of leaves and curvatures and proliferations of the stems and leaves. Ten days later cross sections of the stem disclosed that adventitious root primordia had formed within an eighth of an inch of the tip. At the extreme tip the root primordia originated inside the cambium ring and grew into the pith (Fig. 4). One-half inch or more back of the tip the primordia arose outside the cambium and grew into the cortex (4). This type of response is illustrated in Figure 2 A. The leaves of plants treated around the stem with lanolin preparations showed swelling and proliferations on the upper side of the petiole, indicating the direction of transport of the active substances.

There were indications that concentrations of dichlorophenoxyacetic

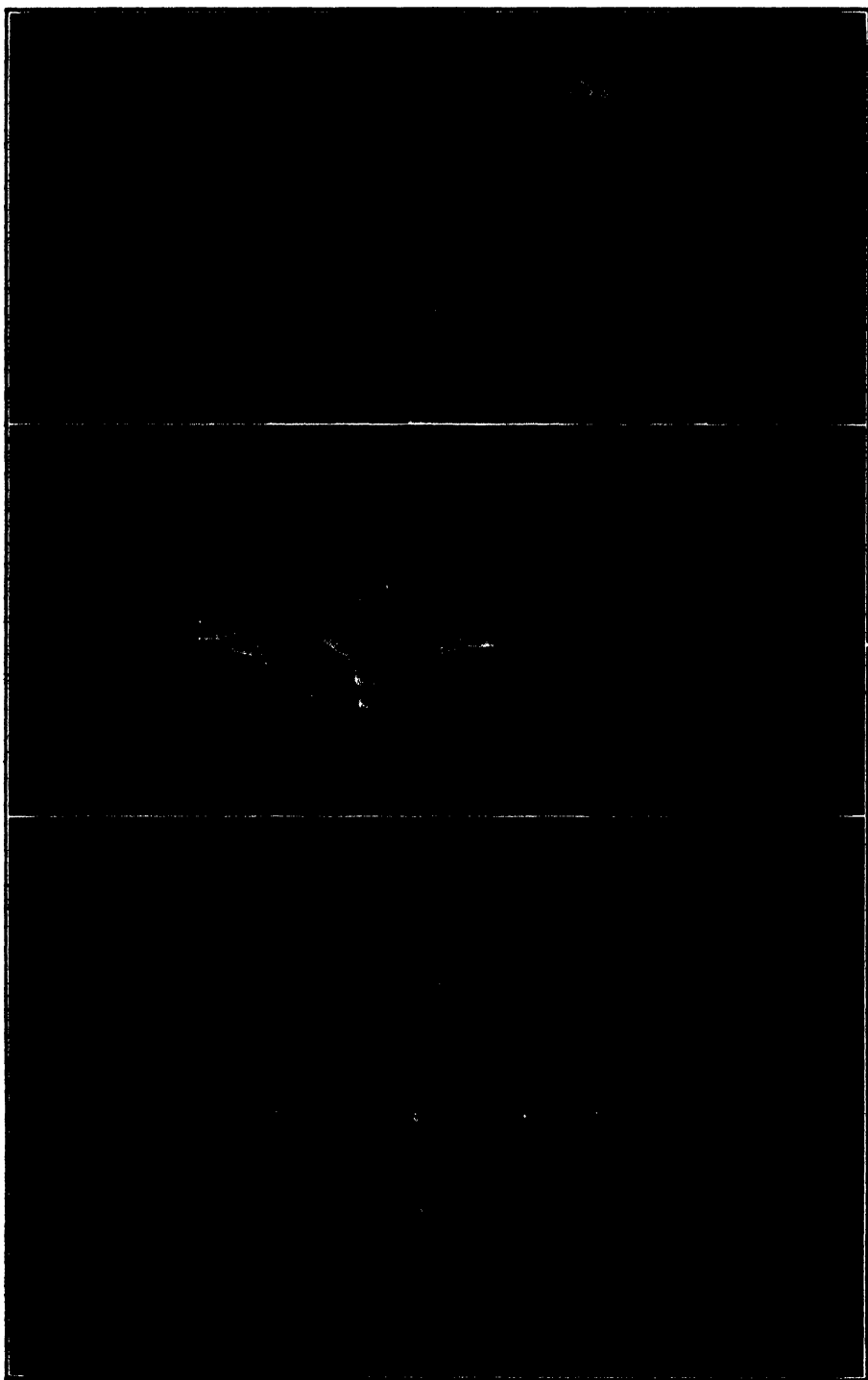


FIGURE 6. A. Y-shaped tomato plant. Left branch treated near middle with *p*-chlorophenoxyacetic acid 20 mg./g. of lanolin. Note modified leaves and flowers of shoot on right. B. Enlargement of "A" showing parthenocarpy before flower opened. C. Tomato leaves. Upper row from one plant; lower row, shoots and leaves taken at random from plants treated with chlorophenoxyacetic acid compounds.

acid too low to cause epinasty and proliferations also moved up the stem. The tips of tomato plants were removed and two axillary buds were allowed to grow, making Y-shaped plants. When the branches were approximately six inches long one of each plant was treated around the middle of the stem with a dichlorophenoxyacetic acid preparation containing 10 mg./g. of lanolin. The treated branch showed epinasty of leaves but the opposite branch did not. However, within six days the new growth of the non-treated shoots grew modified leaves and flowers, a characteristic influence for chlorophenoxyacetic acid (Fig. 6 A). The minimum concentration necessary to cause formative effects has not been determined but it is estimated to be approximately one part of the chemical to 50 million of the solvent. This assumption is based on the fact that one part of dichlorophenoxyacetic acid in 50 million of water induces slight epinasty of leaves when the root system of a tomato is immersed in the solution.

When flower clusters of tomato were sprayed with dichlorophenoxyacetic acid (25 to 300 mg./l.) the chemical influenced the new growth of stem tips at least 20 inches away.

Tobacco plants approximately ten inches in height, which were sprayed at the tip with 200 mg./l. of *p*-chlorophenoxyacetic acid, continued to produce modified leaves for 30 days until the plants were 30 inches in height and flower buds were visible (Fig. 3).

Ten-inch tomato plants treated on the cut surface of the stump after the tip had been removed produced many new shoots with modified leaves. Figure 5 C illustrates various degrees of modification on shoots two to seven inches in length 30 days after treatment. It is not known whether the growing tips were still under the influence of the chemical applied to the stump or whether genetic changes occurred as with colchicine. If the influence is of a direct chemical nature the substance must continue to move downward in the old stem and then upward to the tip of the new shoot (Fig. 6 A).

Chemical structures and biological activity. It is not the purpose of this paper to present theories or a lengthy discussion on the structural requirements for biological activity. It is rather our purpose to point out the inconsistencies and the difficulties encountered in attempting to arrive at a rational basis for selecting physiologically active substances by means of a study of their structural formulae. Biological activity of hormone-like substances extends from simple molecules, like carbon monoxide gas, to complex structures like naphthaleneacetic acid. In spite of the "minimum requirements" laid down by Koepfli *et al.* (7, p. 779), a comparison of the molecular structures of active and inactive compounds shows that we are still far from a satisfactory understanding of what constitutes a growth substance. There are no logical bases developed to date for excluding carbon monoxide, acetylene, propylene, and ethylene from the

list of growth substances. One of the "minimum requirements," "d. a carboxyl group (or a structure readily converted to a carboxyl) on this side chain at least one carbon atom removed from the ring, . . ." eliminates compounds which have a side chain made up of only a carboxyl group attached directly to the ring. An example is benzoic acid. It happens that benzoic acid is inactive, but 2-bromo-3-nitrobenzoic acid is active, though the carboxyl is linked directly to the ring.

Koepfli *et al.* (7) also considered the stoichiometric relationships and concluded that mole for mole all the active substances were equal for inducing a minimum growth response. This relationship does not hold for chlorophenoxy compounds as shown in Table I. For example the lower limits for inducing minimum responses are as follows: *o*-chlorophenoxyacetic acid, 0.5 mg. per gram of lanolin; *p*-chlorophenoxyacetic acid, 0.125 mg. per gram of lanolin; and 2,4-dichlorophenoxyacetic acid, 0.015 mg. per gram of lanolin.

Ortho and para acids have equal molecular weights though the latter is the more active. The activity of dichlorophenoxyacetic acid is relatively high and out of proportion when considered from the standpoint of molecular weights.

Similarly, *p*-chlorophenoxyacetamide is active down to 0.25 mg./g. while 2,4-dichlorophenoxyacetamide is active down to 0.015 mg./g.

It would seem, therefore, that the chlorophenoxy as well as some other compounds (5) do not follow a stoichiometric relationship for growth activity (3).

After *p*-chlorophenoxyacetic acid proved active for growth responses it seemed a safe assumption that *p*-nitrophenoxyacetic acid should also be active. However, tests involving several methods showed that the nitro group did not activate the molecule. In fact it inactivated, since phenoxyacetic acid, without the NO₂ group, possessed slight activity. *p*-Bromochlorophenoxyacetic acid, however, had growth activity though less than *p*-chlorophenoxyacetic acid. 2,4,6-Tribromochlorophenoxyacetic acid was inactive.

It is interesting to note that 2-bromo-3-nitrobenzoic acid is active for growth while 2-chloro-5-nitrobenzoic acid is inactive though both possess approximately equal capacity for inducing formative effects.

2-Chloro-3-nitrobenzoic acid has not been tested for activity. It seems logical to assume that it should be at least equal to and probably more active than 2-bromo-3-nitrobenzoic acid. However, it has not been possible safely to predict activity for new substances without biological tests.

DISCUSSION

The new growth substances mentioned in this paper are the first to show activity in relation to chlorine and bromine groups substituted in

the ring. They immediately suggest many other possibilities for other ring compounds. Perhaps the activity of indole, naphthalene, anthracene, and naphthoxy compounds can be improved by substituting halogens in various positions in the rings. The data presented in this paper show that the position in the ring where the chloro or bromo groups are substituted or combinations of these with the nitro group greatly affect the activity of the molecule.

All of the chlorophenoxy compounds that were active for growth also had a morphogenetic influence. Phenoxyacetic acid was slightly active for growth but did not induce formative effects. It would appear, therefore, that the chlorine group is at least in part responsible for the capacity of chlorophenoxyacetic acid to induce morphogenetic effects. *p*-Nitrophenoxyacetic acid is inactive for both types of response. 2-Chloro-5-nitrobenzoic acid has morphogenetic activity, but lacks growth activity (cell elongation), while 2-bromo-3-nitrobenzoic acid has activity for both responses. *p*-Bromophenoxyacetic acid is active for both growth and morphogenesis, while tribromophenoxyacetic acid is inactive for both. From these facts it appears that it is not enough to have bromine groups substituted in the ring, but that the molecular configuration as a whole determines the activity.

To date three groups of substances are known to have the power to induce morphogenetic effects on plants. They are β -naphthoxy, phenoxy, and benzoic acid derivatives. Our tests indicate that wherever the acid form is active the esters, salts, and amides also are active. If all these are counted there are some 40 substances possessing the peculiar capacity to induce formative effects when applied to plants. A study of structural formulae suggests many other possibilities and most likely many new substances will be found to possess this interesting characteristic. It is not clear at present how formative influences are linked up with growth, but complex interrelationships with natural substances in the plant are suggested. There is little or no effect on the form of organs or parts of organs already present. The modifications appear on the new growth which develops after the plants are treated with the chemicals.

SUMMARY

Several new growth substances have been described and their effects on plants compared and contrasted with those reported for other hormone-like compounds. Of the new substances, 2,4-dichlorophenoxyacetic acid and its derivatives were the most active for growth, inducing cell elongation of tomato with concentrations as low as 0.0007 per cent in lanolin. This compares favorably with the activity of naphthaleneacetic acid. *p*-Chlorophenoxyacetic acid was less active than the dichlorophenoxyacetic acid and *o*-chlorophenoxyacetic acid was less active than the para

form. When a bromine group was substituted in the para position in the ring the resulting compound was less active than chlorine in the same position. 2-Bromo-3-nitrobenzoic acid showed growth activity down to 0.05 per cent in lanolin. This is the first active compound having the carboxyl group linked directly to the ring.

All of the chlorophenoxy compounds having growth activity also induced another response having to do with morphogenesis. The new organs which developed under the influence of the chemicals were modified as to size, shape, pattern, and venation. 2-Bromo-3-nitrobenzoic acid and 2-chloro-5-nitrobenzoic acid induced formative effects though the latter was inactive for cell elongation.

The new compounds varied in their capacity to induce adventitious roots, the *p*-chloro and 2,4-dichlorophenoxyacetic acids and amides being the most active.

The chlorophenoxy compounds induced parthenocarp when applied to flowers of tomato and cucumber. Dichlorophenoxyacetic acid, at 10 to 25 mg. per liter of water, sprayed on open flowers of tomato, was very effective for inducing seedless fruit.

Considering the activity of halogen substituted derivatives, it appears likely that additional research in this new field would bring forth many new active compounds.

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AGRONOMIC VALUE OF KITCHEN WASTE

M. M. McCool

There is great need for improvement in the methods of disposal of garbage or organic waste in municipal areas and the conservation of much of it may be practicable. According to the American Public Works Association, which has brought together much information with respect to waste collected in several cities, such waste is termed garbage. It "originates primarily in kitchens, stores, markets, restaurants, hotels, and other places where food is stored, cooked, or consumed" (1, p. 4). It is also reported that the yearly per capita production of such waste amounts to about 300 pounds in Los Angeles, California, 152 pounds in Madison, Wisconsin, and 203 to 222 pounds in Rochester, New York. Some of the kitchen waste or garbage is fed to swine but much of it is either burned, placed in dumps, or thrown into rivers or into the sea. Thus, the total aggregate of organic matter wasted is great. This report deals with the agronomic value of some of the materials utilized in or prepared during earlier investigations (4).

REVIEW OF LITERATURE

An examination of the available literature which deals with the decomposition and fertilizing value of waste or garbage, as defined above, reveals a paucity of reports. There are several, however, which consider the composting and utilization on the land, of garbage to which other organic materials have been added. Bodler (3) early reported results of tests on the fertilizing value of sifted garbage and also of sewage sludge. He considered them to be of greater value generally than commercial fertilizer when added to sandy, gravelly, clay loam, and clay soils deficient in humus.

According to Tripp (7) field trials of one year's duration with kitchen waste which had been fermented 16 days, were conducted by the Rothamsted Experiment Station at five locations. Kale, sugar beets, potatoes, and mangold roots were included in the tests. Ammonium sulphate, rape dust, and farmyard manure were compared with the new product. The value of the nitrogen in the latter was about one-half that in ammonium sulphate. In addition it was superior to dung three times out of four. The author expresses the view that if the residual effect of the materials had been ascertained the processed waste would have had a higher rating. He also calls attention to the variations in the final products at the beginning of the venture and their correction later on. Although the results do not reveal whether fermentation of the waste improved it, they indicate the agronomic value of kitchen waste.

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Along with other reports which deal with studies on mixtures of garbage and other organic materials may be considered that of Anstead (2). He discusses the work of Fowler and others at Cownpore. Town refuse was easily converted into a fine humus of considerable value as a fertilizer by running activated sludge on to heaps of refuse and turning the mass from time to time.

Wood (8) emphasizes the losses entailed from not making use of habitation waste as manure and describes methods of procedure in composting mixtures of village refuse, house garbage, street refuse, estate rubbish, and hedge trimmings. Each batch or collection was mixed with that which had been composting one week. He concludes that the procedure was successful but uneconomic owing to the poor quality of the raw materials.

Wood (9) calls attention to the wastage of street and household refuse. He describes a process invented by Pico, an Italian scientist, which was first operated at Calabria in 1932. The process, which since has been modified, improved and patented in England, consists of an anaerobic fermentation followed by an aerobic fermentation. The waste is sprayed with a fluid containing bacteria and dumped loosely into a concrete cell which is sealed as soon as filled. Seven days later air is pumped through the mass by means of pipes. Aerobic fermentation proceeds for an additional seven days. The material is then turned out as a finely disintegrated cellular mass easily handled and without unpleasant odors.

Subrahmanyam (6) reviews the earlier literature on the conversion of different forms of waste into manures and gives results of experiments in which town refuse was sprayed with sewage. The tests were conducted in open air in heaps which ranged in weight from 744 to 1,534 pounds. The form of nitrogen and phosphate carriers added to the different heaps varied. One-tenth of one per cent of nitrogen, 0.03 per cent each of phosphate and potash were added throughout. The results show losses of much nitrogen except where activated sludge was added. Since the availability of the phosphate and potash did not change it was considered not necessary to add these to the composts.

Vegetation experiments which were conducted in the field showed the refuse manure to be as good as farmyard manure for the first crop and superior to it in its residual effects.

Night soil inoculum, prepared by mixing it with raw sewage and leaving it 10 days in a drum, was used successfully in breaking down the refuse. The hot fermentation process also was utilized. Here town waste was mixed with the starter in large aerated cisterns. After a few days the cisterns were sealed and so remained for three months. There was no loss of nitrogen, and very little decrease in the organic matter content. Unless the aerobic fermentation was allowed to proceed, the odor of the mass was offensive.

Plimmer (5) calls attention to the value of humus in maintaining suitable soil and plant relationships, the lowered resistance of plants to diseases, viruses, and insect pests when grown on infertile soils, and the likelihood of improperly nourished animals and human beings when fed on plants thus grown. The author also discusses the conservation of refuse, its pulverization and sale to farmers for composting with manure, and also the sale of mixtures of pulverized house refuse and sewage sludge to farmers after the demand for its use on taxpayers' land has been met.

MATERIALS AND METHODS

Most of the tests were conducted in the greenhouse in two-gallon glazed jars. Gloucester soil was used. It was taken directly from the field and screened without drying. Five grams of superphosphate (16%) and 0.5 gram of KCl were thoroughly mixed with the soil in each pot. For the outside experiments galvanized rings two feet in diameter and eight inches deep were used and the top four inches of the soil was screened and fertilized as described for the pot cultures.

The cultures in which millet (*Echinochloa frumentacea* Link) was grown were arranged in replicated blocks and those which carried corn (*Zea mays* L. var. Funk Bros. hybrid G-169) and tobacco (*Nicotiana tabacum* L.) in replicated rows on the central bench in the greenhouse. In order to reduce the variations in the different replicate cultures about 25 millet and 6 of the hybrid corn seed were planted in each container. All but 10 uniform millet and 2 of the corn seedlings were removed. Galvanized rims two feet in diameter and eight inches deep were utilized as the containers for the screened, moist, Gloucester soil in the field trials. The manures were passed through a $\frac{1}{4}$ -inch sieve and the waste samples through a 10-mesh sieve. All materials were mixed throughout the soil in the greenhouse tests and with the top four inches of that in the rims. The latter was accomplished by removing the soil and placing it on canvas. The hybrid corn seedlings were thinned to four in each of the galvanized rims.

The shredded stockyard manure, which came from Chicago, Illinois, contained 2 per cent nitrogen and 35.09 per cent ash. The cow manure, nitrogen content 2.08 per cent, was obtained from a local dairy. The sources of the organic waste employed are given in a previous report (4). The Dobbs Ferry waste was that collected from 40 household kitchens in an apartment on two different dates. The materials from Hastings were collected from a restaurant on each of five successive days. Each lot of Yonkers waste was that taken from 35 restaurants. These were selected for study because they differed in composition (4), and in the rate at which they nitrified in the soil, thus affording what were considered to be representative materials.

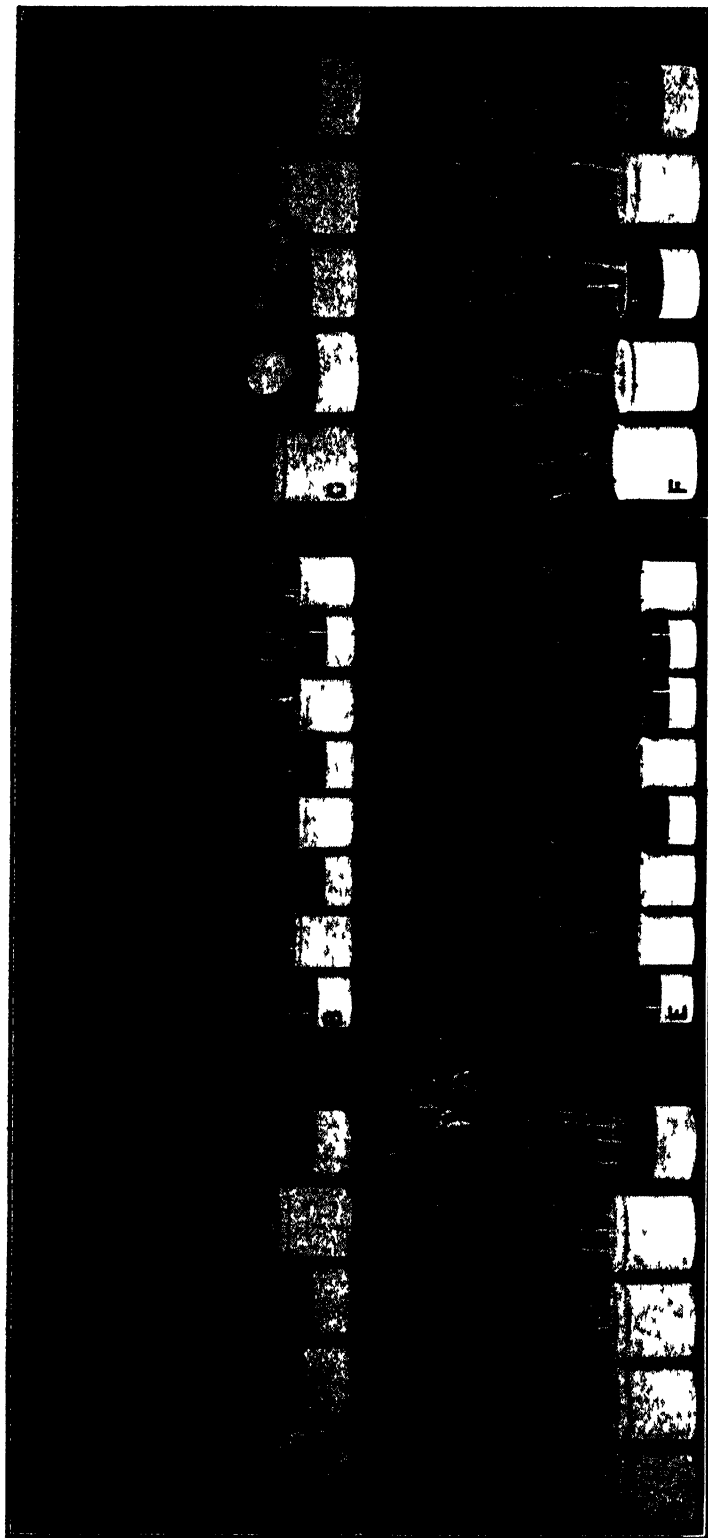


FIGURE 1. Greenhouse tests on availability of kitchen waste. A. Corn, left to right: control, 0.8 gram nitrogen as stockyard manure, 0.8 g. nitrogen as Dobbs Ferry composite waste, 0.8 g. nitrogen as Hastings composite waste, 0.6 g. nitrogen as stockyard manure, 0.6 g. nitrogen as Dobbs Ferry waste 1, 0.6 g. nitrogen as Hastings composite waste, 0.6 g. nitrogen as Yonkers, July 1940, waste, 0.6 g. nitrogen as Yonkers, September 1940, waste, 0.6 g. nitrogen as Yonkers, May 1941, waste. C. Tobacco, cultural treatments as (A). D. Millet. Control, 25 grams Hastings composite waste applied at seeding, 25 g. Dobbs Ferry composite waste applied at seeding, 25 g. Hastings composite waste applied 30 days before seeding, 25 g. Dobbs Ferry composite waste applied 30 days before seeding. E. Corn following millet in cultures (B). F. Millet following corn in cultures (A).

EXPERIMENTAL RESULTS

The first growth tests which are to be considered had to do with the availability of air-dried unprocessed waste from different sources, commercial process tankage, and stockyard manure. In these studies the materials were mixed with the soil, the mixture kept moist, and seeded or planted seven days later. The results derived from the use of millet as the plant growth indicator together with their statistical analysis comprise Table I. They are also illustrated by means of Figure 1 B. The process

TABLE I
AVAILABILITY OF KITCHEN WASTE. CROP INDICATOR, MILLET. DURATION OF GROWTH PERIOD, 55 DAYS. RESULTS EXPRESSED IN GRAMS, DRY WEIGHT BASIS

Cultural treatment	Yield of replicates			Totals	Mean
No nitrogen, control	4.1	3.6	3.1	10.8	3.6
0.2 Gram nitrogen as process tankage	9.5	10.8	7.8	28.1	9.4
0.4 " " " " "	17.5	17.2	17.1	51.8	17.4
0.6 " " " " "	19.5	21.6	20.5	61.6	20.5
0.3 " " " stockyard manure	2.3	2.3	4.4	9.0	3.0
0.6 " " " " "	3.8	4.6	3.6	12.0	4.0
0.9 " " " " "	4.4	7.0	3.9	15.3	5.1
0.3 " " " Dobbs Ferry waste 1	5.0	3.3	5.5	13.8	4.6
0.6 " " " " " 1	7.4	8.1	9.9	25.4	8.5
0.3 " " " " " 2	5.2	6.5	5.9	17.6	5.8
0.6 " " " " " 2	7.4	5.7	7.5	20.6	6.9
0.3 " " " Hastings composite waste	4.2	3.8	5.7	13.7	4.6
0.6 " " " " " "	6.3	7.7	8.1	22.1	7.4
0.3 " " " Yonkers, July 1940, waste	3.0	3.5	4.8	11.3	3.8
0.6 " " " " " "	5.4	4.9	7.7	18.0	6.0
0.3 " " " " Sept. " "	7.7	6.3	8.0	22.0	7.3
0.6 " " " " " "	15.1	13.3	13.3	41.7	13.9
0.3 " " " " May 1941, " "	11.0	11.9	10.3	33.2	11.1
0.6 " " " " " "	15.0	13.6	—	—	14.3
Block totals	138.8	142.1	147.1	428.0	

Standard error mean = 0.61

Minimum difference between means for significance = 1.74

tankage was more available than were the samples of manure and waste from different sources. A comparison, for example, of the yields derived from the cultures treated with 0.6 gram of nitrogen in process tankage, stockyard manure, and the samples of waste in the order given in Table I show them to be 19, 41, 33, 36, 29, 67, and 69 per cent of that from tankage. It is notable also that the various additions of stockyard manure to the soil and the smaller amount of the waste from sample 1 Dobbs Ferry, Hastings, and Yonkers, July 1940, did not result in significant increases in yields over the control cultures. The same amount of Dobbs Ferry 2, Yonkers, September 1940, and May 1941, and the larger amount of the waste in every instance, however, did so.

The residual or carry-over effect of the different materials as measured

by the growth of corn in the same cultures after the removal of the roots of the millet plant may be seen by an examination of the data which comprise Table II and Figure 1 E. Process tankage which was so effective with the first crop, or millet, did not augment the yield of corn over the control cultures significantly. The cultures to which waste was added, with the exception of those treated with the smallest amount of Yonkers, September 1940 material, produced significantly larger yields than did the control cultures. Furthermore those samples of waste which were most

TABLE II
RESIDUAL EFFECT OF KITCHEN WASTE, GROWTH INDICATOR, CORN FOLLOWING
MILLET. DURATION OF GROWTH PERIOD, 55 DAYS. RESULTS
EXPRESSED IN GRAMS, DRY WEIGHT BASIS

Cultural Treatment	Yield of replicates			Totals	Mean
No nitrogen, control	1.9	2.1	1.7	5.7	1.90
0.2 Gram nitrogen as process tankage	1.7	1.4	2.1	5.2	1.73
0.4 " " " " "	2.3	2.0	2.2	6.5	2.17
0.6 " " " " "	2.4	2.0	3.1	7.5	2.50
0.3 " " " stockyard manure	3.0	2.5	2.6	8.1	2.70
0.6 " " " " "	2.7	3.2	2.1	8.0	2.67
0.9 " " " " "	3.1	5.0	2.6	10.7	3.57
0.3 " " " Dobbs Ferry waste 1	3.4	3.7	3.9	11.0	3.67
0.6 " " " " " 1	5.1	7.7	6.6	19.4	6.47
0.3 " " " " " 2	4.7	3.4	3.4	11.5	3.83
0.6 " " " " " 2	5.8	5.8	6.5	18.1	6.03
0.3 " " " Hastings composite waste	4.7	3.8	3.5	12.0	4.00
0.6 " " " " " "	5.5	4.1	5.4	15.0	5.00
0.3 " " " Yonkers, July 1940, waste	2.7	4.2	2.8	9.7	3.23
0.6 " " " " " "	3.8	4.3	4.3	12.4	4.13
0.3 " " " " Sept. " "	3.2	2.3	2.7	8.2	2.73
0.6 " " " " " "	4.3	4.1	3.6	12.0	4.00
0.3 " " " " May 1941, " "	3.0	3.5	3.9	10.4	3.47
0.6 " " " " " "	4.1	5.0	—	9.1	3.03
Block totals	63.3	65.1	63.0	200.5	

Standard error mean = 0.38

Minimum difference between means for significance = 1.06

active in the promotion of the growth of millet were less so with respect to corn. On the other hand, those which were less available for the millet, with one exception, had greater residual effects than did those which were most active in the first series of tests. It is also to be noted that stockyard manure was the least active of the materials added to the soil.

The results derived from the use of the tobacco plants as the growth indicator comprise Table III and Figure 1 C. Tankage, and the larger quantity of Dobbs Ferry composite and Hastings composite samples and each of the quantities of the Yonkers, May 1941, waste or garbage increased the yield over the control cultures. The Yonkers material was notably effective in this respect. The cultures which received 0.4 gram of nitrogen in stockyard manure, Dobbs Ferry composite waste, Hastings

TABLE III

AVAILABILITY OF KITCHEN WASTE. CROP INDICATOR, TOBACCO. DURATION OF GROWTH PERIOD, 55 DAYS. RESULTS EXPRESSED IN GRAMS, DRY WEIGHT BASIS

Cultural Treatment	Mean of 5 replicates
No nitrogen added	2.42
0.4 Gram nitrogen as process tankage	9.36
0.4 " " " stockyard manure	2.84
0.8 " " " " "	6.30
1.2 " " " " "	5.10
0.4 " " " Dobbs Ferry composite waste	4.76
0.8 " " " " "	9.90
0.4 " " " Hastings " "	4.18
0.8 " " " " " "	8.66
0.4 " " " Yonkers, May 1941, " "	9.00
0.8 " " " " " "	16.56

Standard error mean = 1.57

Minimum difference between means for significance = 4.44

composite waste, and Yonkers, May 1941, waste produced 30, 50, 44, and 95 per cent respectively of that derived from the same amount of nitrogen in process tankage.

Corn (Fig. 1 A) was also used to measure the availability of the waste from different sources. According to the data which comprise Table IV,

TABLE IV

AVAILABILITY OF KITCHEN WASTE CROP INDICATOR, CORN. DURATION OF GROWTH PERIOD, 55 DAYS. RESULTS EXPRESSED IN GRAMS, DRY WEIGHT BASIS

Cultural Treatment	Mean of 3 replicates
No nitrogen, control	3.70
0.2 Gram nitrogen as process tankage	7.0
0.4 " " " " "	11.93
0.6 " " " " "	12.90
0.4 " " " stockyard manure	5.13
0.8 " " " " "	6.36
1.2 " " " " "	7.03
0.4 " " " Dobbs Ferry composite waste	5.03
0.8 " " " " "	9.60
0.4 " " " Hastings " "	5.86
0.8 " " " " " "	6.56
0.4 " " " Yonkers, May 1941, " "	6.56
0.8 " " " " " "	12.13

Standard error mean = 0.58

Minimum difference between means for significance = 1.64

all treatments with the exception of those to which 0.4 gram of nitrogen in stockyard manure and in Dobbs Ferry composite waste was added, increased the yield significantly above that derived from the control cultures. The yield of corn from cultures treated with 0.4 gram of nitrogen

in Dobbs Ferry composite, Hastings composite, and Yonkers, May 1941, waste was 42, 49, and 55 per cent respectively of that derived from the same amount of nitrogen in tankage.

In addition, millet was seeded in the above cultures after the removal of the roots of the corn plants. The results obtained make up Table V and Figure 1 F. Here again the residual effect of the tankage was markedly

TABLE V
RESIDUAL EFFECT OF KITCHEN WASTE. CROP INDICATOR, MILLET FOLLOWING
CORN. DURATION OF GROWTH PERIOD, 44 DAYS. RESULTS
EXPRESSED IN GRAMS, DRY WEIGHT BASIS

Cultural Treatment	Mean of 3 replicates
No nitrogen, control	1.73
0.2 Gram nitrogen as process tankage	2.03
0.4 " " " " "	2.76
0.6 " " " " "	3.13
0.4 " " stockyard manure	2.80
0.8 " " " " "	4.93
1.2 " " " " "	5.57
0.4 " " Dobbs Ferry composite waste	5.47
0.8 " " " " "	9.10
0.4 " " Hastings " "	6.33
0.8 " " " " "	7.53
0.4 " " Yonkers, May 1941, " "	4.57
0.8 " " " " " "	7.43

Standard error mean = 0.45

Minimum difference between means for significance = 1.28

lower than was its influence on the previous crop of corn, only the cultures to which was added 0.6 gram of nitrogen from this material yielded more than did the controls. All other treatments except the smallest amount of

TABLE VI
AVAILABILITY OF KITCHEN WASTE. FIELD TEST GROWTH INDICATORS, SWEET CORN
AND MILLET. DURATION OF GROWTH PERIOD, CORN 70 AND MILLET 42 DAYS.
RESULTS EXPRESSED IN GRAMS, FRESH WEIGHT BASIS

Cultural treatment	Sweet corn	Millet*
Control, no nitrogen	372 300 269 328	16.4 19.2 17.8
60 lbs. nitrogen per acre as stockyard manure	421 346 435 496	23.5 19.0 24.2
120 lbs. nitrogen per acre as stockyard manure	623 432 543 762	32.1 39.3 36.4
60 lbs. nitrogen per acre as waste**	585 516 577 450	20.6 23.1 24.6
120 lbs. nitrogen per acre as waste	635 1058 1066 —	30.1 37.0 30.8

* Greenhouse tests with soil taken from rims which produced corn.

** Waste composed of composite samples of Hastings, Dobbs Ferry, and Yonkers, May 1941, collections.

stockyard manure increased significantly the growth of the millet. Those cultures which received 0.4 gram of nitrogen in Dobbs Ferry, Hastings, and Yonkers waste, for example, yielded 202, 364, and 165 per cent respectively more dry matter than was derived from the controls.

Sweet corn (var. Golden Bantam) was planted in soil which was placed in rims in the field. After the harvest and weighing of the entire above-ground portions, soil was removed, placed in jars, taken to the greenhouse, and millet seed planted in it. The availability of a mixture of equal amounts of Hastings composite and Dobbs Ferry composite samples and Yonkers, May 1941, was compared with that of stockyard manure. The results obtained comprise Table VI, and are illustrated by Figure 2. The

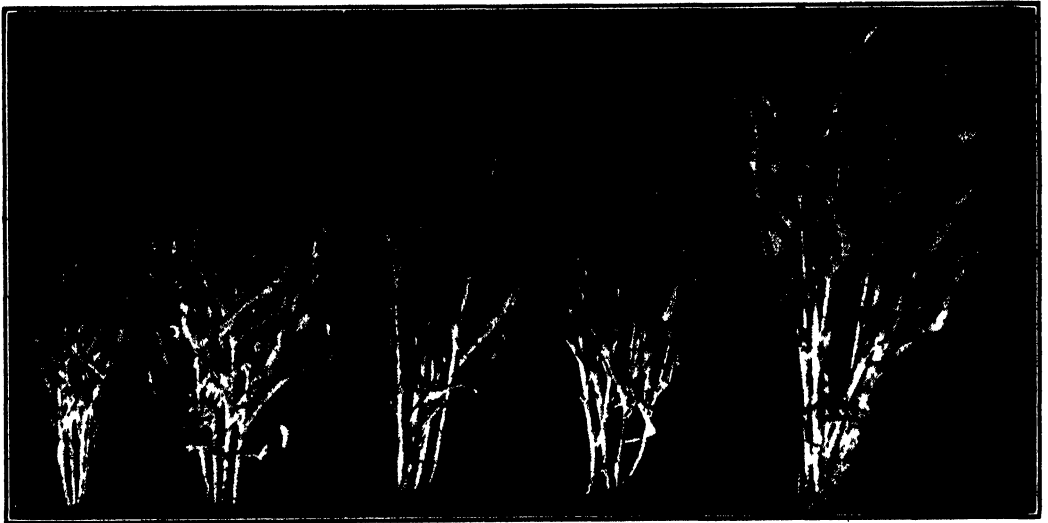


FIGURE 2. Field trial, availability of kitchen waste. Left to right: Control; 60 lbs. nitrogen per acre as stockyard manure; 120 lbs. nitrogen per acre as stockyard manure; 60 lbs. nitrogen as kitchen waste composite of Hastings, Dobbs Ferry, and Yonkers, May 1941, collections; 120 lbs. nitrogen per acre as kitchen waste, composite of Hastings, Dobbs Ferry, and Yonkers, 1941, collections.

waste was more effective than was stockyard manure for the production of the corn. The residual effects of these on the growth of millet did not differ significantly.

Throughout the conduct of these tests it was observed that with the exception of Yonkers, May 1941, collection, the addition of waste or garbage to the soil cultures retarded temporarily the growth of plants and did not result beneficially until several days after planting. A series of cultures was arranged in which waste was mixed with the soil 30 days prior to and at the time of seeding of millet. The yields derived from the growth cultures comprise Table VII and Figure 1 D. The yield of millet in the control cultures after 22 days was not significantly different from those

TABLE VII

EFFECT OF TIME OF APPLICATION ON AVAILABILITY OF KITCHEN WASTE. GROWTH INDICATOR, MILLET. RESULTS EXPRESSED IN GRAMS, FRESH WEIGHT BASIS

Cultural treatment	Growth period in days			
	22		42	
No nitrogen, control	6.5	5.9	14.0	12.6
25 Grams Hastings composite waste applied at seeding	5.5	5.0	24.1	21.3
25 Grams Dobbs Ferry composite waste applied at seeding	7.5	8.4	45.0	48.4
25 Grams Hastings composite waste applied 30 days before seeding	35.5	39.4	113.2	103.4
25 Grams Dobbs Ferry composite waste applied 30 days before seeding	19.5	23.5	58.5	64.4

to which organic materials were added at the time of seeding, but much less than that derived from those with which they were mixed 30 days prior to it. Twenty days later the waste had resulted in greatly increased growth of the millet, the early applications being the most effective.

Although the data are not presented, the addition of nitrate of soda to

TABLE VIII

EFFECT OF FIELD COMPOSTING ON AVAILABILITY OF KITCHEN WASTE. GROWTH INDICATOR, MILLET. DURATION OF GROWTH PERIOD, 44 DAYS. RESULTS EXPRESSED IN GRAMS, FRESH WEIGHT BASIS

Cultural treatment*	First harvest			Second harvest			Totals
Control, no nitrogen	4.5	3.4	3.9	6.4	7.0	6.2	20.4
0.2 Gram nitrogen as cow manure	8.9	6.0	7.4	6.2	6.6	6.5	41.6
0.4 " " " " "	15.4	11.6	12.3	10.2	10.0	8.6	68.1
0.8 " " " " "	14.2	11.6	13.4	11.0	8.4	9.6	68.2
0.2 " " " waste compost 1	5.5	4.1	4.6	22.1	21.8	19.4	77.5
0.4 " " " " " 1	6.5	6.1	5.8	27.2	34.9	29.6	100.1
0.2 " " " " " 2	4.2	3.9	4.4	18.5	16.4	17.6	67.0
0.4 " " " " " 2	4.1	3.5	3.8	25.1	23.0	21.2	81.7
0.2 " " " " " A	6.5	6.2	7.1	17.3	21.4	18.7	77.2
0.4 " " " " " A	6.0	5.5	6.1	27.2	24.6	23.4	92.8
0.2 " " " " " B	9.1	6.5	7.6	16.7	18.9	17.8	86.6
0.4 " " " " " B	5.4	5.1	4.8	30.3	29.2	27.3	101.1
0.2 " " " Yonkers, Oct. 1939, waste	18.4	16.8	17.2	18.0	18.1	20.2	108.7
0.4 Gram nitrogen as Yonkers, Oct. 1939, waste	13.3	10.4	12.1	33.1	30.2	28.3	127.4

* Compost 1 = Yonkers, Sept. 1939, waste field composted 10 days.

Compost 2 = Yonkers, Sept. 1939, waste + limestone and manure field composted 10 days.

Compost A = Yonkers, Oct. 1939, waste field composted 10 days.

Compost B = Yonkers, Oct. 1939, waste + limestone and manure field composted 10 days.

cultures along with different samples of waste and waste composts was successful in counteracting the delayed benefits of the materials.

The effect of composting different samples of waste is next to be considered. The first series of experiments involves Yonkers, September and October 1939, materials. These were composted 10 days in the field as described. At the termination of this period the materials which comprised each heap were mixed, a portion removed, prepared, and utilized in soil cultures. After the harvest of the first crop of millet the roots were removed, the soil returned to the original container and reseeded to millet. The data obtained comprise Table VIII. The composts were less readily available than cow manure as evidenced by the lower yields of the first

TABLE IX

EFFECT OF COMPOSTING ON AVAILABILITY OF KITCHEN WASTE, INCUBATED AT 40° C. GROWTH INDICATOR, MILLET. DURATION OF GROWTH PERIOD, 45 DAYS. RESULTS EXPRESSED IN GRAMS, DRY WEIGHT BASIS

Cultural treatment*	Yield of replicates		
Control, no nitrogen	2.1	2.8	2.4
Dobbs Ferry waste composite	3.7	3.2	3.1
“ “ “ “ composted 2 days	3.8	4.2	—
“ “ “ “ “ 4 “	3.7	4.5	4.1
Hastings waste composite	4.7	5.4	4.9
“ “ “ “ composted 2 days	6.6	6.7	6.2
“ “ “ “ “ 4 days	6.2	6.8	6.0
Hastings, Dobbs Ferry, and Yonkers, May 1941, composite	6.8	7.2	8.2
Hastings, Dobbs Ferry, and Yonkers, May 1941, composite, composted 2 days	8.6	9.5	8.2
Hastings, Dobbs Ferry, and Yonkers, May 1941, composite, composted 4 days	5.9	5.5	4.8

* 0.5 Gram nitrogen added in waste.

harvest of millet. The carry-over or residual effect of the composts, however, was much larger than that of the manure. The availability of the Yonkers, October 1939, waste was reduced markedly by composting.

The effect of incubating for two and four days at 40° C. inoculated composite samples from Hastings, Dobbs Ferry, and also a mixture of equal amounts of these and waste collected in Yonkers, May 1941, on their availability was studied. The results obtained are presented in Table IX. The availability of the samples of waste was not raised by composting. The value of the waste comprised of equal amounts of the mixtures from each source, however, was lowered by composting it four days.

The next set of growth tests dealt with materials which were composted three days at 55° C. Here tobacco plants followed by white mustard, *Brassica alba* (L.) Boiss., were utilized as the growth indicators. The data, together with their statistical analyses, comprise Table X. Since a difference of 12 between the means is acceptable for significance it is to be

TABLE X

EFFECT OF COMPOSTING KITCHEN WASTE 3 DAYS AT 55° C. ON AVAILABILITY.
GROWTH INDICATORS, TOBACCO AND MUSTARD. DURATION OF GROWTH
PERIOD, TOBACCO 50 AND MUSTARD 45 DAYS. RESULTS EXPRESSED
IN GRAMS, FRESH WEIGHT BASIS

Cultural treatment	Mean of 5 replicates	
	Tobacco	Mustard
Control, no nitrogen	14.76	3.6
20 Grams Hastings composite, not composted	43.42	15.0
20 " " " composted at 55° C.	44.12	15.8
20 " Dobbs Ferry composite, composted at 55° C.	41.20	16.7
20 " Yonkers, May 1941, not composted	74.24	17.5
20 " " " " " composted at 55°C.	84.06	17.5

Standard error mean for tobacco = 4.1, for mustard = 0.78

Minimum difference between means for significance for tobacco = 12.0, for mustard = 2.2

concluded from these results that composting did not affect the rate of availability of the samples as measured by the growth of tobacco. The residual effects are shown by the yields of mustard. Here again composting did not alter the availability of the waste samples.

DISCUSSION

The results derived from growth tests in the greenhouse afford evidence that the samples of waste collected with one exception were less readily available than was process tankage. Those samples of waste which were least available to the first crop became more so to the subsequent crop grown, whereas the residual effect of the tankage was negligible. They also demonstrate that the value of the waste materials as soil amendments varied with source and time of sampling. It would appear, however, that such variation would be minimized by the mixing of large volumes of the collections (7).

Thus if composting is to be justified it is to be done on the basis of other effects induced during the process. Those given (4) are darkening in color, structural changes, loss of obnoxious volatile materials, and reduction in the moisture content. A decrease in the content of the latter from 70 to about 40 per cent within 72 hours may take place. This is important when it is considered that inoculated waste when placed in an insulated chamber, heated with warm air moving through it, would soon give off heat due to the activity of microorganisms and thus lose water at a relatively small cost. The cost of the removal of water from the waste or waste composts appears to be the key to the solution of this problem. If the cost were too great it would not be practicable from the agronomic standpoint.

Owing to its agronomic possibilities it would appear to be advisable to set up an experimental or pilot plant to determine the practicability of

processing kitchen waste for use as a manure on the land. It appears, furthermore, that the mass should be ground, an inoculum from rapidly decaying compost added, and placed in an insulated chamber which is divided into several connecting compartments. These should be brought to 40° C. by passing heated air through them. Decomposition should be stopped about 72 hours later and the mass dried and ground. It further appears that large volumes should be mixed in order to provide a less variable product for distribution.

SUMMARY AND CONCLUSIONS

The soil-improving value of air-dried and ground samples of kitchen waste or garbage has been determined by means of greenhouse and field cultures. Tobacco, millet, and corn were employed as growth indicators in the former and sweet corn in the latter. In addition, the effect of composting at 40° and 55° C. on the availability of representative samples has been studied.

The results derived from the use of samples of waste in greenhouse tests showed them to be less effective for the first crop than tankage and to be superior to cow manure and shredded stockyard manure, when compared on the basis of the addition of equal amounts of nitrogen to the soil cultures. The residual effects, however, were greater than were those from tankage and the manures employed.

In a field test where sweet corn was grown, waste comprised of a mixture of those from different sources was superior to stockyard manure. The residual effect on millet, which was grown in jars of soil taken from the field cultures, did not differ.

The beneficial effects as indicated by plant growth in general were not obvious for several days after planting. This condition was corrected by the addition of a small amount of nitrate of soda and also by mixing the waste with the soil 30 days before planting.

As measured by growth effects, incubating the waste to which was added material from active composts did not increase its availability. When other ameliorating phases, such as changes in color, structure, loss of obnoxious volatile materials, and a marked reduction in the water content during decomposition, are taken into consideration, incubation appears to be practicable.

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INDUCED FORMATION OF A β -GLUCOSIDE IN THE RADISH

LAWRENCE P. MILLER

The absorption of certain chemicals by various species of higher plants has been shown to lead to the formation of β -glycosides with the introduced chemical serving as the aglycon (1). Insofar as information is available at this time it seems that such glycoside formation can occur quite generally among higher plants even in species or families not known to contain natural glycosides. The present paper reports results obtained with radish (*Raphanus sativus* L.) treated with chloral hydrate. These experiments were undertaken because the radish belongs to the Cruciferae, many species of which contain mustard oil glucosides with an enzyme system different from that associated with the ordinary glycosides. The results have shown, however, that the fate of chloral hydrate in the radish is no different from that in species not containing mustard oil glucosides and that in both root and top β -2-trichloroethyl-D-glucoside is formed from the absorbed chemical.

EXPERIMENTAL

Radish roots (2630 g., Scarlet Globe variety) which had been grown in the greenhouse were cut into pieces about one inch long and were immersed for 1.5 hours in a solution containing 1.5 g. of chloral hydrate per 100 cc. After the treatment period the solution was drained off and the roots placed in a desiccator through which a current of moist air was drawn at the rate of about 15 liters per hour. After six days the material was ground through a food grinder, the juice expressed through cheesecloth, centrifuged, heated to 80° C. and again centrifuged. Analysis showed this extract to contain non-ionic chlorine equivalent to 0.83 millimol of a trichloro-compound per 100 cc. Of this amount 0.48 millimol was recovered on distillation while an additional 0.12 millimol was released after hydrolysis by emulsin. Increased recovery of a compound containing non-ionic chlorine after emulsin hydrolysis indicated the presence of a β -glycoside involving the added chemical as aglycon. Following the same procedure used with tomato plants treated with chloral hydrate (2) this β -glycoside was obtained as the crystalline acetate and was found to be identical with synthetic β -2-trichloroethyl-D-glucoside tetraacetate. The preparation acetylated contained non-ionic chlorine equivalent to 2.0 millimols of a trichloro-compound and gave 1.11 g. of crude acetyl derivative melting at 139° to 140° C. Three recrystallizations from absolute alcohol gave 0.45 g. of the pure product melting at 144.5° to 145.5° (corr.) and with a specific rotation of $[\alpha]_D^{26} = -29.6^\circ$ (concn., 3.55 g. in 100 cc., CHCl_3). Syn-

thetic β -2-trichloroethyl-D-glucoside (3) has been found to have a melting point of 144.5° to 145.5° C. and a specific rotation of $[\alpha]_D^{25} = -29.0^{\circ}$. No depression was observed in a mixed melting point determination.

Analysis: Calcd. for β -2-trichloroethyl-D-glucoside tetraacetate, $C_{16}H_{21}O_{10}Cl_3$: Cl, 22.17. Found: Cl, 22.06, 22.09.

In another experiment β -2-trichloroethyl-D-glucoside (as the tetraacetate) was obtained from the leaves of radish plants grown in sand culture with chloral hydrate added to the nutrient medium. The plants were started from seed in sand in gallon containers (20 seeds per container). For the first month nutrients were added by the drip culture method (4) after which the containers were supplied with saucers and a nutrient solution previously described used (1). Two months after the start of the cultures 0.5 millimol of chloral hydrate in 50 cc. of distilled water was added to each container six times weekly for ten applications followed by the addition of 1.0 millimol six times weekly until a total of 13.5 millimols had been added to each culture. The plants were then sampled and the expressed juice obtained. The juice from the tops and roots was found to contain non-ionic chlorine equivalent to 0.63 and 0.24 millimol of a trichloro-compound per 100 cc. respectively and of this amount 0.12 and 0.08 millimol were volatile on distillation while hydrolysis by emulsin released an additional 0.10 and 0.01 millimol per 100 cc. Acetylation of a purified preparation (2) of the β -glycoside from the tops yielded 0.46 g. of crude product. After several recrystallizations from absolute alcohol the substance melted at 144.5° to 145.5° C. and gave no depression in a mixed melting point determination with synthetic β -2-trichloroethyl-D-glucoside tetraacetate. Specific rotation was found to be $[\alpha]_D^{25} = -28.8^{\circ}$ (concn., 3.735 g. in 100 cc., $CHCl_3$).

SUMMARY

Through its isolation as the tetraacetate, β -2-trichloroethyl-D-glucoside was shown to be formed in both roots and leaves of the radish from absorbed chloral hydrate.

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INTERRELATIONSHIP OF STORAGE TEMPERATURE, CONCENTRATION, AND TIME IN THE EFFECT OF CARBON DIOXIDE UPON THE SUGAR CONTENT OF POTATO TUBERS

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That the rapid increase in reducing sugar content which occurs in potato tubers stored at 5° C. could be prevented by adding carbon dioxide in the amount of 5 per cent by volume to the air surrounding the tubers was shown in a previous article (3).

For the further tests here reported upon, the temperatures 2° and 7° C. were included, and the CO₂ concentrations were increased up to 20 per cent or even to 40 per cent. The previous experiment was with one variety only and did not extend beyond 60 days of storage, whereas in the present tests tubers of three varieties were included and the duration was 90 days.

The general effect of CO₂ in retarding reducing sugar development at these low temperatures was again clearly evident from the newer tests, but it was found that, although at 2° C. 20 per cent CO₂ had a greater retarding influence than 5 per cent, at 5° and 7° C. the reverse was true; and, further, that at 7° C. 20 per cent CO₂ at first retarded reducing sugar development during about 30 days and then hastened it, so that after 90 days the reducing sugar content of the CO₂-treated lots was three to four times that of the controls.

As previously reported, the effect of CO₂ upon sucrose at 5° C. is just the opposite to that upon reducing sugar, sucrose formation being favored by the presence of CO₂. This result was confirmed in the present tests and was obtained not only at 5° but also at 2° and 7° C. However, this behavior occurred in storage at 2° C. only provided the period of storage was long enough, i.e., 60 to 90 days. The first effect of CO₂ at 2° C. was to retard the rate of sucrose formation, so that at the end of 30 days the sucrose in the CO₂-treated lots was only about one-third of that in the controls; but CO₂ then began to favor the formation of sucrose, so that at the end of 90 days the sucrose in the treated was about twice that in the controls (although with Katahdin such a response was obtained with 5 per cent and not 20 per cent CO₂).

METHODS

The potato (*Solanum tuberosum* L.) tubers were harvested in August, 1941, and were stored at room temperature, approximately 20° to 25° C.,

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until November 12, 1941, when they were distributed into lots of 16 tubers each in cheesecloth bags.

The containers for maintaining the proper gas mixtures in the atmospheres surrounding the tubers were tin cans, capacity approximately five gallons. The method of sealing the cans, preparing the gas mixtures, transferring the mixtures into the cans, and analyzing the gases in order to maintain the desired concentration of oxygen and carbon dioxide was discussed in a previous report (6). The selected concentrations of carbon dioxide and oxygen were maintained by replacing the gas mixtures every two to three days. Gas analyses of the atmospheres were made frequently in order to check the gas concentrations in the containers during the period of storage. The carbon dioxide content of the control treatment was maintained at zero by absorbing the CO_2 produced by the tubers with a 4 per cent sodium hydroxide solution. The 5 per cent CO_2 concentration when tested by gas analysis was never found to be below 4.2 nor above 5.6 per cent CO_2 , and the 20 per cent CO_2 concentration was maintained between 19.0 and 22.2 per cent.

The oxygen concentration was adjusted at 21 per cent when the gas mixtures were replaced, and during the two- to three-day period this percentage was reduced usually to about 17 but never below 16.5, except in a few cases at the start of these tests; in those tests after the tubers were exposed to 20 per cent CO_2 , the oxygen supply was found to have been reduced to 14.8 per cent for two periods totaling 6 days; this condition was corrected thereafter by more frequent changing of the gas mixtures in the containers.

At each sampling period the tubers were cut in two and slices were removed for the preparation of potato chips; the balance of the tissue was passed through a food grinder and juice was pressed by squeezing the tissue in cheesecloth bags. The procedure for frying the slices for chips and for carrying out the sugar analyses are described in a previous report (2).

RESULTS

The analytical values are shown mainly in Table I, data for reducing sugar per cc. of juice being entered in columns 3 to 11, and those for sucrose in columns 12 to 20. Since the effect of CO_2 upon reducing sugar is quite different from that which it has upon sucrose, the results for these two types of sugar will be dealt with separately.

REDUCING SUGAR

General response to CO_2 . Examination of the values in Table I, columns 3 to 11, shows that the effect of CO_2 upon the reducing sugar content of the juice has varied with the concentration of CO_2 , variety of potato, duration of the storage, and especially upon the temperature. In order

TABLE I
EFFECT OF CO₂ UPON THE SUGAR CONTENT OF POTATO TUBERS STORED FOR DIFFERENT LENGTHS
OF TIME AT DIFFERENT TEMPERATURES

Variety	Dura- tion, days	Reducing sugar, mg. per cc. of juice						Sucrose, mg. per cc. of juice					
		2° C.		5° C.		7° C.		2° C.		5° C.		7° C.	
		% CO ₂		% CO ₂		% CO ₂		% CO ₂		% CO ₂		% CO ₂	
		0	5	20	0	5	20	0	5	20	0	5	20
Irish Cobbler	30	6.3	0.0	0.0	5.0	0.0	0.0	29.3	24.3	10.4	8.1	28.0	26.4
	60	20.3	4.6	0.0	9.6	0.0	4.9	18.9	38.9	20.8	6.0	23.2	38.9
	90	23.9	8.1	3.5	9.4	0.0	4.1	16.4	42.8	35.2	5.8	31.3	41.1
Green Mountain	30	6.6	1.1	1.8	7.3	2.5	1.5	29.7	18.2	11.7	13.4	32.0	23.1
	60	30.9	5.8	2.7	12.2	4.1	5.2	14.2	33.9	23.2	9.9	33.1	35.8
	90	24.3	8.5	4.2	10.4	4.4	10.8	18.4	35.3	32.0	7.5	31.8	46.7
Katahdin	30	2.3	0.0	0.0	2.7	0.0	0.0	22.5	9.6	8.2	3.8	12.6	11.4
	60	15.2	3.9	1.0	5.6	2.6	2.2	12.9	26.7	14.2	2.8	13.4	15.1
	90	17.7	9.7	2.7	5.2	6.5	4.7	11.2	33.5	15.0	3.2	20.2	24.5

Note: (a) The values at the start were: Red. sug. 0.0, 0.1, 0.1, and sucrose 0.3, 3.0, 0.1 for Irish Cobbler, Green Mountain, and Katahdin respectively; (b) a zero value means that the sugar value was less than 0.1 mg. per cc. of juice by the method of sugar analysis used.

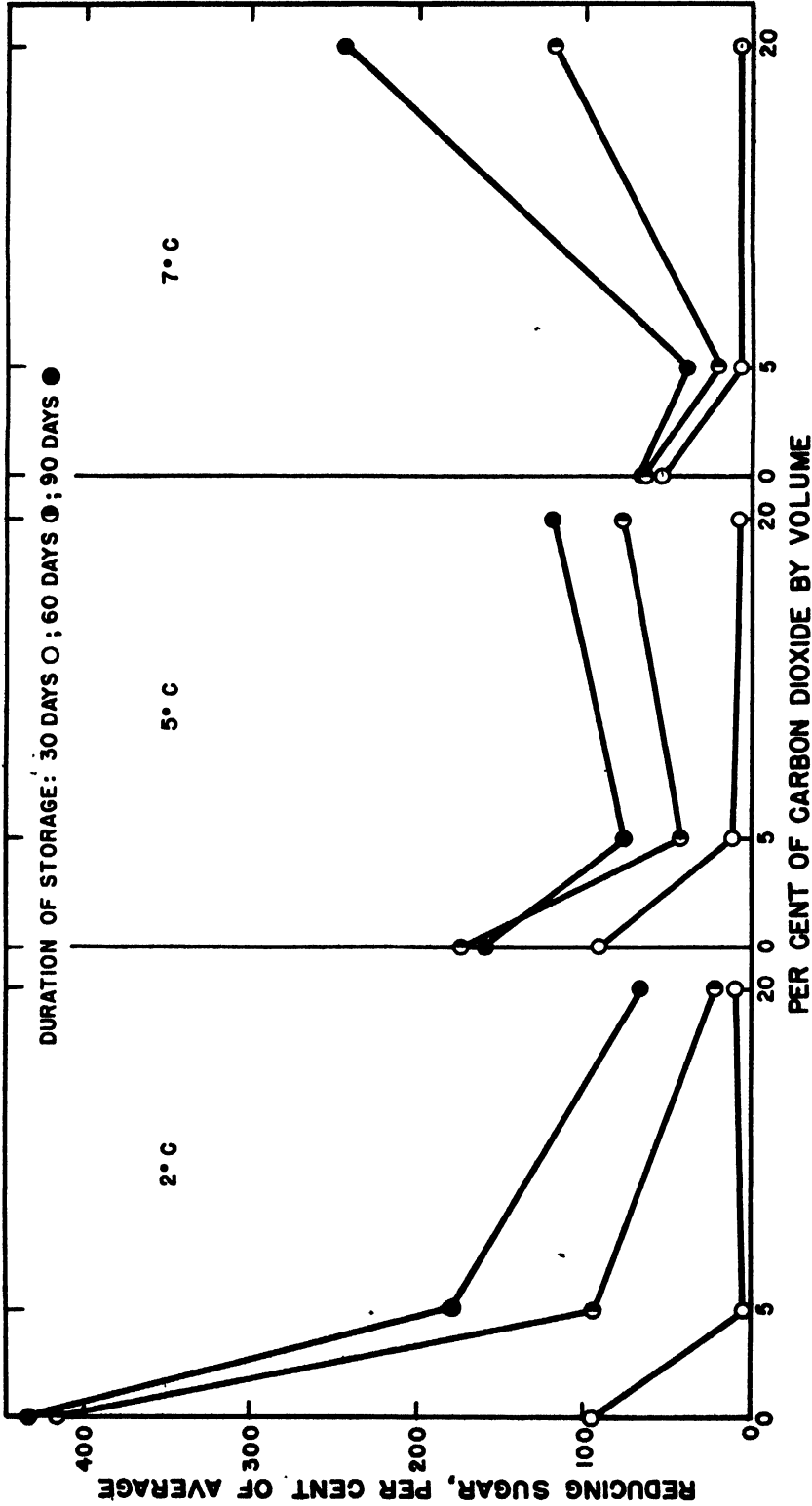


FIGURE 1. Effect of CO₂ treatment upon reducing sugar of potato tubers stored at different temperatures. Each plotted point is the average for three varieties in Table I after all of the values for each variety in the table were expressed as a percentage of the average value for that variety including the values for all temperatures, durations, and treatments.

to obtain a general view of the situation before proceeding to a consideration of the details, the curves in Figure 1 will be referred to first. These graphs were prepared from the data in Table I by expressing all values for each variety as a percentage of the mean value for each variety over all temperatures, treatments, and durations. For example, the average value for Irish Cobbler, lines 1 to 3, columns 3 to 11, is 4.7 and the percentage corresponding to 6.3 in line 1, column 3, is 134; for 6.6 in line 4 it is $100 \times (6.6 \div 4.7) = 93$, and for 2.3 in line 7 it is $100 \times (2.3 \div 3.9) = 59$. After all of the reducing sugar values in Table I had been converted into percentages, the three percentages representing the three varieties at each sampling period were averaged and these values were plotted in Figure 1. For example, the value for 2°, 30 days, control lot, is one-third of $134 + 93 + 59 = 95$. The other points used in plotting Figure 1 were obtained in like manner.

The question whether the combining of these observations is justifiable and gives a dependable picture was approached by testing the homogeneity of the variances of the "replicates" obtained in this way. There were three percentage values for each plotted point in Figure 1, i.e., one for each of the three varieties. The sum of the squares of the deviations of each member of the triplicate from the average value of the three was computed, and a table (not shown here) similar to that described by Snedecor (5, p. 207) was set up. The corrected chi-squared value obtained by this test was 42.89, which for 26 degrees of freedom gives a probability between 0.01 and 0.05 and indicates a lack of homogeneity. This failure, however, rested heavily on the value 6.5 for the Katahdin variety, line 9, column 7 in Table I, a value which is clearly inconsistent with the other values in the table. When this one value out of a total of 81 was omitted the chi-squared value became 34.97, which for 26 degrees of freedom (omitting one value having an effect upon Snedecor's designation $k-1$ but not upon the sample number, m , see Snedecor's table 10.14) corresponds to a probability a little larger than 0.10. This was taken to indicate a group of variances sufficiently homogeneous to justify the averaging of values for the preparation of the graphs. The value 6.5 was not omitted from any computation in this report except that involved in testing for the homogeneity of the variances in preparing the graphs for Figure 1.

Figure 1 shows that 5 per cent CO₂ retarded the increase in reducing sugar at all three temperatures of storage and at all durations. When the amount of CO₂ was increased to 20 per cent, however, the response depended upon the temperature, this concentration retarding sugar development at 2° C. and hastening it at 7° C.; at 5° C. the values for 20 per cent CO₂ were higher than those for the 5 per cent but lower than those for the control; 5 per cent CO₂ was less efficient in retarding than 20 per cent at 2° C. but more effective at 5° and 7° C.

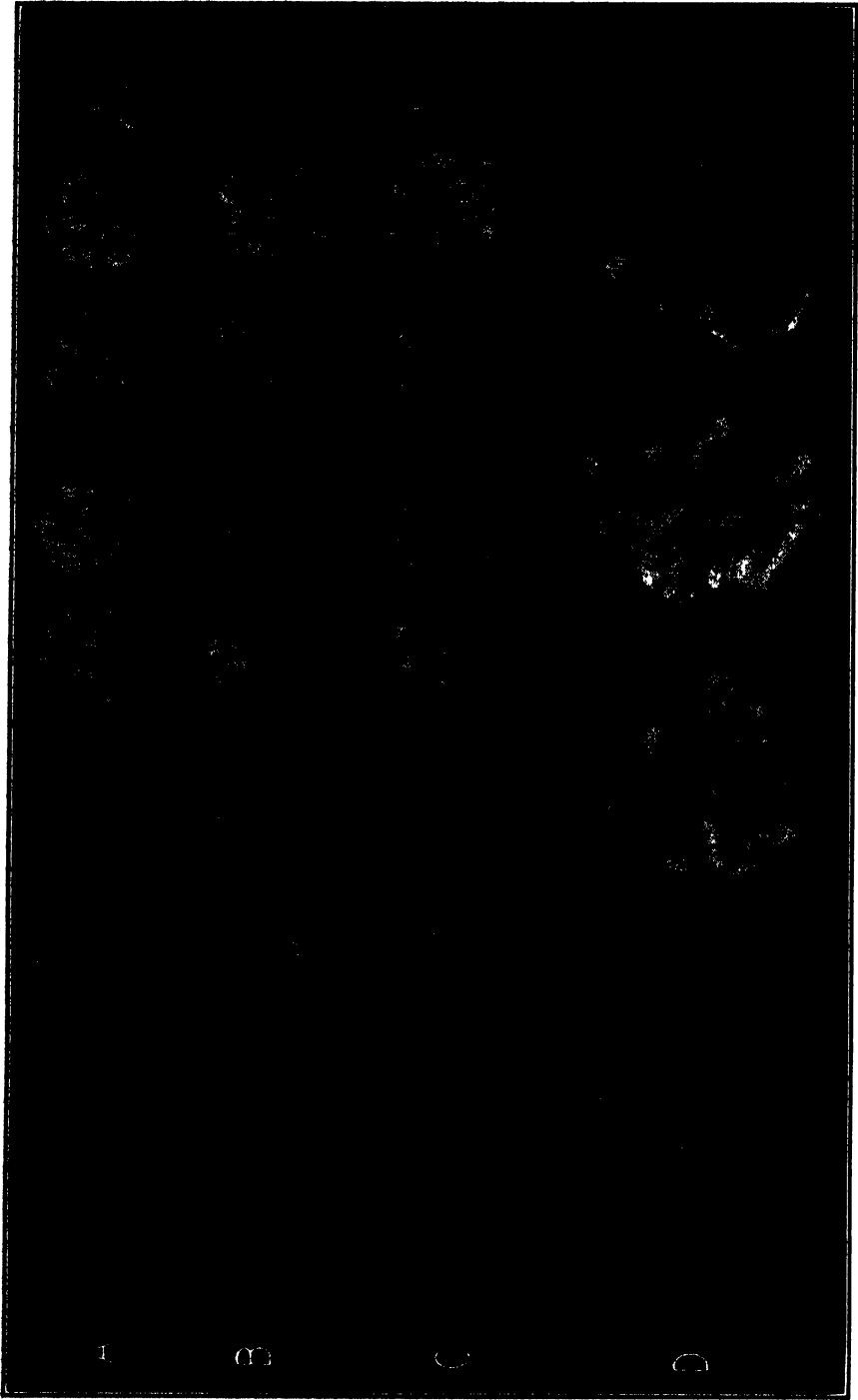


FIGURE 2. A, B, and C. Potato chip samples for all of the Irish Cobbler samples in Table I and arranged in the same order as in the table. Row A. 30 days; row B. 60 days; row C. 90 days. Files 1, 2, and 3, stored at 2°; files 4, 5, and 6, stored at 5°; files 7, 8, and 9, stored at 7°. Files 1, 4, and 7, controls; files 2, 5, and 8, treated with 5% CO₂; files 3, 6, and 9, treated with 20% CO₂. D. Potato chip samples of Green Mountain potatoes after 30 days' storage at 2° C. Left to right: 0%, 5%, 20%, 40% CO₂. Reducing sugar values in the same order are: 10.6, 2.5, 1.7, 3.8 mg. per cc. of juice.

The graphs in Figure 1 show a gradual shift in going from 2° to 7° C. At 2° the curves slant steeply downward, at 5° they become broken with 5 per cent CO₂ at the low point, at 7° the minimum is still maintained at 5 per cent but the 20 per cent CO₂ curve now becomes reversed and rises sharply. There is, thus, a contrast in the shape of the curves for 2° and 7° because of this difference in the effect of 20 per cent CO₂ at 2° and at 7° C.

Potato chip colors. The effect of the CO₂ treatments upon reducing sugar development is also shown in Figure 2 A, B, and C. This is an attempt to show in black and white the extent of the development of the brown color in potato chips made from the Irish Cobbler tubers the analyses of which are given in Table I, lines 1, 2, and 3. The darkness of the color is an indication of the reducing sugar content (2), the light colors being associated with the absence of reducing sugar, or at least with amounts preferably lower than 3.0 mg. of reducing sugar per cc. of juice. Particular attention is directed to the color of the following lots of potato chips in Figure 2: (a) row A, file 2, 5 per cent CO₂ for 30 days at 2°; (b) rows A and B, file 3, 20 per cent CO₂ for 30 and 60 days at 2°; (c) rows A, B, and C, files 5 and 8, 5 per cent CO₂ at all duration periods at 5° and 7° C. The higher sugar values of some of the 20 per cent CO₂ lots as compared to the corresponding lots with 5 per cent CO₂ are also reflected in the slightly darker colors of the potato chips, rows A, B, and C, files 5 and 6, 8 and 9, in Figure 2.

Higher amounts of CO₂. Since as shown in Figure 1 and Table I, at 2° C. the higher of the two concentrations of CO₂ used, 20 per cent, was the more effective in retarding reducing sugar formation, it might be suspected that even higher amounts of CO₂ might be still more effective. That this is not the case is shown by Figure 2 D which reproduces the color of the potato chips after exposure to 0, 5, 20, and 40 per cent CO₂ for 30 days at 2° C. The variety was Green Mountain and the experiment started October 7, 1941. The reducing sugar values, reading left to right, were 10.6, 2.5, 1.7, and 3.8 mg. per cc. of juice. It is seen that the CO₂ concentration of 40 per cent was not more but less effective than 20 per cent or 5 per cent in depressing the reducing sugar rate of increase. The chip colors also were in accordance with the chemical analyses.

Possible tissue injury. In his experiments with storage of potatoes at low temperatures in atmospheres to which CO₂ was added, Barker (1) observed injurious effects by 20 per cent CO₂ at 1° C. as shown by the odor of the tubers. This point was given consideration in these tests by examination of the tubers at the time of removal from storage, when cutting slices for the preparation of potato chips and grinding the tissue for the extraction of juice. None of the lots had a bad odor, and in all lots the tubers were sound and furnished firm, brittle slices. However,

many (19 out of 54) of the lots receiving CO₂, and especially those (7 out of 9 lots) removed after a storage interval of 90 days showed some black spots ranging from a mere speck to a ring 5 mm. in diameter. Many of these spots disappeared in frying the slices for potato chips but some remained in the completed chip.

However, no spotting was observed in any of the lots with 5 per cent CO₂ stored at 5° C. for any duration period, and since these lots show the principal features of this experiment, i.e., retardation of reducing sugar development and increase in amounts of sucrose as a result of CO₂ treatment, it is believed that the main features are not dependent upon the

TABLE II
ANALYSIS OF VARIANCE OF DATA IN TABLE I

Source of variation	D.F.	Variance	
		Red. sugar	Sucrose
Treatments	2	307.82	1767.39
Temperatures	2	113.10	321.20
Varieties	2	83.21	867.60
Days	2	265.30	279.41
Treat. × Temp.	4	181.54	555.86
Treat. × Var.	4	18.05	60.71
Treat. × Days	4	20.76	288.44
Temp. × Var.	4	1.01	32.52
Temp. × Days	4	24.30	16.92
Var. × Days	4	2.90	0.71
Treat. × Temp. × Var.	8	4.33	15.93
Treat. × Temp. × Days	8	39.62	60.87
Treat. × Var. × Days	8	4.31	15.70
Temp. × Var. × Days	8	1.31	4.65
Treat. × Temp. × Var. × Days (Error)	16	2.43	11.51
Req. diff. for significance between single items		4.7	10.2
" " " " " " sums of 3 items		8.1	17.6
" " " " " " " " 9 "		14.0	30.5

occurrence of spotting. In any event, the sugar values are brought about by the effect of CO₂ on the tissue under the conditions of the test; how this is brought about is a problem the solution of which was not attempted in these experiments.

Tests of significance. The curves in Figure 1 suffice to show the general effect of the temperature, CO₂ treatment, and duration of storage. For a more critical examination to determine the significance of differences among the various conditions and varieties, attention is turned to Tables I and II. Table I shows the reducing sugar values for each variety and experimental condition at each sampling period, and Table II shows the analysis of variance for the values in Table I. The variances for treatments, temperatures, varieties, and days (i.e., duration) are all highly

significant. The interactions, treatment $\times$ variety, treatment $\times$ days, treatment $\times$ temperature, and days $\times$ temperature are also significant, as is the triple interaction, treatment $\times$ temperature $\times$ days. It thus appears that the response to treatment has been marked but has varied with the variety, duration of treatment, and temperature, and that the effect of temperature has been different for different durations of storage.

The error variance for reducing sugar was found to be 2.43 (Table II, line 15) and the least significant difference between items in Table I is $\sqrt{2.43} \times \sqrt{2} \times 2.120 = 4.7$. Using the value 4.7 to search through Table I, columns 3 to 11, for significant differences, it is found that this difference was reached for all of the comparisons of the CO₂ treated and control lots at 2°; in comparing the values for 5 per cent with those for 20 per cent the required difference is not usually reached; however, when the sums of the three values representing each variety are obtained and the differences taken, these differences between 5 per cent and 20 per cent CO₂ are all greater than the required difference between the sums of three entries, which is given by the expression: $\sqrt{2.43 \times 3 \times 2} \times 2.120 = 8.1$ (4, p. 49, and p. 248, line 16 under $P = 0.05$). This shows that 20 per cent CO₂ has been more effective than 5 per cent CO₂ in retarding reducing sugar development at 2° C.

For 5° C., columns 6 to 8, Table I, the value 4.7 was reached in the series for Irish Cobbler in all comparisons of either 5 per cent CO₂ or 20 per cent CO₂ with the control lots, and the differences between 5 per cent and 20 per cent CO₂ are significant if the three entries are summed and compared with the value 8.1. Significant differences between 5 per cent CO₂ and the control are found in all of the Green Mountain tests but with 20 per cent CO₂ the sugar values tend to rise with the duration of storage until there is no difference between the CO₂-treated lot and the corresponding control. With Katahdin significant differences between control and treated were not reached in any of the comparisons at 5°. As stated in a previous paragraph the correctness of the value 6.5 in column 7, line 9, Table I, is suspected; but since replicate juice samples were available it is known that this is not due to an analytical error; it may have been due to a sampling deviation among different lots of tubers, the chance of the occurrence of this large a departure from the expected being estimated at about 1 in 70.

At 7° C. the reducing sugar values reached by the control lots even after 90 days' storage were not high, and there was consequently less opportunity for any retarding effect of CO₂ to become manifest. In examining the data for 5 per cent CO₂ and the corresponding controls it is seen that the individual values for 5 per cent CO₂ with one exception are lower than those for the controls although the differences do not reach the value 4.7 in any case. However, the sum of the nine values for the controls

in column 9 is 30.7, and for the 5 per cent CO₂ values in column 10 is 11.2. The difference is 19.5. The required difference between the sums of nine items is $\sqrt{2.43 \times 9 \times 2 \times 2.120} = 14.0$, and on this basis 5 per cent CO₂ retarded the reducing sugar value at 7° C. These differences are furnished mainly by Irish Cobbler and Green Mountain, the Katahdin data contributing only 2.2 to the total difference, 19.5.

In examining the 20 per cent CO₂ values at 7° C. in column 11, Table I, we are now interested not in testing for decreases in reducing sugar but for increases above the values of the corresponding controls. The required increases were reached by the 90-day period with each variety. Thus, the 20 per cent CO₂ values which showed at 2° definite decreases, and at 5° a tendency to furnish values as high as the corresponding controls, finally at 7° gave reducing sugar values higher than those reached by the untreated lots.

SUCROSE

The required difference for testing for differences in sucrose between comparable entries in Table I, columns 12 to 20, is 10.2, and since the effect of the CO₂ treatments upon sucrose content was so extensive, there is little difficulty in finding differences of this amount in most of the comparisons between treated and control. The effect of CO₂ was, in general, to cause an increase in sucrose, with the interesting exception that in storage at 2° C. there was first a decrease at the end of the 30-day period followed by an increasing gain over the control until by the end of the 90-day period the CO₂ lots were much higher than the control, in all cases with 5 per cent CO₂ but not in all with 20 per cent CO₂. Although 5 per cent CO₂ was more effective than 20 per cent at 2° C. (see columns 13 and 14), the reverse was true at 5° and 7° (see columns 16 and 17, 19 and 20). By the end of the 90-day period at 2° C. the sucrose values for the 5 per cent CO₂ lots were approximately double or treble those of the control lots, and at 5° and 7° the 20 per cent CO₂ lots showed sucrose values 6 to 10 times those of the controls.

It will be noted that the control lots at 2° show a temporary maximum in sucrose at the 30-day period (see also curve C in Fig. 3), the values rising from nearly zero (or 3.0 for Green Mountain) at the start to 20 to 30 mg. per cc. at 30 days, then show decreasing values at the end of the 60- and 90-day periods. The lots treated with CO₂, however, did not show this temporary increase in sucrose (see also curve F, Fig. 3); the sucrose values rose steadily and did not reach the maximum until the end of the experiment.

DISCUSSION

Barker (1) carried out an experiment similar to this one in many respects. He used CO₂ at concentrations up to 20 per cent at 1° C. and up

to 15 per cent at 5° and 7.5° C. Unfortunately, Barker reported results for total sugar only and in order to obtain a comparison of these results with those of Barker it is necessary to use only the total sugar values obtained by adding the percentages for reducing sugar and sucrose. At the 1° temperature he employed 20 per cent CO₂ and 20 per cent O₂ and this same gas combination was used in these tests although at 2° instead

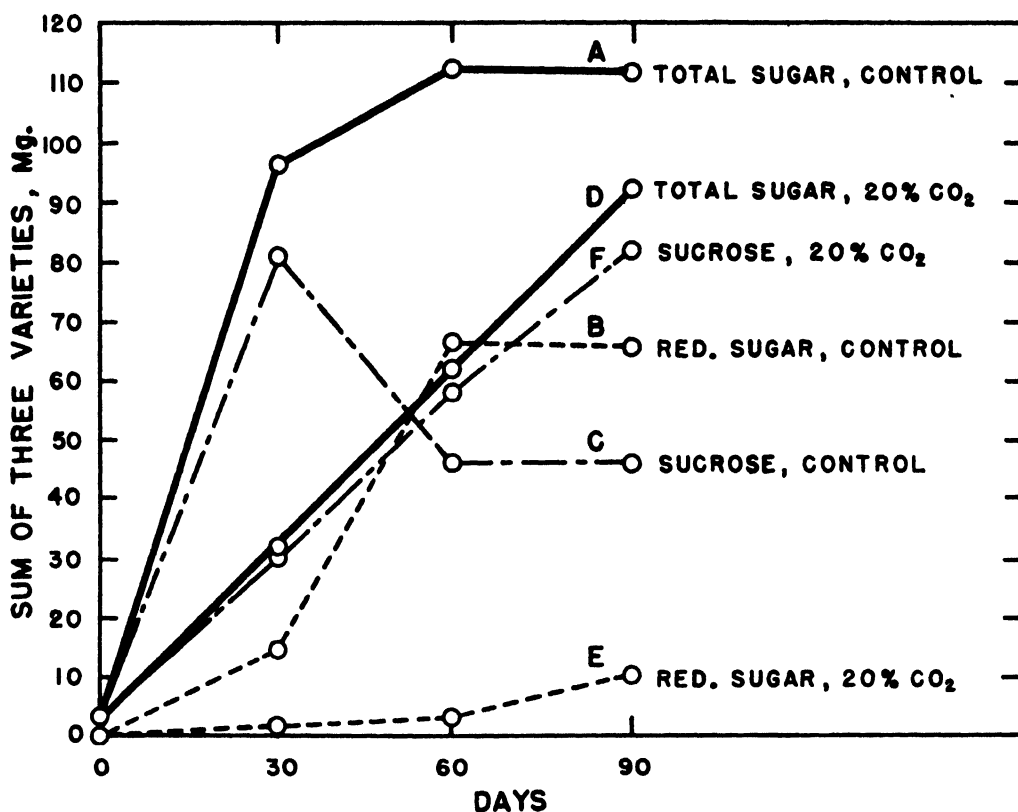


FIGURE 3. Graphs obtained by plotting the data for 2° in Table I to demonstrate the unsuitability of a total sugar curve for showing the effect of CO₂ upon the sugar content of potato tubers. Curve A fails to furnish the information contained in its constituent curves B+C; and Curve D, made by combining E and F, would not disclose the effect of CO₂ in depressing the reducing sugar development (compare E and B) and increasing that of sucrose (compare F and C).

of 1° C. Barker found that 20 per cent CO₂ reduced the amount of total sugar as compared with the control, and the results of the present tests are in accord. However, in order to indicate the unsuitability of the total sugar values for showing what changes in the sugar constituents were really taking place, Figure 3 was prepared from the data for the control and 20 per cent CO₂ lots at 2° in Table I. The values plotted are the sums obtained by adding the corresponding values for the three varieties. The heavy lines in Figure 3 show that the total sugar values for the CO₂-

treated lot are lower than those for the check, but from this one should not conclude that the effect of the CO_2 was to retard sugar development. The broken lines in Figure 3 indicate the separate values for reducing sugar and sucrose for the control and treated lots. Curve A is merely the resultant curve obtained by adding the ordinates for curves B and C, and curve D is the resultant likewise of E and F. These curves for the separate constituents show that the real effect of the CO_2 treatment was to decrease the rate of accumulation of reducing sugar and increase that of sucrose. Curves such as A and D, although smooth and indicating regularity of change with time, cannot successfully delineate the rather complicated features shown by curves B, C, E, and F. Barker tested 5 per cent CO_2 at 5° and 7.5° C. in combination with 15 per cent O_2 and so the results are not strictly comparable with the present tests in which the O_2 concentration was 21 per cent. He found that the CO_2 treatment increased the total sugar, a result which was also obtained in these tests; but Table I shows that this fact is the resultant of two opposite actions occurring simultaneously: a decrease in reducing sugar and an increase in sucrose, the latter merely furnishing larger numerical values than the former, which results in a computed increase when the two values are summed.

SUMMARY

Potato tubers were stored at 2° , 5° , and 7° C. in atmospheres containing 0, 5, and 20 per cent CO_2 and 21 per cent O_2 . At intervals of 30, 60, and 90 days, samples were removed for the determination of the reducing sugar and sucrose contents of the juice, and for the preparation of potato chips.

The rapid increase in reducing sugar which occurs in potato tubers stored at 2° C. was inhibited for 30 days by 5 per cent CO_2 and for 60 days by 20 per cent CO_2 ; after 90 days the values for the CO_2 -treated lots were one-half to one-sixth of those for the controls.

At 5° C. the reducing sugar increase was prevented by 5 per cent CO_2 for 90 days with Irish Cobbler, and for 30 days with Katahdin; with Green Mountain the 5 per cent CO_2 values were kept down to about one-half of those of the controls. A retarding effect of 5 per cent CO_2 was also obtained at 7° C.

Treatment with 20 per cent CO_2 , although exerting a retarding effect over a period of 30 days at 5° and 7° , then had the reverse effect, so that at the end of 90 days at 5° the reducing sugar values were equal to the controls, and at 7° were three to five times those receiving no CO_2 . Changing the temperature from 2° C. to 7° C. reversed the effect of 20 per cent CO_2 upon reducing sugar content.

The general effect of CO_2 upon the sucrose content was to greatly increase it, about six-fold at 5° and 7° C. At 2° C., treatment with CO_2 first

retarded sucrose increase as compared with the untreated, and then greatly increased it. Part of this anomalous effect is due to the fact that the temporary maximum, which is formed in the sucrose curve of the juice from untreated tubers after about 30 days' storage at 2° C., does not occur in the presence of suitable amounts of CO₂.

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EFFECT OF A FEW HOURS OF CHILLING UPON THE GERMINATION OF GLADIOLUS CORMS SUBJECTED TO AN ARTIFICIALLY PROLONGED REST PERIOD

F. E. DENNY

The rest period of corms of some varieties of gladiolus can be prolonged merely by replanting them in moist soil soon after harvest and storing at warm temperatures, such as at room temperature or at 27° C. (2). By such means, corms of many varieties have been held in storage in good condition without sprouting for many months, even for one year or longer.

When corms which had been held in the dormant condition in this way for several months were treated with the vapor of ethylene chlorohydrin, growth of buds was induced (2). However, since it had been found (1, p. 475) that the corms of some varieties, at least when freshly-harvested, required a preliminary cold storage period before the chemical treatment could be applied successfully, a portion of the bulbs in this prolonged dormant condition was removed from the soil and stored at 5° C. for one week before the ethylene chlorohydrin treatment was applied. The unexpected result was that the corms receiving this relatively short cold storage period germinated promptly, in fact nearly as well as the lots which received both the cold storage and the chemical treatment.

Inasmuch as practically complete germination of these dormant corms was obtained with seven days of storage at 5° C., experiments were undertaken to determine the shortest period of exposure to low temperature needed to cause a definite increase in germination. The object of the present paper is to show the results of these tests. It was found that with suitable varieties, after a certain minimum period of prolonged dormancy, exposure to low temperature for 12 to 24 hours, and probably for only 5 to 6 hours, was enough to increase the germination rate significantly.

Since the time during which the corms were at the low temperature was so short, the term "cold storage period" seemed hardly properly descriptive and the term "hours of chilling" was regarded as corresponding more nearly to the actual situation.

METHODS

The gladiolus corms were harvested in October of each year, and after the corms had remained at room temperature for 7 to 10 days the husks were removed, and the corms were planted in moist soil in flats. These were piled in tiers and stored in a room the temperature of which varied from

20° to 27° C., usually being about 25° C. This is referred to in the paper as room temperature (R.T.). The flats were examined each week or two, and the water needed to keep the soil moist was added.

For exposure to temperatures of 5°, 10°, or room temperature, the corms, after removal from the soil, were brushed off and were spaced widely apart on boards placed in the rooms at the desired temperature. For treatments at 0° C., the corms were placed in one-gallon, wide-mouthed jars, the number of corms per jar being adjusted to fill the jar not more than half full. The jars were then sealed and immersed in pails of chopped ice, water being added to just cover the ice.

After the treatments were completed the corms were planted in flats in soil which was watered copiously at the start. Thereafter, the flats were piled in tiers, stored at room temperature, and examined frequently as to the need of additional water. As sprouts appeared they were recorded and usually discarded. However, in some tests they were allowed to make full growth in the flats in which they were planted and in other tests the newly-sprouted corms were transplanted individually into clay pots for observations as to further growth.

RESULTS

DEVELOPMENT OF THE SENSITIVE CONDITION

A test made with *freshly-harvested* corms showed no effect upon germination upon exposure of corms of Senorita up to eight days at 0° C., or of those of Salmon Star for a like period at 5° C., or of Minuet at 5° C. for four days.

The corms were not found to have the capacity to respond to short periods of exposure to low temperature at harvest time nor soon thereafter, but the sensitive condition was developed in them after a certain period of storage in moist soil at room temperature. This minimum time was found to be different for the various varieties involved in the experiments. Figure 1 shows the response of different varieties at two different stages after harvest. The bulbs were harvested in October, 1939, and were stored in moist soil at room temperature (approximately 25° C.) until they were removed for the tests which in this case were made on two dates, January 8, 1940 and May 1-15, 1940, i.e. after the bulbs had been held dormant in the moist soil for periods of three months and seven months, respectively.

The results with the two most dormant varieties, Salmon Star and Senorita, are shown in the two sets of graphs at the top of Figure 1. When corms of these two varieties were removed from the soil, exposed to a temperature of 5° C. for 48 hours starting on January 8, and then replanted in soil, none of the corms germinated over a period of 126 days; the controls likewise failed to germinate. When, however, the corms were allowed to remain in the soil until May 15, 1940 and then were subjected

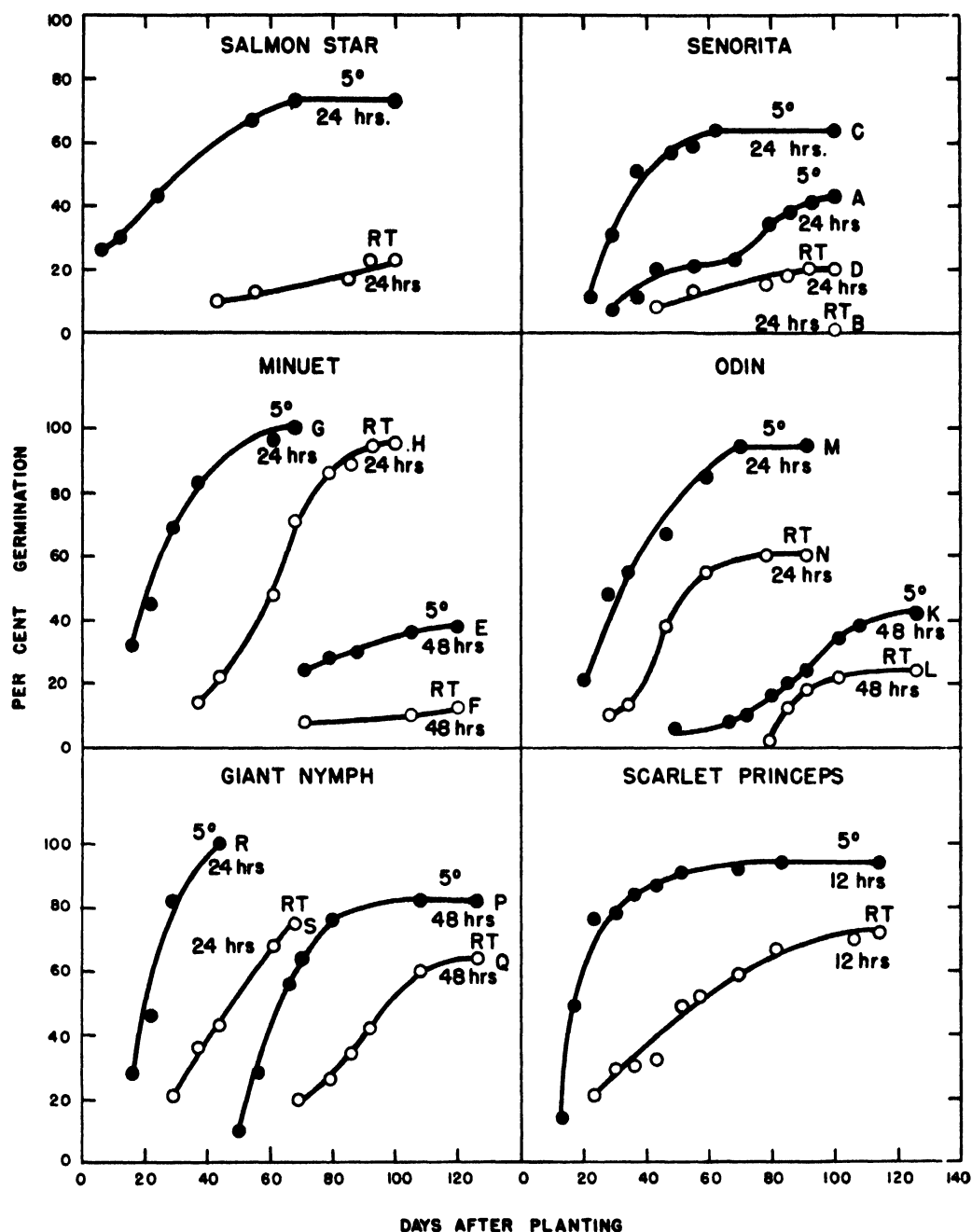


FIGURE 1. Effect of short periods of chilling upon the germination of gladiolus corms, harvested in October 1939 and held in the dormant condition in moist soil at room temperature (R.T.) until January 8, 1940 and May 1-15, 1940. Then corms were removed from the soil and stored at 5° or R.T. for 12 to 48 hours, replanted in soil and held at R.T. Treatments of Salmon Star and Senorita applied on May 15; of Scarlet Princesses on May 1. Treatments for E, F, K, L, P, and Q applied on January 8; those for G, H, M, N, S, and P on May 15. A, B, P, Q corms exposed to air; C, D, R, S corms soaked in water. No. of corms per lot = 28 to 61, av. 49.5.

to the temperature treatments, those exposed at 5° C. for 24 hours germinated freely as shown in the curves for Salmon Star and Senorita in Figure 1. With Salmon Star (Fig. 1, upper left) the percentage germination reached 73 after about 70 days, while that for the control lot was only 23 after 100 days. The results with Senorita are shown in Figure 1, upper right. In the control lot (curve B, Fig. 1) only one bulb out of 61 germinated within 100 days after replanting, while in the lot exposed to an air temperature of 5° C. for 24 hours (curve A, Fig. 1) the value 43 per cent was reached. Curves C and D in Figure 1 show the results when the treatment was applied by soaking the corms in water at 5° C. and at room temperature. The 24-hour treatment in water at 5° C. increased the germination, the final percentage germination being 64 for the 5° lot, and 20 for the control. This might seem to indicate that soaking in water is much more effective than exposure to air at the same temperature, but other experiments, for example those described in Table I, did not show this large difference in the method of treatment.

The curves for Minuet and Odin (Fig. 1) show the results with less dormant varieties. When treated on January 8, gains in rate of germination were obtained by treatment of corms of Minuet for 48 hours at 5° (curves E and F), but the germination reached only 38 per cent after 120 days; a 24-hour treatment at 5° (results not shown in Fig. 1) gave a germination response practically identical with that of the control (curve F). However, when the treatment was applied on May 15, the germination response was much more rapid and more nearly complete as shown by curves G and H. The treatment at 5° C. for 24 hours produced a gain of 30 to 40 days in the initial stages of germination. The results with Odin were similar, at least qualitatively, to those with Minuet, the number of days gained by 24 hours at 5° being somewhat less, i.e. about 20 days, but the difference in the final percentages of germination being considerably greater with Odin than with Minuet, 93 per cent for 5° C. and 60 per cent for room temperature.

The corms of Giant Nymph were emerging from the rest period even by January 8, as shown by curve Q, Figure 1. Nevertheless, a treatment at 5° for 48 hours improved the rate of germination (curve P, Fig. 1). The results of the 24-hour treatment on May 15 are given by curves R and S, Figure 1, and, although the control corms (curve S) germinated promptly, a gain in the rate of emergence of sprouts was obtained by exposure of the corms to 5° C. for 24 hours (curve R).

Special attention is directed to the curves for Scarlet Princess in Figure 1, since in this case the exposure to the low temperature was for only 12 hours. The corms of this variety were found to be quite dormant on January 8, since a treatment of 48 hours at 5° C. at that time failed to improve the germination, the values after 126 days from planting being

8 per cent for the treated and 12 per cent for the control at room temperature. By May 1, however, the corms had become sufficiently sensitive to the low temperature treatment to furnish the result shown by the curves for Scarlet Princeps in Figure 1, lower right. The gain due to 12 hours' exposure to 5° C. over the control at room temperature was quite definite, both as to rate of emergence and final percentage.

The results in Figure 1 show that after the corms remained in the soil in the dormant condition for a period of a few months, they became amenable to the low temperature treatment, and that finally an exposure to 5° C. not longer than 24 hours improved the germination of the corms of each of the varieties in the test.

TREATMENTS AT DIFFERENT TEMPERATURES

Not only 5° C. but also 0° and 10° C. were tested, at least in a limited way, and the results are shown in Table I. The period of exposure to the

TABLE I

EFFECT OF A 24-HOUR CHILLING PERIOD AT DIFFERENT TEMPERATURES

Temp. of treatment	Per cent germination, 55 days after planting					
	Purple Glory		Senorita		Salmon Star	
	1938 crop		1938 crop	1939 crop	1939 crop	
	In air	In water	In air	In water	In air	In water
0°	71	73	82	65	71	33
5°	76	61	45	53	54	60
10°	73	76	11	—	—	—
R.T.	22	34	11	26	17	27

R.T. = room temperature, approx. 25° C.

No. of corms per lot = 28 to 43, av. 35.

temperatures in this test was 24 hours, and the values in Table I are percentages of emergence at the end of 55 days from planting. The treatment was applied on July 25, 1940; therefore the corms from the 1938 crop had been stored in the moist soil at room temperature for one year and nine months, and those from the 1939 crop for nine months, at the time the treatment was started.

With Purple Glory, columns 2 and 3, Table I, all three of the lower temperatures were more favorable than room temperatures, doubling or trebling the percentage of germination and with no choice among the three. Senorita, columns 4 and 5, showed higher values with treatments at the lower temperatures. The germination of Salmon Star corms was considerably improved by a treatment at 5° C., and also at 0° if the treatment was applied in air rather than by soaking in water.

The results were not extensive enough to furnish a definite conclusion as to the most favorable temperature, but they suggest that 0° may be more favorable for treatments with corms of Senorita.

Because of the favorable results with Senorita corms at 0° C., an experiment the results of which are shown in Table II was started September 18, 1940, with corms of the 1938 crop, i.e. after the corms had remained dormant in the soil at room temperature for nearly two years. In this test the exposure periods were reduced to 12 and 6 hours. The results in Table II show that the germination rates and final values were improved by the treatment at 0° C., whether the exposure period was for 12 or 6 hours.

TABLE II
EFFECT OF A CHILLING PERIOD OF 6 TO 12 HOURS ON THE
GERMINATION OF CORMS OF GLADIOLUS, VAR. SENORITA

Days after planting	Per cent germination		
	12 hrs. at 0° C.	6 hrs. at 0° C.	12 hrs. at R.T.
12	55	49	13
19	85	67	16
29	89	69	18
41	91	69	24

R.T. = room temperature, approx. 25° C.
No. of corms per lot = 45.

TREATMENTS FOR DIFFERENT LENGTHS OF TIME AT DIFFERENT TEMPERATURES

The results of further tests to determine the lower limits for the duration of exposure of corms to low temperatures are shown in Tables III and IV. The corms used were from the 1940 crop, and the experiments were started on July 30, 1941 and November 12, 1941, respectively for Tables III and IV, i.e. after storage periods of 9 and 13 months in soil following harvest.

In the test the results of which are shown in Table III, the duration of exposure was varied from 24 to 6 hours, and the values in columns 4 and 5 show the number of corms germinated and not germinated in the different lots 79 days after planting. The germination counts are, in all comparisons, higher for the lots receiving 0° or 5° treatments than for the controls at room temperature. An attempt was made to use the chi-squared test to determine which of the comparisons gave significant differences and which did not.

The value of chi-squared (χ^2) is shown separately for each variety in lines 7, 14, and 24, and was computed according to the method given by Paterson (3, p. 77). Each value in column 6 is the ratio of the square of

TABLE III
EFFECT OF SHORT PERIODS OF CHILLING UPON THE GERMINATION
OF GLADIOLUS CORMS

Variety	Temp., ° C.	Hours stored	79 days after planting, number of corms		(Dev.) ² Exp.
			Germinated	Not germinated	
Senorita	0	24	16	9	14.2
		12	17	8	17.7
		6	10	14	1.6
	R.T.	24	1	24	7.8
		12	0	25	10.5
		6	0	25	10.5
Req. χ^2 , 5 D.F., for prob. 0.05 = 11.1; χ^2 = 62.3					
Salmon Star	5	24	7	13	0.0
		12	15	4	18.0
		6	10	7	5.6
	R.T.	24	1	15	4.9
		12	3	16	2.3
		6	0	21	10.0
Req. χ^2 , 5 D.F., for prob. 0.05 = 11.1; χ^2 = 40.8					
Purple Glory	0	24	15	8	17.2
		12	7	16	0.1
		6	12	11	7.5
	5	24	7	18	0.0
		12	6	17	0.0
		6	5	19	0.4
	R.T.	24	2	21	3.9
		12	2	20	3.7
		6	0	23	8.5
Req. χ^2 , 8 D.F., for prob. 0.05 = 15.5; χ^2 = 41.3					

Summary of χ^2 Values Computed from the Above Data

Variety	R.T. vs.	For each separate comparison at each duration			After combining the R.T. values for 24, 12, and 6 hrs.		
		24 hrs.	12 hrs.	6 hrs.	24 hrs.	12 hrs.	6 hrs.
Senorita	0°	17.5	22.8	10.6	47.8	52.0	26.0
Salmon Star	5°	2.7	12.8	13.9	7.1	34.5	19.3
Purple Glory	0°	13.4	2.0	13.6	33.2	7.6	22.3
	5°	1.6	1.2	3.4	6.6	5.2	3.0

Required χ^2 for 1 D.F. for a probability of 0.05 = 3.84

Note: R.T. means room temperature, approx. 25° C.

the deviation from the expected value to the expected value itself, and the sum of these is χ^2 . The χ^2 value found for each variety is much larger than the requirement for a probability of 0.05, and, in fact, larger than the requirement for 0.01 (3, p. 250). This shows that the temperature treatments as a whole produced an effect upon the germination.

The χ^2 values for the different temperature comparisons for different durations are shown in the section at the bottom of Table III. In columns 3 to 5 the comparisons were made separately for each pair of entries in Table III at each duration period. For example, in comparing R.T. and 0° for Senorita at the 24-hour period, the values 16 and 9 in line 1, and 1 and 24 in line 4, in Table III, were used to set up a 2 × 2 contingency table (not shown here). Since only one degree of freedom is involved in such a case, and since the expected numbers were usually small, the method of computing χ^2 which applies Yates' correction for continuity, as described by Snedecor (4, p. 168), was used for all of the computations reported in the bottom portion of Table III. Values of χ^2 larger than 3.84 indicate a significant effect and this result was obtained with the 0° treatment for Senorita at all durations; with Salmon Star at 5° storage for 12- and 6- but not for the 24-hour period; and with the 0° lots in Purple Glory at 24 and 6 but not at 12 hours. Treatment at 5° with Purple Glory did not show significant differences on this basis for making comparisons.

Since the room temperature lots showed little or no effect of duration of exposure, the germination being about the same for the 24, 12, and 6-hour lots for each variety (as is also the case for Senorita in Table IV), the R. T. values for each variety were combined giving a new base value to represent the germination of the control corms. Using these totals for comparison with the germination obtained after the different low temperature treatments, the χ^2 values shown in columns 6 to 9 at the bottom of Table III were computed. For example, corresponding to 16 and 9 for Senorita in line 1 we have 1 + 0 + 0 = 1, and 24 + 25 + 25 = 74, as the germination of the control, lines 4, 5, and 6, columns 4 and 5. This gives a new 2 × 2 table (not shown here) which gives a corrected χ^2 value of 47.8 (see appropriate entry opposite Senorita at the bottom of Table III). In like manner, the other values in columns 6, 7, and 8 at the bottom of Table III were obtained. On this basis, the χ^2 values for all of the comparisons of the different temperature treatments at all duration periods reached significance, except that with Purple Glory at 5° for 6 hours.

A similar experiment was carried out with corms of the variety Senorita and the results are shown in Table IV. The temperature treatments were made on November 12, 1941, i.e. one year and one month after harvest. The periods of exposure to the low temperatures were 10, 5, and 2.5 hours. The χ^2 value for the experiment as a whole was 20.8, line 10, Table IV, which is greater than 15.8, the required value for a probability of 0.05,

showing that the temperature treatments were effective in increasing the germination.

The values at the bottom of Table IV were obtained in a manner similar to that described for Table III. Treatment at 0° C. for either 10 or 5 hours improved the germination of the corms of Senorita, an exposure of 2.5 hours, however, being without effect.

TABLE IV
RESPONSE OF CORMS OF GLADIOLUS, VARIETY SENORITA,
TO SHORT PERIODS OF CHILLING

Hours stored	Temp., ° C.	150 days after planting, number of corms		(Dev.) ² Exp.
		Germinated	Not germinated	
10	0	11	8	4.9
	5	7	13	0.3
	R.T.	1	15	4.1
5	0	11	10	5.4
	5	7	15	0.0
	R.T.	2	18	3.6
2.5	0	4	17	1.1
	5	6	14	0.0
	R.T.	3	15	1.4

Required χ^2 for 8 D.F. = 15.5; $\chi^2 = 20.8$

Summary of χ^2 Values Computed from the Above Data

R.T. vs.	For each separate comparison at each duration			After combining the R.T. values for 10, 5, and 2.5 hrs.		
	10 hrs.	5 hrs.	2.5 hrs.	10 hrs.	5 hrs.	2.5 hrs.
0°	9.8	6.7	0.0	14.7	12.4	0.3
5°	2.7	1.8	0.3	4.2	3.4	2.6

Required χ^2 value for 1 D.F. for a probability of 0.05 = 3.84.

The result in Table IV corroborates those in Tables II and III in showing that a temperature treatment as short as 5 or 6 hours had a definite effect in increasing the germination of gladiolus corms.

GROWTH OF PLANTS AFTER SPROUTING

The main object of the experiment related to the germination of corms and not to the subsequent growth of the plants. In some of the tests, however, the sprouted plants were put in the greenhouse to note whether

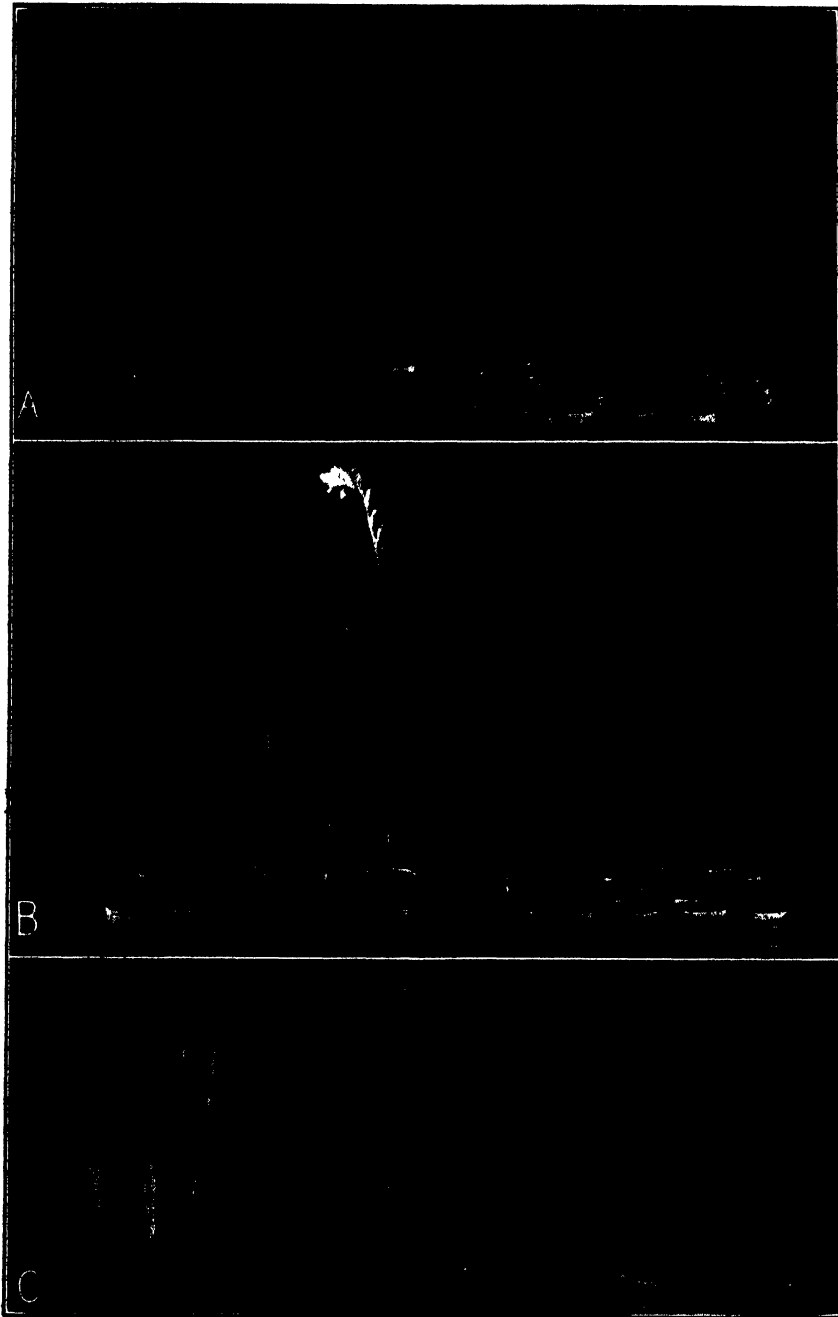


FIGURE 2. Effect of short periods of cooling on the germination of gladiolus corms. A. Senorita after 9 months' storage in soil following harvest; left group, corms removed from soil and exposed to 0°C . for 12 hours and replanted; right group, same but exposed to room temperature 12 hours approximately 25°C .; this photograph taken 3.5 months after planting. B. Same as A, but variety is Salmon Star and temperature was 5°C . C. Left two flats, Senorita corms, harvested in October 1938, held in soil until July 25, 1940, removed from soil and exposed to 0° for 24 hours and replanted; right two flats, same but exposed to room temperature for 24 hours. This photograph taken 48 days after planting.

growth would continue in a manner approaching the normal behavior. The growth was often slow in the early stages, and with a certain amount of curvature of leaves in an arc sideways in the plane of the blade, and with wrinkling of the leaf-blade. These abnormalities were later outgrown, and the subsequent growth was good, as shown in Figure 2. Special attention is directed to Figure 2 C, since these plants were grown from corms after the dormant period had been prolonged until nearly two years after harvest before the chilling treatment was applied.

DISCUSSION

It is not suggested that these short periods of chilling are more effective than some other method of treatment might be, such as increasing the temperature rather than decreasing it, or bruising the corms, or applying a chemical treatment. That the initiation of growth in many types of buds including those of gladiolus, and in the embryos of seeds of numerous plant species can be accomplished more rapidly by lowering the temperature than by increasing it, although now a well established fact, is, nevertheless, a result contrary to expectation based on the response of nearly all other processes to change in temperature. The results of the present tests furnish an example of a case in which the period of exposure to the low temperature need not be a matter of months or weeks, as is usually required, but may be a matter merely of days, or even of hours.

Indeed, these corms become so sensitive after this long period of enforced dormancy, that the application even of low temperature is not needed to produce an effect upon the germination. This is shown by the behavior of the control lots in some of these tests. In these cases the corms were merely removed from the soil, exposed to the air for a few hours, returned to the same soil from which they had been removed, and again stored at the same temperature. The curves in Figure 1, and the data in the tables, especially Tables I and II, show many instances in which corms after remaining many months in the dormant condition in the soil started into growth when merely removed from the soil and returned to it again with only the moderate intervening change of conditions involved in the handling of the control lots in these tests.

However, with the most dormant varieties, such as *Senorita* and *Salmon Star*, in Figure 1, and especially after a period of enforced dormancy of only 9 to 13 months as shown in Tables III and IV, the renewed germination brought about in the controls was not extensive, with percentages of germination remaining at 20 or below (and sometimes practically at zero) after 55 to 150 days.

SUMMARY

Gladiolus corms after having been held in enforced dormancy for several months by storage of the freshly-harvested corms in moist soil at

warm temperatures, were induced to germinate by merely removing the corms from the soil, exposing them to a temperature of 0° or 5° C. for a few hours, and then returning them to the soil.

Corms were not found to respond to these short periods of low temperature at harvest time, or soon thereafter, but they acquired this sensitive condition after some months' storage in warm moist soil.

The duration required for the exposure to low temperature varied with the variety and the length of the period of enforced dormancy at which the treatment was applied, but good results were obtained with periods of 24 and 12 hours; periods of even 5 or 6 hours definitely improved the germination.

Gains of 20 to 150 days in the time of initiation of germination, and many-fold increases in the percentage of germination were obtained by a treatment of the corms for not more than 24 hours at temperatures of 0° and 5° C.

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THE USE OF METHYL ESTER OF α -NAPHTHALENEACETIC ACID FOR INHIBITING SPROUTING OF POTATO TUBERS, AND AN ESTIMATE OF THE AMOUNT OF CHEMICAL RETAINED BY TUBERS

F. E. DENNY

The original report of Guthrie (2) showed the effectiveness of the vapor of methyl ester of naphthaleneacetic acid in inhibiting the sprouting of potato tubers. Subsequent experiments (1) determined the approximate concentrations of the chemical needed, and the temperature range within which successful storage could be accomplished without the development of sprouts and without serious shrinkage. The treatment was found to be without important effects upon the sugar content of the tubers. These results were obtained in experiments using only small quantities of tubers in each test, treatments being carried out in jars of one- to two-gallon capacity, or in five-pound paper bags.

In the tests here reported upon, larger quantities of tubers were used, 20 bushels in each lot in the main tests, and 50 pounds per lot in the subsidiary trials. It was intended that the conditions should simulate those that prevail in practical potato storage, when wooden bins, or heavy paper boxes or bags are used.

Tubers were stored in such containers without sprout development from October 1941 (shortly after harvest) until the experiment was terminated in May 1942, at temperatures which ranged from room temperature (approx. 22° to 25° C.) down to 10° C. Potatoes in the control lots without chemical sprouted freely under the same conditions.

When the treatment was applied by impregnating paper towels with the methyl ester of α -naphthaleneacetic acid ($C_{10}H_7CH_2COOCH_3$) and distributing these towels rather evenly among the stored potatoes, an amount of 50 to 100 mg. of the methyl ester per kilogram of tubers was sufficient to inhibit sprouting practically completely, and an amount of 33 mg. permitted only moderate sprouting. Later tests using talcum powder as a carrier indicated that when the chemical was distributed over the surface of the tubers in this medium, the amount of chemical required could be reduced at least to 25 mg. per kg. of tubers. Other carriers, such as confetti and chopped dried plant residues were tested in a preliminary way.

The amount of chemical which was absorbed by the tissue was estimated by a hyponasty test using the tips of young potato plants as indicators of the presence of the vapor of the methyl ester of α -naphthaleneacetic acid. Hyponasty of the upper leaves of the potato cutting was ob-

tained within four hours when the amount of the methyl ester incorporated into filter papers was as low as 0.02 to 0.01 mg. in a closed container having a volume of one liter. By extracting weighed amounts of the treated tissue with acetone, transferring the extracted methyl ester to filter papers, placing such impregnated papers in closed vessels with potato tips, and comparing the response with that obtained with papers containing known amounts of methyl ester, an estimate of the amounts in the sample of potato tissue was obtained. These measurements indicated that the amount of methyl ester taken up by the tissue of tubers treated five months with an amount of methyl ester equal to 100 mg. per kg. of potato tubers was not more than 5 mg. of the methyl ester per kilogram of tissue, of which amount at least four-fifths was in or on the skin.

Tubers treated for five months with the methyl ester, even in amounts as low as 33 mg. per kg. of tissue, did not germinate satisfactorily when cut and planted, nor even when treated with ethylene chlorohydrin which induces 90 to 100 per cent germination in freshly-harvested and therefore very dormant tubers of this variety. When such tubers were first washed thoroughly with soap and water, the treatment with ethylene chlorohydrin was more successful, especially with tubers previously treated with 33 mg. per kg., but with incomplete emergence in the case of tubers treated with 100 mg. per kg. The dormancy of tubers inhibited with the methyl ester seems to be deeper than that which occurs in tubers at any stage of the natural rest period.

MATERIALS AND METHODS

The potato tubers (*Solanum tuberosum* L.), variety Irish Cobbler, were obtained from a commercial potato producer on Long Island, were harvested on September 8, 1941, and were delivered at the Institute in Yonkers, New York, on September 9, 1941. They were allowed to stand in burlap bags under conditions of adequate ventilation at a temperature of 22° to 23° C. until October 3, 1941. In the meantime, paper towels had been impregnated with the methyl ester of α -naphthaleneacetic acid by dissolving the chemical in acetone, distributing the solution uniformly throughout the papers, suspending them on a cord, and allowing the acetone to evaporate. The amount of the solution and therefore the chemical in each paper was adjusted so that one paper would treat ten pounds of tubers with the desired amount of the methyl ester, i.e. 100, 50, 33, and 11 mg. of methyl ester per kg. of potato tubers.

For the main experiment, wooden bins 2.5' by 3.5' by 3.0', made with one-inch tongue and groove material, were used. These held approximately 20 bushels each. The potatoes were poured into these bins in layers, about two tubers deep for each layer. As the layers were formed the paper towels were spread so as to give alternate layers of tubers and papers. The cover

for each bin consisted of two layers of wrapping paper, with rolls of paper tucked in at the top periphery, one layer of burlap covering the whole, and this being held in position by short pieces of boards.

For the subsidiary experiments, heavy-walled paper shipping bags, made with three thicknesses of heavy paper, and holding 50 pounds of potatoes each, were used. In some of the tests with these paper bags, the paper towels impregnated with the methyl ester were distributed evenly in layers with the potatoes throughout the bag; in other tests all of the papers needed for the tubers in an entire bag were placed at the top of the bag; while in other tests, the chemical was distributed over the inner surface of the paper bag in acetone solution, and the acetone was allowed to escape from the bag by evaporation, which left a coating of the methyl ester on the inner surface of the bag.

The storage space for all lots whether in bins or paper bags was a basement room, without automatic temperature control, but with ventilators leading to the outside air. When the air temperature fell to 15° C. or lower, the ventilators were adjusted to maintain a temperature not higher than 15° C. nor lower than 10° C. At the start of the test the temperature was 22° to 23° C.; it decreased gradually to about 15° C. by November 15, to 12° C. by December 15, to 11° C. by January 15; it then increased to 12° by February 15, 14° C. by March 15, 15° C. by April 15, and to 22° C. by May 6 when the experiment was discontinued.

The chemical treatments used for hastening the germination of the tubers after sprout development had been inhibited for several months were as follows: The tubers were cut into approximately one-ounce pieces, each having at least one bud; the pieces were distributed evenly into four lots of 25 pieces each in wide-mouthed glass fruit jars. For the lots marked EC in Table I, a solution of ethylene chlorohydrin ($\text{ClCH}_2\text{CH}_2\text{OH}$), made by adding 30 cc. of 40 per cent ethylene chlorohydrin to 970 cc. of water, was poured into the glass jar containing the potato-seed pieces until the potatoes were covered; the liquid was decanted at once and the excess was removed by inverting the jar and shaking it; a small whole tuber was put on top to provide for drainage; the fruit jar was then inverted and allowed to stand in this position for 16 hours at room temperature (20° to 25° C.); the dipped pieces were removed from the jar and planted at once in soil in the greenhouse bench. For the lots marked "none" in Table I, the procedure was the same except that water instead of ethylene chlorohydrin was used. For the lots marked EC+NaSCN in Table I the treatment proceeded as described for EC until the dipped pieces were removed from the fruit jars after the 16-hour treatment period, then the pieces were soaked for one hour in a solution made by dissolving 10 g. of sodium thiocyanate (NaSCN) in one liter of water, were rinsed with three successive portions of water, and planted. For the lots marked EC+ thiourea in Table I the

process was the same as for EC+NaSCN except that the solution for the one-hour period of soaking was made by dissolving 10 g. of thiourea (NH_2CSNH_2) in one liter of water. The subsidiary treatments with NaSCN and thiourea were employed because in previous tests with freshly-harvested tubers there was some indication that the combination treatments were more effective than a treatment with ethylene chlorohydrin alone. Such an effect was not obtained in the present tests, as no important differences among the chemical treatments for breaking dormancy were noted.

RESULTS

TREATMENTS IN WOODEN BINS

The results of the treatments of potato tubers in wooden bins, using approximately 20 bushels in each bin, are shown in Figure 1. The treatment for tubers shown in Figure 1 A was started on October 3, 1941 (at which time small sprouts were showing with many of the tubers), and the methyl ester of α -naphthaleneacetic acid was applied by spreading paper towels (previously impregnated with the proper amount of chemical) in layers among the tubers. The amount of chemical in the paper towels was adjusted to give a series of concentrations of chemical, i.e. 100 mg., 33 mg., 11 mg. of the methyl ester per kg. of potato tubers undergoing treatment. The photograph for Figure 1 A was taken April 13, 1942 and it shows the sprout development of tubers removed from the bins by accumulating small samples taken from different levels in the bin. Only a few small sprouts were formed in the lot receiving the methyl ester at the rate of 100 mg. per kg. of tubers; sprouts were formed sparsely in the lot receiving 33 mg. per kg.; the 11 mg. lot, however, showed about the same amount of sprouting as that of the control lot.

Figure 1 B shows the result of a treatment using 50 mg. of methyl ester per kg. of tubers, starting December 8, 1941. Previous to December 8 these tubers had been stored at 5° C., and so at the time of the start of this test they were not dormant, sprouting of tubers in the control lots starting at once at the temperature of the treatment. The temperatures in this test are those shown by the schedule given above for the tubers in the bins treated with 100 mg., 33 mg., etc. Figure 1 B shows that, although these tubers were nearly non-dormant, treatment with 50 mg. of methyl ester per kg. of tubers inhibited sprouting successfully over the period from December 8, 1941 to April 13, 1942. At the end of the test on May 6, 1942 the tubers of the treated lot were still in about the same condition as that shown in Figure 1 B.

TREATMENTS IN PAPER BAGS

The results of treatments in the triple-walled heavy paper bags (contents approx. 50 lbs. of potatoes) are shown in Figure 2. The test, the re-

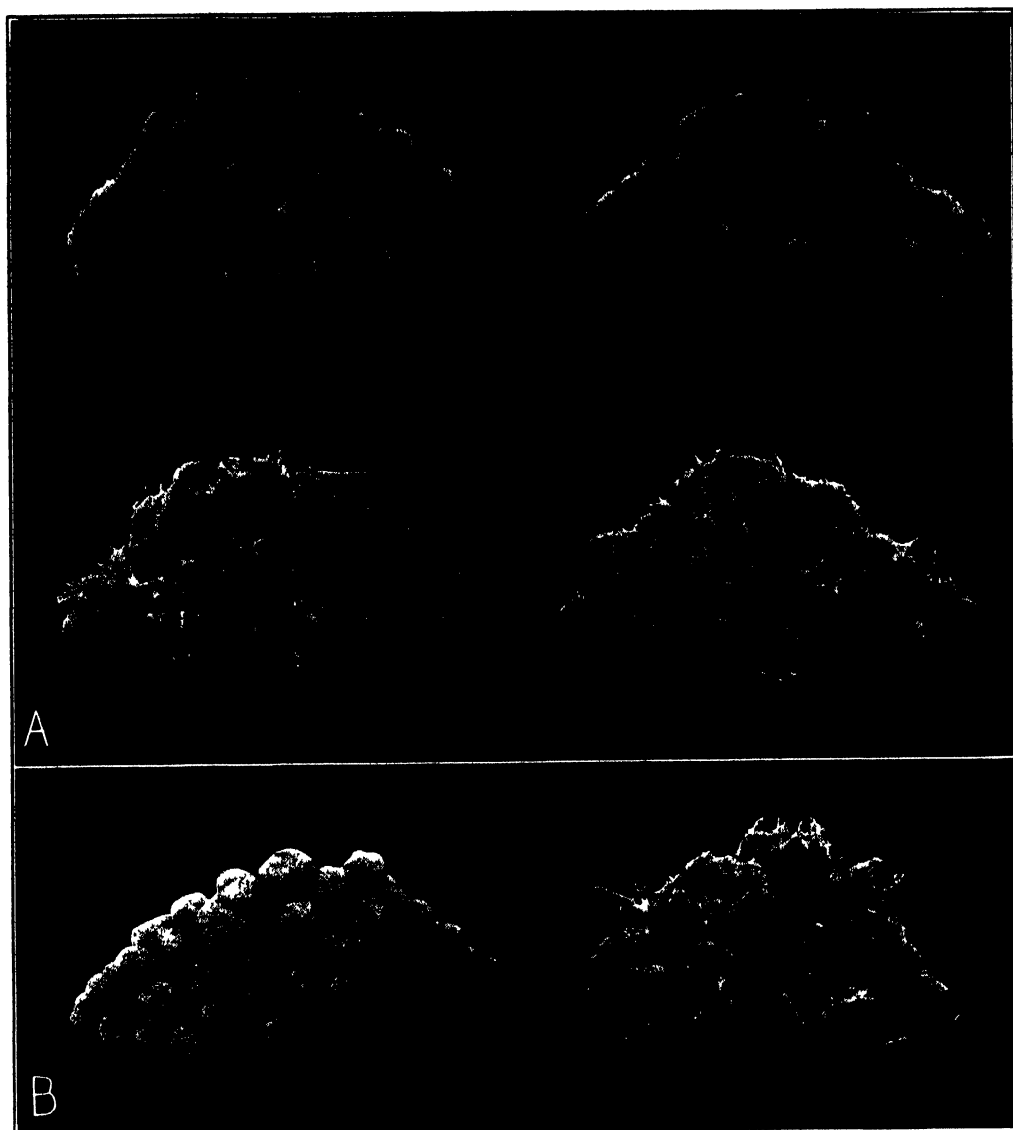


FIGURE 1. Effect of vapor of methyl ester of α -naphthaleneacetic acid in inhibiting sprouting of potatoes stored in wooden bins, approx. 20 bu. in each bin. A. Treatment started Oct. 3, 1941; top left, treated with methyl ester at rate of 100 mg. per kg. of tubers; top right, 33 mg.; center left, 11 mg.; center right, control. B. Treatment started Dec. 8; bottom left, treated with 50 mg. per kg.; bottom right, control. These photographs taken Apr. 13, 1942.

sults of which are shown in Figure 2 A, B, and C, was started October 3, 1941. The amounts of chemical used were the same as those applied to tubers in bins, i.e. 100, 33, and 11 mg. per kg. of tubers. The tubers for the photograph for Figure 2 A were obtained on April 13, 1942, by removing samples at various levels in the paper bag. They show the results of the treatment when the paper towels impregnated with methyl ester were

distributed among the tubers at various levels from the top to the bottom of the bag. The results were about the same for the treatments in the paper bags as in the wooden bins. Sprouting was inhibited by 100 mg. and 33 mg. but not entirely so by 11 mg., although with the latter sprouting occurred less freely in the paper bags than in the corresponding wooden bin.

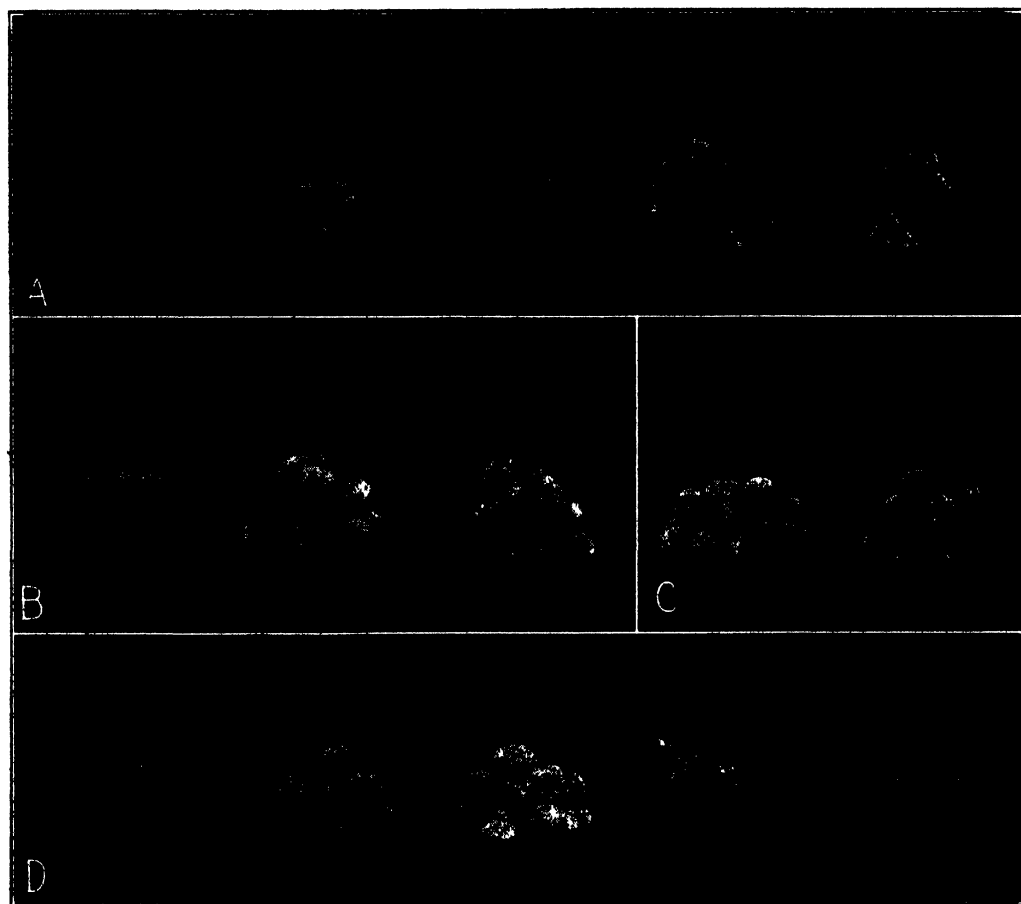


FIGURE 2. Results of treatments made with tubers stored in triple-walled paper bags each holding 50 lbs. of potatoes. A. Paper towels containing the methyl ester scattered evenly among the tubers in the paper bag; left to right, treatment with 100 mg., 33 mg., 11 mg., control papers prepared originally with acetone only (without methyl ester), control without papers at all. B. Paper towels containing the methyl ester at the 100 mg. rate not scattered among the tubers but merely piled on top of them; left to right, tubers just below the papers, tubers from the second layer below, tubers from the bottom of the bag. C. Methyl ester at the 100 mg. rate applied to inner surface of the paper bag; left pile, tubers touching the bag; right pile, tubers from the central core of the bag. D. Methyl ester incorporated into talcum powder which was then applied to the surface of the tubers; left to right, 100 mg., 50 mg., 25 mg. of methyl ester per kg. of tubers, control with talcum only, control without either talcum or methyl ester. A, B, and C treatments started Oct. 3, 1941; D treatment started Dec. 13, 1941. These photographs taken Apr. 13, 1942

Figure 2 B shows the result when the papers impregnated with 100 mg. of methyl ester per kg. were not distributed evenly throughout the bag but were merely placed on the tubers at the top of the bag. The expectation that the vapor from the papers would diffuse downward and reach the tubers at the bottom of the bag was not fulfilled. The photographs show that only the tubers in the top layer just below the papers received sufficient chemical for inhibition of sprouting, and that as the distance from the papers increased the effectiveness of the treatment decreased, until at the bottom layers in the bag sprouting occurred freely.

Figure 2 C furnishes a further illustration of the fact that the vapor from the impregnated paper diffuses from the paper for only a short distance. In this experiment the methyl ester was not incorporated into paper towels but was applied to the inner layer of the large paper bag. Examination of the contents of the bag showed even as early as December 1941 that the sprouting of the outer layer of tubers (those touching the paper) was inhibited, but that tubers in even the second layer inward showed considerable sprout development. Indeed, in many instances, when the tubers were removed from the bags at the end of the experiment, a given tuber with a flat side touching the paper would show no sprout development from the buds touching the paper, although sprouting occurred freely on the opposite side of the same tuber.

TREATMENTS WITH CHEMICAL INCORPORATED INTO TALCUM POWDER

Since the tests with treatments in paper bags had indicated that nearness to the tuber was an essential condition for the success when the chemical was applied by allowing evaporation from impregnated papers, attention was turned to the use of a powder for applying the methyl ester, the expectation being that at least some of the dust particles would lodge in the eyes of the tuber, and in this way the chemical would be deposited upon the buds themselves.

In a preliminary test to determine the amount of talcum powder that would adhere to the surface of tubers a number of tubers of known weight were rolled in talcum powder and each tuber tapped to dislodge the excess powder. The powder was then washed off, filtered through a tared filter paper, dried, and weighed. It was found that 1.65 g. of the powder adhered to 1 kg. of tubers. This determined the amount of the acetone solution of the methyl ester to be added to talcum powder in order that the concentration of the chemical applied in the dry powder after evaporation of the acetone would be equal to 100 mg., 50 mg., and 25 mg. per kg. of tubers. The control lot with talcum was dusted in like manner by talcum to which acetone had been added and then removed by evaporation.

The results of the treatments with methyl ester incorporated into talcum and applied as a powder are shown in Figure 2 D. After the powder

was applied, the tubers were stored in triple-walled heavy paper bags. The experiment was started December 13, 1941, and the photographs were taken April 13, 1942. It is seen that an amount of methyl ester as low as 25 mg. per kg. of tubers completely inhibited sprouting.

Treatments (not described in detail here) in which bentonite instead of talcum was used showed less favorable results, probably because the physical condition of the bentonite was changed by the acetone in which the methyl ester was dissolved. After the acetone had evaporated the bentonite formed a hard cake and the original degree of fineness of the powder was not reached in preparing the material for dusting the tubers.

In other experiments the methyl ester was incorporated into confetti, again by means of acetone as a solvent. The impregnated confetti, when scattered loosely over potatoes stored in stoneware jars, successfully retarded sprouting in the period from March 21, 1942 until June 5, 1942, at which time this report was being prepared. A similar test using the dried plant tissue fragments such as is commonly employed as litter for small animals gave results that were less favorable than those obtained with confetti. With these carriers consisting of small pieces, there was a tendency for uneven distribution because of the pieces gravitating toward the bottom of the container. This difficulty could be overcome by using shredded newspaper which would furnish short ribbon-like strips not easily dislodged.

AMOUNT OF CHEMICAL RETAINED BY POTATO TUBERS

In order to contribute information that might be of use in feeding-tests with animals in a study of the toxicity of the methyl ester of α -naphthaleneacetic acid, and of tissue from potato tubers treated with it, an effort was made to obtain an estimate of the amount of the chemical absorbed by the tissue, or adhering to the surface of the tubers.

It was found that the hyponastic response which the tips of young potato plants make to the vapor of this chemical could be used for this purpose. Epinasty of potato leaves is also obtained, and at a concentration even lower than that inducing hyponasty, but the end-point in a series of concentrations is much sharper with hyponasty than with epinasty.

The method of testing used is shown in Figure 3. Tips of young potato plants, two tips in each test, are placed in vials of water adjusted in the center of the watch glasses so that when the liter beaker is inverted over them the leaf edges do not touch the sides. Trimming off a bit from the ends of the leaves does not interfere with the test. Tips from tubers of the variety Green Mountain are especially favorable since the first well developed leaves have relatively short leaf blades and petioles. The methyl ester, either as an acetone solution of the pure chemical used in preparing the standards of comparison, or that obtained from the treated tubers by

extraction with acetone, is incorporated into four filter papers (15 cm.), and the acetone is allowed to evaporate at room temperature. The dry filter papers are crumpled and placed in the space around the vials; the liter beaker is inverted over vials, potato tips, and papers; a tight seal is made with modeling clay.

With suitable amounts of methyl ester, and at a temperature of 27° C., hyponasty is obtained in one to four hours, occurring more quickly with



FIGURE 3. Method of testing for methyl ester of α -naphthaleneacetic acid by hyponastic response. The chemical either in the pure form, as used for preparing standards, or as extracted from tissue previously treated with it, in acetone solution is incorporated into four filter papers. After the acetone is allowed to evaporate the dry filter papers are crumpled and placed in the watch glasses. The tips of young potato plants such as those shown at the right are placed in vials of water which are then put in the center of the watch glass. The liter beaker is inverted over the watch glass and a seal is made with modeling clay. Temperature should be 27° C. Hyponasty is shown by the test plants at the left and center after an exposure of one hour to four filter papers containing a total of 0.02 mg. of methyl ester. Control plant at right after an exposure of four hours.

the relatively higher amounts of chemical. The response is much slower and at times uncertain at lower temperatures, especially at those below 24° C. It is not the bending of the very immature leaves at the tip, but that of the well developed leaves at the next two lower levels that is to be looked for in this test.

When a series of decreasing concentrations of the methyl ester was tested, it was found that the critical range for hyponasty was from about 0.04 mg. to 0.005 mg. of the methyl ester in the four filter papers in the volume of the container (approx. one liter). With 0.04 and 0.02 mg. the hyponasty was strong and definite; at 0.01 mg. it usually but not always occurred; at 0.005 mg. it occurred only occasionally. These four concentrations were adopted as standards against which to compare the response from extracts of potato tissue, and were set up each day under the same conditions as those to which the tissue tests were exposed, and with potato tips from the same source of supply.

Since the methyl ester of α -naphthaleneacetic acid is readily soluble

in acetone, and since the acetone evaporates rapidly from the filter papers at room temperature, this solvent was used for extracting the methyl ester from potato tubers. Separate extractions were made for the peel and flesh. After the peel had been removed, small pieces of it were taken, in such manner as to furnish tissue representing all of the tubers in the sample and various positions on each tuber; these were combined to make a total of 7 g., fresh weight. The samples of flesh consisted of thin slices from quartered tubers, outside to center, the total sample being 15 g. The tissue was ground in a mortar, transferred to a filter with acetone, and extracted with successive quantities of acetone until the extract reached a volume such that 5 cc. equaled 1 g. of fresh tissue. Aliquots of the acetone extract equal to 1, 2, and 4 g. of peel, and to 5 and 10 g. of flesh were then transferred with a pipette to four filter papers (15 cm.) on a watch glass; 5 to 10 cc. of the acetone extract were applied at a time, and the acetone was allowed to evaporate in an air current before a second or third amount of extract was transferred to the filter papers. The dry filter papers containing the extracts from known amounts of tissue were used in the hyponasty tests, the response being compared to that obtained from known amounts of methyl ester in the four standard concentrations (0.04, 0.02, 0.01, and 0.005 mg.) of the methyl ester.

When tubers which had been treated for five months in the wooden bins with methyl ester at the rate of 100 mg. per kg. of tubers were tested in the manner described above, it was found that an aliquot equal to 1 g. of peel gave a hyponastic response about equal to the 0.02 mg. standard. The proportion of peel and flesh obtained in these tests was approximately 200 g. of peel and 800 g. of flesh in each kilogram. On this basis the methyl ester in the peel was 200×0.02 mg., or about 4 mg. per kg. of tubers treated with methyl ester at the rate of 100 mg. per kg. The aliquot representing 5 g. of the flesh after peeling was negative in all cases, and the aliquot representing 10 g. of flesh gave weak hyponasty only in occasional tests. It was concluded that the methyl ester from 10 g. of flesh was at least not higher than 0.01 mg. of methyl ester, and on this basis the amount in the flesh was not more than $0.01 \text{ mg.} \times (800 \div 10) = 0.8$ mg. of methyl ester per kg. of tubers. From these tests it appears that the tubers in the lots treated with methyl ester at the rate of 100 mg. per kg. take up not more than 5 mg. of the chemical per kg. of tubers, and that of this amount, four-fifths or more are in or on the skin.

Some of the treated tubers were boiled (peels not removed) for 20 minutes, and the thin brown membranes were stripped off, extracted with acetone, and tested for the presence of methyl ester. An aliquot representing 2 g. of this brown membrane gave a hyponastic response equal to 0.01 mg. of the standard. Since the weight of the brown membrane from 1 kg. of boiled tubers was found to be 65 g., the amount of methyl ester in the

membranes of boiled potatoes was approximately one-third mg. per kg. of boiled potatoes. When tubers were baked for 45 minutes at 500° F., the outer brown membranes, when stripped off from the warm tuber and extracted with acetone, showed the presence of methyl ester by a hyponastic response equal to the 0.01 mg. standard with an aliquot representing 1 g. of membrane. Since 1 kg. of baked potatoes furnished 60 g. of the brown membrane, the amount of methyl ester in the skin of the baked potato was approximately two-thirds mg. per kg. of baked potato. Whether from boiled or baked potatoes, however, the flesh after the removal of the brown membrane showed negative or very weak hyponastic responses in the manner shown by the flesh before boiling or baking.

The peels from tubers treated four months with methyl ester at the rate of 50 mg. per kg. gave a positive test for hyponasty, an aliquot from 1 g. showing a response about equal to that given by the 0.01 mg. standard, and so indicating a methyl ester content in the fresh peel of about 2 mg. per kg. of tissue. A test of the peeling from the tubers treated five months with the chemical at the rate of 33 mg. per kg. indicated a methyl ester content of the peel less than 1 mg. per kg.

Similar tests were made of peel and flesh of the control tubers from the bin not receiving a treatment with the chemical. The extractions with acetone, the incorporation of the extracts into filter papers, and the details of the procedure of testing these filter papers in the liter beakers with potato tips were the same as those used in testing tissue from tubers treated with methyl ester. In no case was hyponasty obtained with control tubers. In addition, tests were made of the residue of tissue of treated tubers after the acetone extraction had been completed in order to note whether all of the methyl ester had been removed. The residue from 5 g. of peel from the 100 mg. treatment, after acetone extraction and after exposure of a thin layer of the powdered tissue to air to remove the acetone, failed to cause hyponasty.

These estimates made as to the amounts of the methyl ester absorbed by the tissue are based on the assumption that the methyl ester of α -naphthaleneacetic acid was not transformed in the tissue into some other compound which is not volatile, or does not cause hyponasty of potato tips, at least at the same concentrations as those of the methyl ester used as standards of comparison in these experiments.

Since the methyl ester evaporates only very slowly, care had to be taken to remove the last traces from all glassware at the end of a test so as not to vitiate the results of a succeeding experiment. Scrubbing the apparatus with powdered cleaner, soaking overnight in a 2.5 per cent trisodium phosphate solution, rinsing thoroughly in a stream of hot water, and drying, removed the chemical satisfactorily, so that negative tests for hyponasty were obtained with glassware so treated.

INJURY TO TUBERS

In previous tests (1, 2) with tubers of various varieties, and in some cases with amounts of the methyl ester of α -naphthaleneacetic acid much larger than those used in this experiment, no injuries to tubers were noted in any case. In the present test, however, an injury such as that shown in Figure 4 was obtained on about 5 to 10 per cent of the number of tubers in the treated lots, the controls in all cases being without injury. These malformations were in the form of excrescences due to growth of underlying cells. These lumps varied in diameter from 0.5 to 3.0 cm., and the knotty tissue formed in them was much firmer than that of other portions of the tuber. As this abnormal tissue aged it became darker, but no rot developed in these areas. The factors causing these injuries are not known. The tubers

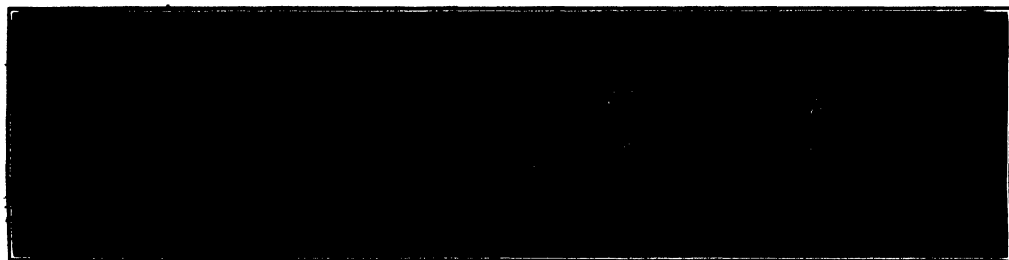


FIGURE 4. An injury occurring to approximately 5 to 10 per cent of the number of tubers in this experiment but not observed in any previous test. The injured portions, usually in the form of lumps, are firmer than other parts of the tuber. Discoloration but no rot occurs.

for this treatment were larger than those in previous tests, being in some instances over-sized, and evidently formed by a rapid enlargement of tubers during the growing season. Also, these tubers were either out of the dormant period completely, or in the early stages of sprouting, at the time the treatments were applied. Possibly the development of these enlargements in non-bud tissue was related to abruptly stopping an active growth which had already been initiated in the buds.

GERMINATION OF INHIBITED TUBERS

Beginning on March 9, 1942, after the tubers had been under treatment for about five months, samples from the 100 mg. and 33 mg. lots were removed for tests of the capacity to sprout when the tubers were cut into pieces and planted. On the assumption that the tubers would be too dormant for prompt sprouting, treatments with ethylene chlorohydrin using the dip method were made, and, in addition, a combination of this treatment with a subsequent treatment made by soaking the cut tubers (after the 16-hour exposure to chlorohydrin vapor) for one hour in a solution either of sodium thiocyanate (NaSCN), or of thiourea (NH_2SCNH_2); see paragraphs on Materials and Methods.

At the time that these samples of tubers were removed from the bins for a sprouting test to be applied at once, two similar samples were removed, put aside in burlap bags, and exposed to air in a storage room at 10° to 15° C., one lot for two weeks and the other for four weeks. At the expiration of these periods of storage after removal from the presence of the vapor (except such are remained on or in the tuber), the same treatments for inducing sprouting were again applied.

Since it had been found that most of the chemical was on the surface of the tuber, or in the external layers, a large sample of tubers was removed on April 3, 1942, scrubbed thoroughly with soap and water, rinsed, dried, and divided into three sub-samples, one to be treated at once, one after storage in air for two weeks, and the other after aeration for four weeks. The storage temperature for these was below 15° C. until about April 15 but rose gradually to 21° C. on May 2.

Finally on May 6 samples were again removed from the bins and the same treatments for inducing sprout development were applied at once.

The results are shown in Table I. The tubers from the lot treated with methyl ester at the rate of 100 mg. per kg. were quite dormant as shown in lines 1 to 4, Table I. Those not given a chemical treatment for breaking dormancy, line 1, showed only 4 per cent germination, i.e. one tuber out of 25, even after an aeration period of four weeks following removal from the treatment bins; washing these tubers with soap and water, line 1, columns 6 to 8, favored the germination slightly, but possibly not significantly, the germination reaching only 32 per cent at the highest. The effects of the chemical treatments on the 100 mg. samples are shown in lines 2 to 4. Since the error variance was found to be 9.6, the required difference for significance between the totals of the six entries in each line of Table I, columns 3 to 8, is $\sqrt{9.6 \times 6 \times 2 \times 2.069} = 22$ (3, p. 49 and p. 248). This difference is easily exceeded in all comparisons of treated and not treated in lines 1 to 4, but the germination was incomplete, being only 69, 68, and 84 out of 150 in each case, or 221 out of 450, or approximately 50 per cent. With the lots that had been washed with soap and water, lines 2 to 4, columns 6 to 8, the chemical treatments were somewhat more helpful, inducing a germination of 137 out of 225, or about 60 per cent, a result, however, which could hardly be regarded as satisfactory.

The tubers from the 33 mg. methyl ester treatment were considerably less dormant than those from the 100 mg. lot as shown in lines 5 to 8 in Table I. Nevertheless, those removed March 9 showed only about 30 per cent germination, and those removed April 3 and washed with soap and water showed only 63 per cent. The chemical treatments improved the germination as shown by the totals in column 9, the differences between treated and control being 39 to 42, while the difference required for significance is 22.

In the above paragraphs it has been assumed that the improvement in germination shown in columns 6 to 8 was due to washing the tubers with soap and water, and not to the fact that the removal date was later, i.e. April 3 instead of March 9. This assumption seems to be justified by the

TABLE I

GERMINATION IN THE SPRING OF TUBERS TREATED OVER-WINTER WITH METHYL ESTER OF α -NAPHTHALENEACETIC ACID, WITH AND WITHOUT CHEMICAL TREATMENTS TO BREAK DORMANCY

Methyl ester amounts used over-winter	Treatment in spring to break dormancy	No. of sprouts (out of 25 planted) emerged after 4 weeks from planting						Total No. germinated	Tubers re-moved May 6 and treated at once. No. germ. in 4 wks.
		Tubers removed Mar. 9, not washed			Tubers removed Apr. 3, washed with soap and water				
		Treated at once	Let stand in air		Treated at once	Let stand in air			
			2 wks.	4 wks.		2 wks.	4 wks.		
100 mg. per kg.	None	1	1	1	2	5	8	18	0
	EC	6	13	9	16	10	15	69	6
	EC+ NaSCN	4	12	7	17	13	15	68	5
	EC+ Thiourea	7	13	13	18	19	14	84	5
33 mg. per kg.	None	7	6	11	11	19	17	71	9
	EC	16	19	19	16	24	19	113	12
	EC+ NaSCN	17	13	16	19	23	22	110	13
	EC+ Thiourea	17	16	18	16	24	19	110	18
Total No. germ.		75	93	94	115	137	129	643	68

Analysis of Variance of Data in Columns 3 to 8, Table I

Source of variation	D.F.	Variance	Source of variation	D.F.	Variance
Total	47	—	Treatments $\times$ Washing	3	11.0
Treatments	3	194.6	Treatments $\times$ Delay	6	3.5
ME amounts	1	567.0	Washing $\times$ ME	1	2.0
Washing	1	295.0	Washing $\times$ Delay	2	1.5
Delay periods	2	28.5	Delay $\times$ ME	2	2.5
Treatments $\times$ ME	3	10.3	Error	23	9.6

Note: EC = ethylene chlorohydrin; EC + NaSCN = ethylene chlorohydrin treatment followed by treatment with NaSCN; EC + Thiourea = treatment with ethylene chlorohydrin followed by treatment with NH_2CSNH_2 (see directions under paragraph on Methods); ME = methyl ester of α -naphthaleneacetic acid.

values in column 10 for the lot removed May 6. A comparison of the germination counts in columns 3 and 10 do not indicate any important change in the capacity to germinate in the interval from March 9 to May 6 (the tubers, of course, having been in contact with the methyl ester vapor in the bins during that period).

In comparing the totals of the eight entries in the columns in Table I the required difference for significance is $\sqrt{9.6 \times 8 \times 2 \times 2.069} = 26$. The gains in germination due to washing the tubers with soap and water are significant as shown by comparing the totals of columns 3 and 6, 4 and 7, 5 and 9, the required difference 26 being exceeded in each comparison. This effect of washing is consistent with the previous results in showing that a good share of the chemical was adhering to or was absorbed by the periderm. The methyl ester incorporated in the particles of soil on the surface of the tuber was removed in washing, and so exerted no further inhibiting influence. Probably at least some of the chemical on or in the periderm was dissolved by the soak solution.

It might have been expected that a delay after removal of the tubers from the treatment bins would have had a favorable effect upon the germination, since during the two to four weeks of aeration the loss of chemical might be sufficient to allow the processes of germination to get started. The results in Table I, however, show that gains due to allowing the tubers merely to stand in air after removal from the bins were not large, and probably not significant. The variance for delay was 28.5 with 2 D.F., and so the F value is $28.5 \div 9.6 = 2.97$, whereas the required F value for a probability of 0.05 with 2 D.F. and 23 D.F. is 3.42 (3, p. 254). It is true that if the chi-squared test (3, p. 77, and 4, p. 168) is applied to the values in Table I, significant differences are obtained in some of the comparisons of the "at once" with the "delay" lots; nevertheless, this does not eliminate the fact that no outstanding gain in germination was obtained by aerating the treated tubers for a considerable period before planting, or treating and planting.

Among the factors responsible for this slow increase in the capacity to sprout after removal of tubers from the treatment may be mentioned: slow evaporation of the methyl ester from the tubers, incomplete removal of the chemical in washing, and possibly a slow emergence of buds from the enforced dormant condition even if most or all of the chemical had been removed.

The effects of the treatments with ethylene chlorohydrin, or with combinations of chlorohydrin and NaSCN or thiourea, were less pronounced than would have been expected with freshly-harvested tubers of this variety. It is believed that the dormancy induced by the methyl ester of α -naphthaleneacetic acid is deeper than that which occurs in the normal rest period of Irish Cobbler potatoes.

SUMMARY

Potato tubers (*Solanum tuberosum* L.), in lots of 20 bushels each, placed in wooden bins shortly after harvest, were exposed to the vapor of the methyl ester of α -naphthaleneacetic acid ($C_{10}H_7CH_2COOCH_3$) incorporated into paper towels which were interspersed evenly among the tubers in the bins. An amount of the methyl ester equal to 100 mg. per kg. of tubers inhibited completely the sprouting of the tubers from October 3, 1941 (shortly after harvest) until the end of the experiment, May 6, 1942. Only a few sprouts were formed by tubers treated with 33 mg. per kg. The storage temperature was 10° to 15° C. from November 15 to April 15, ranging up to 22° to 23° C. in the intervals previous to and subsequent to these dates. Control tubers sprouted freely under the same conditions.

A similar treatment starting December 8, 1941 with 50 mg. per kg., using tubers that were practically out of the natural dormant period at the time, successfully inhibited sprout development until the end of the experiment.

Treatments applied to tubers stored in triple-walled paper bags (50-lb. capacity), using the same concentrations of chemical at the same temperature and over the same interval, gave responses about the same as those obtained with treatments in bins, provided that the paper towels impregnated with the methyl ester were distributed among the tubers. When the chemical was applied only to the inner layer of the paper bag itself, or to paper towels placed on top of the tubers in the paper bag, inhibition of sprouting was obtained only with the tubers touching the sides of the bag, or in the layer of tubers just below the paper towels.

When the methyl ester of α -naphthaleneacetic acid was incorporated into talcum powder which was then applied as a dust to the surface of each tuber, sprout inhibition was obtained at the rate of only 25 mg. of methyl ester per kg. of tubers.

The hyponastic response to the vapor of the methyl ester of α -naphthaleneacetic acid given by the first well developed leaves at the tips of young potato plants was used to form an estimate of the amount of the chemical taken up by the potato tubers as a result of the treatments. The acetone-extracted material from treated tissue was incorporated into filter papers and the response of potato tips enclosed with these in liter beakers was compared to that given by standards containing 0.04, 0.02, 0.01, and 0.005 mg. of methyl ester. At 27° C. a test is completed in one to four hours.

From such comparisons it was estimated that potato tubers, exposed for five months in wooden bins to methyl ester applied in paper towels at the rate of 100 mg. of methyl ester per kg. of tubers, retained not more than 5 mg. of methyl ester per kg. of tissue, and that of this amount at least four-fifths were in the peel. Positive tests for the presence of methyl

ester in small amounts were obtained from the thin brown membranes removed from whole tubers after boiling them for 20 minutes, or baking them for 45 minutes at 500° F., but the flesh from the boiled or baked potatoes gave negative or very weak tests for the methyl ester, indicating amounts at least less than 1 mg. per kg. of the cooked flesh.

An injury occurring on 5 to 10 per cent of the number of tubers in the lots chemically treated was noted in this experiment although none of any kind had been observed in any previous test. It consisted of hard, lump-like excrescences due to enlargement of underlying tissue. These varied in size from 0.5 to 3.0 cm. in the diameter of the injured portion. Darkening of these areas sometimes occurred but there was no incidence of rot at or near them.

Tubers removed from the treatment bins in March to May, 1942, cut into pieces and planted in soil, germinated poorly, especially in the lots treated with methyl ester at the 100 mg. rate, and with less than 50 per cent of germination with lots treated with 33 mg. per kg. Washing the treated tubers with soap and water favored germination. Treatments with ethylene chlorohydrin ($\text{ClCH}_2\text{CH}_2\text{OH}$), or combinations of treatments with ethylene chlorohydrin and sodium thiocyanate (NaSCN) or thiourea (NH_2CSNH_2) brought about a further improvement in germination. However, even then complete emergence of sprouts was not obtained. It is believed that the sprout dormancy induced by the vapor of methyl ester of α -naphthaleneacetic acid is more pronounced than that which occurs in the natural rest period of the tubers of this variety of potatoes.

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THE THIRD YEAR'S RESULTS ON STORAGE OF POTATO TUBERS IN RELATION TO SUGAR CONTENT AND COLOR OF POTATO CHIPS

F. E. DENNY AND NORWOOD C. THORNTON

In these experiments, which have been carried out primarily with regard to the color of potato chips, the behavior of different varieties has been stressed, mainly because this phase of the problem had not received extensive attention previously. In the experiments of the first year (6) there were 11 varieties, in those of the second year (7) there were 25, and in those here reported upon there were 19. The varieties have shown on the whole a consistently uniform response, and, although the conditions of storage have been such as to lead to differences in the amounts of sugar, and rates of increase with time, the varieties have maintained about the same rank in the various tests. This is true of both the reducing sugar and sucrose values. The varieties have definite characteristics with regard to their content of these two types of sugar.

The superiority of the varieties of the Rural group (Stuart, 9), at least for the production of potato chips of good color, was clearly shown in all tests, these varieties being low in reducing sugar, which is the form of sugar controlling the color of potato chips (10), the sucrose content being without effect (6, p. 295). Varieties outside of the Rural group had distinctly higher amounts of reducing sugar, but tubers of some of them could be held in good condition for potato chip production for several months by the use of suitable conditions of storage.

Fair agreement had been reached among previous workers as to the most favorable temperature range within which tubers may be stored if the sugar content is to be maintained over a considerable period at a sufficiently low value for culinary purposes. Wright, Peacock, Whiteman, and Whiteman (11) put the dividing line at between 40° and 50° F. Appleman (1) suggested that the most favorable temperature was a compromise between 38° and 44° F. Butler (4) recommended 46° F. and warned against the use of a lower temperature. Barker (2) proposed 7.5° to 10° C. (45.5° to 50° F.). Barmore (3) placed the range at 4.4° to 7.2° C. (40° to 45° F.) but recognized that this might necessitate a period of de-sugaring at a higher temperature.

Our results tend to narrow these limits, to emphasize the large differences among varieties in their response to temperatures in this range, and to show the differences that may be obtained with tubers of the same variety from the same harvest, depending on the time after harvest at which storage is started. The range from 7° to 8° C. (44.6° to 46.4° F.) was

found to be critical, both as to the amount of reducing sugar formed, and as to the time of initiation of sprouting and length of sprouts. Less than one-half as much reducing sugar was formed at 8° C. as at 7° C. Tubers of some of the varieties were maintained at 7° C. for several months at satisfactory reducing sugar values, and certain other varieties, although forming too much sugar at 7° C., responded well at 8° C., furnishing tubers with low reducing sugar values, at least over a period of three to four months.

The sprouting capacity of the tubers also changed rapidly at this range of temperature, sprouts developing at an earlier date and in a much larger amount at 8° C. than at 7° C. Photographs of tubers of several varieties stored at these temperatures for several months are presented, affording the reader an opportunity to judge whether the amount of sprouting should be considered excessive.

Although it was recognized that 5° C. (41° F.) was a temperature too low for continuous storage of potato tubers for culinary purposes, this temperature was included in each year's tests in order to obtain information on the characteristics of the various varieties with regard to the amounts of the two types of sugar developed and maintained at this temperature (which is a favorable one for the formation of sugar). And even though the sucrose content has no effect upon the color of potato chips, the amount of this sugar constituent was determined in each test. This required only one additional analytical determination, and the inclusion of the sucrose values has been helpful in showing differences between varieties, temperatures of storage, and dates for the starting of cold storage.

One of the most important factors influencing the sugar content during cold storage, both as to the rate of increase and the amount accumulated over a considerable period, has been the time after harvest at which the cold storage at 5° C. (41° F.) was started. The results of two years' tests with many varieties agree in showing that starting storage soon after harvest resulted in a relatively rapid increase in reducing sugar; the sucrose, however, tended to remain low with early starting, the rate of sucrose accumulation increasing progressively with later starting dates. However, the situation is not clear as to the most favorable starting date for retarding the reducing sugar increase; in one year's tests low reducing values were associated with a late start of storage, while in the other year's tests an intermediate starting date was more favorable for obtaining low reducing sugar values. There is need of more experiments on this phase of the problem.

MATERIALS AND METHODS

The tubers for planting in May, 1941 were obtained partly from a commercial seed supply company, and partly from the United States

Department of Agriculture, from Eastern States Farmers' Exchange, Springfield, Mass., and from William Kroemer, Hicksville, N. Y., these three having kindly donated a generous supply of several varieties for planting. We are indebted to Dr. Charles F. Clark, Presque Isle, Maine, to Dr. Roland B. Dearborn, Springfield, Mass., and to Mr. I. H. Vogel, Hicksville, N. Y., for arranging the cooperation.

The tubers were dug August 6 to 15, 1941, were allowed to stand in burlap bags indoors, under conditions of good aeration for seven to ten days, and were sorted into sizes. They were then distributed into lots of 16 tubers each, care being taken to get an equal distribution of the sizes of tubers. Each lot was put in a cheesecloth bag and labeled appropriately. The various lots in cheesecloth bags were distributed into burlap bags each one of which contained the samples that were to be placed under a given storage condition and to be removed at the expiration of a given time. From this time until the date for the start of storage at low temperature, all lots were held in the burlap bags in a basement room at a temperature of approximately 21° C. (70° F.).

For storage at 5° C. and 7° C., rooms approximately 8'×9'×11', equipped with automatic temperature regulation, were available. The lots at 8° C. were in a well insulated incubator, inside measurements 24"×30"×54", with thermostatic control, and with slow air circulation, this incubator being wholly within the room maintained at 7° C. De-sugaring was carried out in a steam-heated room 7'×7'×7', also with automatic control. The thermometer bulbs were immersed in 250 cc. of water in an Erlenmeyer flask (500 cc.). The temperature was read to the nearest quarter-degree C., and the thermostat adjustment was corrected when a consistent deviation from the desired temperature was found. The temperatures were read and recorded by an attendant, at least once each day, except Sunday, and during a portion of the period, twice daily. In addition, watchmen in control of the storage rooms observed the temperature at intervals of two to three hours, although no records of temperatures were made by them.

During the course of the experiment, 180 to 184 thermometer readings were recorded for each room. The average values and the errors of these mean temperatures for the four rooms intended to be maintained at 5°, 7°, 8°, and 27° C. were: 5.011 ± 0.022 ; 7.018 ± 0.008 ; 7.993 ± 0.015 ; 26.832 ± 0.032 . Over a part of this period, temperatures in the top and bottom portions of the incubator at 8° C. were recorded, 102 temperature entries showing the following values: top, 7.973 ± 0.021 ; bottom, 8.039 ± 0.020 .

The methods for the preparation of potato chips and for the analysis of the sugar contents of juices were described in a previous paper (6, pp. 293, 294).

RESULTS

STORAGE AT 7° AND 8° C. (44.6° AND 46.4° F.)

Since continuous storage at 5° C. (41° F.) leads to the accumulation of so much reducing sugar that, with all of the varieties so far tested, potato chips of good color were not obtainable, and since at 10° C. (50° F.) excessive sprouting and shrinkage ensued, certain intermediate temperatures were tested in the previous experiment with the crop of 1940 (7, pp. 222, 237), with good results at least with some varieties. The temperatures within this range tested in the present series with the crop of 1941 were 7° and 8° C.; tubers from nine varieties were included, five of these at each temperature, two of the others at 7°, and two at 8°. The results are shown in Table I.

Reducing sugar. The reducing sugar values in the table are indicative of suitability for potato chip manufacture, values approximately 3.0 (mg. per cc. of juice) or below being associated with good chip color, and values higher than 5.0 with an objectionably dark color. For continuous storage at 7° over a long period up to five months or more, only two of the varieties in Table I maintained sufficiently low reducing sugar values to furnish a constant supply of tubers suitable for chips. They were Carman No. 3 and White Rural (both in the Rural group). Sebago was within the limit for more than four months and Katahdin was near it for six months. The other varieties at 7° C., Irish Cobbler, Green Mountain, and Houma, did not provide chips of good color at any storage duration used in this test. However, as will be shown in detail in a later section of this report, de-sugaring of tubers transferred from 7° C. (44.6° F.) to 27° C. (80.6° F.) occurred quite rapidly in most cases, so that although 7° storage fails to hold the reducing sugar of some varieties to sufficiently low levels, the tubers could probably be brought into proper condition by a short period of storage at a warm temperature.

The reducing sugar values for continuous storage at 8° C. (46.4° F.) are shown in Table I, columns 5, 7, 9, 11, 13, 16, and 17. This change of only 1° C. in the temperature of storage had an important effect upon the reducing sugar content as shown by comparing the data in columns 4 and 5, 8 and 9, 10 and 11, 12 and 13. At this temperature Katahdin failed to form reducing sugar except in traces over a storage period of six months; Irish Cobbler furnished good chips during a storage period of three to four months, and even with Houma, the favorably low sugar value of 3.0 mg. per cc. of juice was approached or reached and maintained over an interval of three to six months. Only Green Mountain among the varieties tested in this experiment failed to reach satisfactorily low sugar levels at 8° C. The result with Blue Mercer at 8° C. is especially interesting in view of the fact that, as shown in later paragraphs, this variety is characteristically unusually high in reducing sugar at a storage temperature of 5° C. However, as

TABLE I
SUGAR CONTENT (MILLIGRAMS PER CC. OF JUICE) OF POTATO TUBERS STORED AT 7° AND 8° C. (44.6° AND 46.4° F.)

Kind of sugar	Start of storage	Days stored	Irish Cobbler		Carman No. 3		Green Mountain		Katahdin		Houma		Sebago	White Rural	Blue Mercer	Russet Rural
			7°	8°	7°	8°	7°	8°	7°	8°	7°	8°	7°	7°	8°	8°
Reducing sugar	Oct. 25	0	0.0	0.0	0.0	0.0	1.1	1.1	0.0	0.0	0.5	0.5	0.0	0.0	0.2	0.0
		30	3.4	0.9	0.0	0.0	3.7	2.3	0.5	0.0	3.8	1.4	1.9	0.4	1.4	0.0
		59	4.6	1.4	0.1	0.0	5.6	3.6	3.3	0.0	5.9	3.7	2.9	0.3	1.9	0.0
		128	8.1	1.5	0.0	0.0	6.7	2.9	3.1	0.0	7.0	3.2	2.7	0.0	2.3	0.0
		178	8.0	3.2	0.1	0.0	7.8	4.4	3.2	0.0	6.2	2.7	4.3	0.2	3.3	0.0
		221	10.2	5.6	0.0	0.0	10.0	6.1	7.0	1.7	10.0	6.8	5.3	0.0	4.0	0.0
Reducing sugar	Dec. 1	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		30	3.7	1.0	0.3	0.0	4.0	2.8	1.7	0.0	4.7	2.4	1.6	0.0	2.3	0.0
		60	5.3	2.3	0.0	0.0	6.1	3.4	2.2	0.2	4.2	1.6	2.3	0.0	2.7	0.0
		100	6.0	2.0	0.0	0.0	7.6	4.1	3.7	0.6	4.6	2.4	1.4	0.0	1.4	0.0
		150	5.7	4.0	0.0	0.0	8.9	6.2	3.7	1.0	8.5	4.3	5.9	0.0	3.0	0.0
Sucrose	Oct. 25	0	1.6	1.6	1.3	1.3	2.5	2.5	1.0	1.0	2.6	2.6	0.3	1.8	2.8	2.4
		30	5.5	4.2	3.6	2.4	8.0	5.0	4.4	2.4	3.5	3.0	5.5	4.0	2.6	2.3
		59	5.2	4.4	3.2	1.3	8.0	4.8	2.8	2.8	2.8	2.2	3.6	2.9	2.1	2.0
		128	4.4	3.3	2.0	1.0	4.7	3.8	2.6	1.3	1.7	2.9	3.0	2.3	0.7	1.0
		178	7.6	6.6	0.2	0.0	4.9	4.0	3.7	2.7	2.8	3.1	3.9	1.2	1.0	1.0
		221	11.5	9.1	1.1	0.0	3.1	6.3	5.3	3.1	4.8	4.8	4.9	1.8	2.5	1.9
Sucrose	Dec. 1	0	1.3	1.3	1.1	1.1	2.9	2.9	0.5	0.5	2.1	2.1	0.3	0.5	2.1	1.1
		30	5.3	4.4	3.7	3.6	11.0	7.2	3.2	2.4	2.9	2.7	4.5	3.8	2.1	2.1
		60	5.8	3.3	2.6	1.8	6.8	4.3	2.4	2.3	2.0	1.9	3.8	2.6	1.1	2.1
		100	7.2	3.8	1.0	0.0	7.2	4.3	2.7	2.6	2.8	2.6	3.1	2.0	1.2	1.0
		150	8.8	10.2	0.0	0.0	6.5	7.6	3.6	3.2	3.3	4.6	7.5	1.5	1.9	1.0

shown in column 16, Table I, at 8° C. reducing sugar values were maintained at a low level for at least four months.

Table I, lines 6 and 11, also show that the reducing sugar continued to increase during long-period storage and to reach relatively high values after 221 days with all of the varieties at either 7° or 8° except the three in the Rural group (Carman No. 3, White Rural, and Russet Rural) and possibly Katahdin and Blue Mercer at 8°. This behavior is unlike that shown by tubers in storage at 5° C., at which temperature the reducing sugar increases to a maximum and then decreases on further storage (see Table III).

Sucrose. The sucrose values for storage at 7° and 8° C. are shown by the entries in the lower half of Table I. Starting with relatively low values, lines 10 and 16, the sucrose increased rapidly to a high value at the 30-day duration period. From this date onward the varieties differed in their re-

TABLE II
WEIGHT OF SPROUTS REMOVED FROM TUBERS STORED FOR VARIOUS
PERIODS AT 7° AND 8° C. (44.6° AND 46.4° F.)

Variety	Weight (in grams) of sprouts from 16 tubers			
	Storage started Oct. 25, 1941		Storage started Dec. 1, 1941	
	7° C.	8° C.	7° C.	8° C.
Irish Cobbler	3.2	27.7	12.6	30.8
Carman No. 3	28.6	41.8	17.0	35.5
Green Mountain	25.8	63.8	44.6	73.5
Houma	28.0	55.5	45.0	46.2
Katahdin	3.3	24.1	11.4	36.4
Russet Rural	—	44.3	—	42.6
White Rural	21.5	—	21.9	—
Sebago	24.8	—	25.7	—
Blue Mercer	—	12.3	—	14.6
Warba	—	70.8	—	82.0
Total removed from comparable lots on:				
June 3, 1942	135.2	340.3	178.2	361.6
Apr. 21, 1942	60.6	244.3	114.0*	249.1*
Mar. 3, 1942	9.1	86.5	—	—
Total for first five varieties in this list (i.e. at the June 3 date)	88.9	212.9	130.6	222.4

* May 4, 1942.

sponse; with those in the Rural group (Carman No. 3, White Rural, and Russet Rural) the sucrose declined from this temporary maximum; with the other varieties, however, the sucrose values after reaching a low level after about 60 to 100 days of storage, then again rose, attaining a high value at the end of the experiment.

Sprout development. Although Table I shows that storage temperatures

of 7° or 8° C. (44.6° or 46.4° F.) were favorable for maintaining low reducing sugar values of most of the varieties over considerable periods of time, an important factor for consideration is the amount of sprouting which occurred at these temperatures. Some information on this point was obtained and is shown in Table II. The values in lines 1 to 10 in Table II are the weights of sprouts removed on June 3, 1942, from 16 tubers in each of the lots, and they show the amount of sprout growth from the start of storage on October 25 or December 1, 1941 until June 3, 1942. The totals for each column are given in line 11, and while these column totals cannot be compared with each other (since the entries are not always in pairs) they can be compared with the values in line 12 which show the sprout weights obtained by the corresponding samples removed on April 21, 1942. These show the weights from the same number of tubers representing the same varieties after the interval from the start until April 21. The weights in line 13 are likewise comparable with those in lines 11 and 12, since the

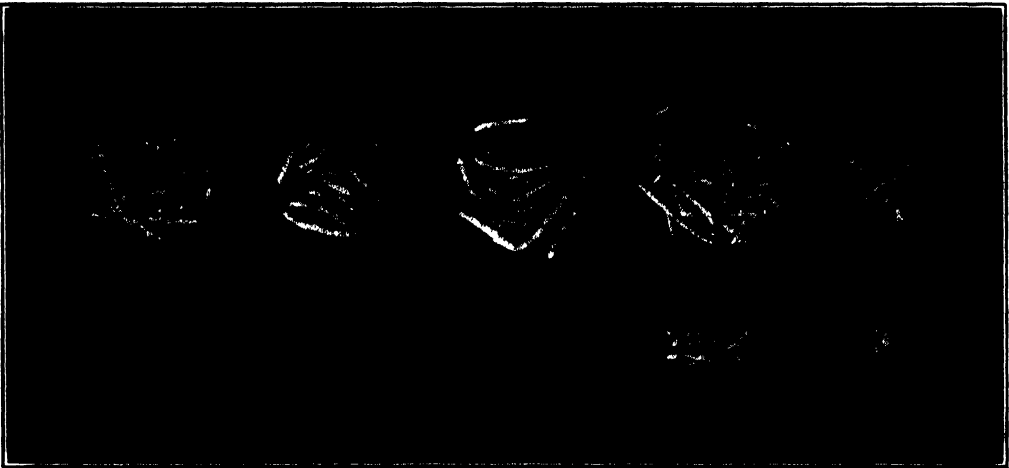


FIGURE 1. Sprouts removed from 16 tubers of each of five varieties after storage from Oct. 25, 1941 to Mar. 3, 1942, at 7° and 8° C. (44.6° and 46.4° F.); top row, 8°, bottom row, 7°; varieties, left to right are: Irish Cobbler, Carman No. 3, Green Mountain, Houma, Katahdin; the zero label (lower right) indicates no detachable sprouts were present.

same number of tubers from the same varieties are represented. The values in lines 11 to 13 indicate the time of year at which sprout development began and became extensive at these two temperatures. At 7° C. sprouting did not begin until March and had not become rapid until June; the values at 8° C. indicate that sprouting at 8° precedes that at 7° by one to two months. The paired values in lines 1 to 5, and the totals of these five varieties in line 14, Table II, emphasize the difference between 7° and 8° C. in the amount of sprout development.

The sprout condition of the varieties Irish Cobbler, Carman No. 3, Green Mountain, Houma, and Katahdin on March 3, 1942 after storage

at 7° and 8° C. starting October 25, 1941, is also shown in Figure 1. On this date, sprouts were developing freely with all five varieties at 8° C. (46.4° F.) but only Houma showed definite sprout emergence at 7° C. (44.6° F.), while with Irish Cobbler, Carman No. 3, and Green Mountain the sprouts were quite small, and with Katahdin no sprouts were visible. On June 3, 1942, the differences between 7° and 8° with regard to sprout development by these same five varieties were still large as shown by line 14 in Table II.

The sprout weights in Table II may not convey a clear idea as to whether the sprout development should be regarded as excessive for a given purpose, let us say for the production of potato chips. Photographs of the samples were taken at intervals from March 3 to June 3, and the condition of the tubers at the beginning and the end of this period are shown in Figure 2.

The sprout condition of all lots on March 3 was satisfactory at both temperatures. On June 3 sprouting was still not extensive with any variety at 7° C., but at 8° Warba showed many sprouts and shriveled tubers, and there was a considerable sprout development with Green Mountain and Houma. The other varieties showed sprouts of only moderate length, and the tubers were sufficiently firm to permit convenient slicing for the preparation of potato chips.

STORAGE AT 5° C. (41° F.)

The sugar contents of the juice of tubers of 19 varieties when stored at 5° C. for various duration periods are shown in Table III. Even though it was known that 5° C. was a temperature too low for continuous storage if potato chips of good color were to be obtained, this temperature was included in the present tests in order to get a comparison of the results of different crops of the same varieties stored at the same temperature in different years. Of these 19 varieties, 16 were the same as those stored in the previous year's tests (7). These are listed in Table III in the approximate order of the amount of reducing sugar per cc. of juice, with the amounts increasing from top to bottom of the table. By comparing the order of the varieties in Table III with the list in the previous article (7, pp. 222, 237), which was arranged on the same basis, it is seen that there is a general agreement in the sequence of varieties in the two years' tests. This question is taken up in greater detail in a later paragraph in this paper.

Reducing sugar. The reducing sugar values are shown in columns 2 to 11, Table III. Storage was continued for 122 to 127 days in this test, about one month longer than in the previous year. It will be noted that the reducing sugar values were practically at zero at the start of each test, and increased steadily to maximum value at 59 to 60 days after start of storage. The sugar then decreased to a lower value at the final sampling period. In

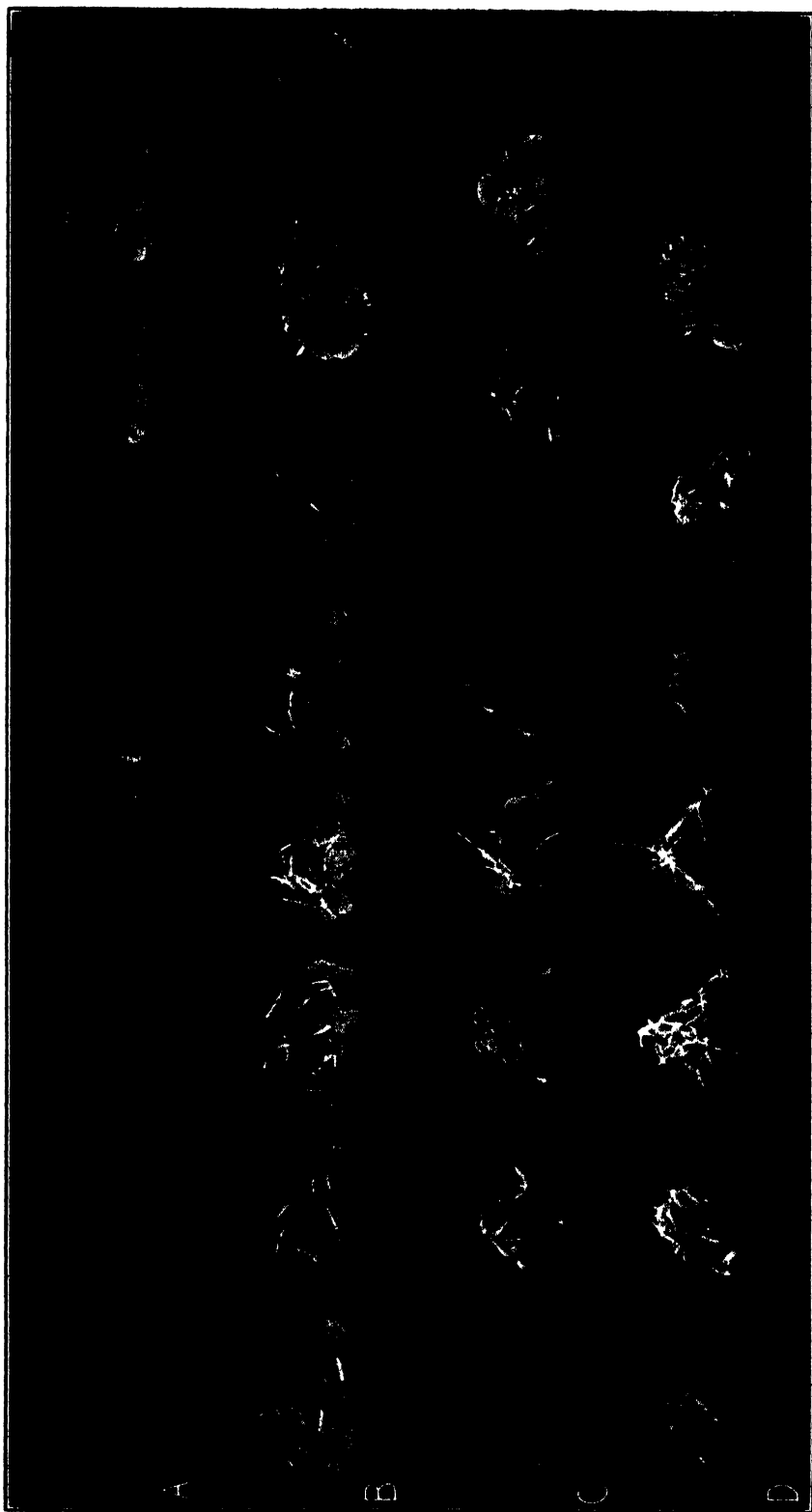


FIGURE 2. Sprout condition of tubers of various varieties after storage at 7° and 8° C. (44.6° and 46.4° F.). Rows A and C stored at 7° C., B and D at 8° C. Rows A and B, storage from Oct. 25, 1941 to Mar. 3, 1942; C and D, storage from Oct. 25, 1941 to June 3, 1942. Vertical columns 1, 2, 3, 4, 5 are in order: Irish Cobbler, Carman No. 3, Green Mountain, Houma, Katahdin; the other varieties are: White Rural (No. 6), and Sebago (No. 7), in rows A and C; Russet Rural (No. 6), Warba (No. 7), and Blue Mercer (No. 8), in rows B and D.

TABLE III
SUGAR CONTENT (MILLIGRAMS PER CC. OF JUICE) OF POTATO TUBERS STORED AT 5° C. (41° F.)

Variety	Reducing sugar												Sucrose											
	Storage started Oct. 25						Storage started Dec. 1						Storage started Oct. 25						Storage started Dec. 1					
	Days of storage						Days of storage						Days of storage						Days of storage					
	0	16	30	59	122		0	16	30	60	127		0	16	30	59	122		0	16	30	60	127	
Carman No. 3	0.0	0.0	1.2	4.4	1.8		0.0	0.0	2.5	6.1	1.3		1.3	5.6	4.9	4.5	2.5		1.1	7.1	4.9	5.6	2.2	
Russet Rural	0.0	0.0	2.6	3.8	2.6		0.0	0.1	3.0	6.5	3.3		2.4	6.1	5.3	3.1	2.3		1.1	9.3	5.7	5.5	2.8	
Number Nine	0.0	0.0	4.3	8.1	3.5		0.0	0.0	4.0	4.6	0.7		1.3	11.2	8.8	0.4	3.8		0.5	13.8	9.0	5.0	3.0	
White Rural	0.0	0.0	4.3	5.6	2.8		0.0	0.0	4.8	7.7	2.7		1.8	8.5	7.8	6.5	3.5		0.5	6.9	6.5	6.8	2.4	
Neverblight	0.0	1.8	4.7	6.9	4.0		0.0	1.3	5.2	6.6	1.7		4.1	9.9	7.6	7.2	4.3		3.3	8.7	8.1	7.8	5.1	
Rural New Yorker	0.0	0.0	4.8	6.7	3.7		0.0	0.3	7.3	9.1	3.0		2.3	7.9	8.8	6.2	3.8		0.8	9.9	7.1	6.3	3.6	
Katahdin	0.0	0.0	3.0	5.7	7.4		0.0	0.0	5.5	8.8	7.6		1.0	5.9	4.4	4.0	5.1		0.5	6.1	4.0	5.3	4.9	
Chippewa	0.0	0.0	3.4	8.4	7.7		0.0	0.1	4.4	11.9	9.7		0.3	11.7	8.5	6.0	3.4		0.1	10.7	7.4	8.0	5.6	
Arran Banner	0.0	0.7	5.5	10.1	5.9		0.0	1.0	0.3	10.0	4.4		2.1	9.1	8.4	8.4	2.6		0.6	7.7	6.9	4.7	2.7	
Spaulding Rose	0.0	4.0	4.9	8.9	7.3		0.0	0.8	7.3	10.4	8.5		2.9	13.4	12.4	10.8	7.4		1.8	14.2	13.3	9.9	6.9	
Early Ohio	0.0	0.1	4.3	8.6	8.2		0.0	0.0	8.9	11.3	8.5		2.4	12.9	11.5	8.0	6.1		1.5	14.0	12.1	10.6	7.3	
Blue Victor	0.0	0.4	4.7	9.3	5.6		0.0	0.5	7.2	14.5	8.5		1.6	10.6	8.7	6.8	3.7		1.2	11.3	8.6	8.2	8.2	
Selago	0.0	0.2	7.2	9.2	8.2		0.0	0.4	9.3	9.7	8.0		0.3	14.3	8.7	4.5	7.7		0.3	16.1	11.3	9.7	10.0	
Irish Cobbler	0.0	2.2	7.4	9.4	9.9		0.0	2.8	10.4	10.9	12.2		1.6	10.1	7.0	6.5	5.5		1.3	12.2	8.9	13.6	12.8	
Green Mountain	1.1	3.1	7.3	9.4	11.4		0.0	7.0	11.0	12.2	12.2		2.5	17.4	14.3	16.2	6.9		2.9	17.0	18.0	15.1	11.6	
Bliss Triumph	0.0	1.6	4.8	10.6	16.9		0.0	3.0	8.7	13.6	16.8		2.8	14.6	11.2	9.2	8.1		1.8	19.5	19.2	22.7	18.7	
Houma	0.5	2.0	8.2	14.0	9.2		0.0	3.4	10.2	19.5	12.0		2.6	7.7	5.4	3.3	3.7		2.1	6.9	3.1	6.4	4.4	
Warba	0.0	1.7	8.1	13.9	10.6		0.0	4.9	13.8	19.6	13.7		2.4	11.9	10.0	7.5	6.6		1.8	10.1	8.1	9.2	5.2	
Blue Mercer	0.2	2.5	11.9	15.2	13.2		0.0	4.5	16.7	21.6	15.1		2.8	9.9	6.2	6.1	5.9		2.1	9.4	4.9	11.8	6.9	
Total	1.8	17.3	102.6	168.2	139.9		0.0	30.1	151.3	214.6	149.9		38.5	198.7	159.9	134.2	92.9		25.3	210.9	167.1	172.2	124.3	

the October 25 series the total for the 19 varieties at 59 days was 168.2 and at 122 days 139.9, the difference being 28.3; the corresponding values for the December 1 series after 60 and 127 days of storage were 214.6 and 149.9, the difference being 64.7. The analysis of variance of the data in Table III is given in Table IV, from which, line 7, it is seen that the error variance for reducing sugar was 0.77. The minimum difference for significance between the totals of 19 items is $\sqrt{0.77 \times 19 \times 2 \times 2} = 10.8$ (8, p. 49). This value was exceeded in both of the comparisons of the totals for 59 to 60 days and 122 to 127 days, i.e. 28.3 and 64.7, showing that the reducing sugar decreased in storage at 5° C. after reaching a maximum at 59 to 60 days' duration.

TABLE IV
ANALYSIS OF VARIANCE OF DATA IN TABLE III

Source of variation	Degrees of freedom	Variance	
		Reducing sugar	Sucrose
Varieties	18	54.58	61.48
Starting dates	1	70.94	30.08
Durations	4	702.88	455.48
Var. $\times$ Dates	18	3.08	9.70
Var. $\times$ Durat.	72	8.96	58.58
Dates $\times$ Durat.	4	13.79	10.93
Error	72	0.77	1.96

Sucrose. The sucrose values for storage at 5° C. are shown in Table III, columns 12 to 21. Starting from low values, the sucrose rose rapidly during the first 16 days, reaching a maximum at that time, and then receding throughout the storage period, the lowest value occurring at the last sampling period. Since the error variance for sucrose was found to be 1.96, line 7 in Table IV, the required difference between totals of 19 items is $\sqrt{1.96 \times 19 \times 2 \times 2} = 17.3$. This value is exceeded in all comparisons between the totals of adjacent columns in Table III, columns 12 to 21, except between the values for 30 and 60 days in the December 1 series.

Comparison with previous year's results. In order to obtain a comparison of the sugar values in the two series of tests, i.e., with the crops of 1940 and 1941 (the data for the first year, 6, p. 300, not being extensive enough to be comparable), Table V was prepared. Advantage was taken of the facts that 16 of the varieties were the same in the two years, and that the duration periods at 5° C. up to and including the 59-day sample were approximately the same. The separate sugar analytical values (mg. per cc. of juice) for each of the varieties at each of the three duration periods were added for each of the four sampling dates and the values are entered in the appropriate cells of Table V. The entries are sums of 16 analyses, one from each of 16 varieties.

Table V shows both similarities and dissimilarities in the sugar values for the samples taken at different dates in the two years. The totals for the separate years are quite similar, 589.0 vs. 555.5 for reducing sugar, and 989.2 vs. 958.6 for sucrose, respectively for 1940 and 1941, these differing by only 3 to 6 per cent. The reducing sugar data for the sample of October 25, 1940, has its almost exact counterpart in the sample of December 1, 1941. Also, the samples on December 24, 1940 and October 25, 1941 are quite similar as to the amounts of reducing sugar at the corresponding intervals. In the case of sucrose the October 25 samples from the two years

TABLE V
COMPARISON OF SUGAR VALUES OF SAMPLES OF THE SAME
VARIETIES IN DIFFERENT YEARS

Sampling date		Combined sugar values (mg. per cc. of juice) for the same 16 varieties at each sampling date									
Year	Month and day	Reducing sugar				Total	Sucrose				Total
		Days of storage					Days of storage				
		o	16-18	30-32	55-59		o	16-18	30-32	55-59	
1940	Oct. 25	1.2	26.9	142.2	178.1	589.0	31.5	179.5	131.1	106.4	989.2
	Dec. 24	0.0	21.4	88.9	130.3		38.2	153.1	166.9	182.5	
1941	Oct. 25	1.3	14.4	81.7	134.9	555.5	33.5	167.6	137.4	118.0	958.6
	Dec. 1	0.0	25.3	122.5	175.4		22.3	180.2	148.2	151.4	

agree closely, but neither of the samples in 1941 showed a type of sucrose development corresponding to that of December 24, 1940.

It is thus seen that while the amounts of sugar found in the juice of these 16 varieties have been about the same in these two years, the progressive change with time in storage has varied with the sampling date. When storage at 5° C. (41° F.) was started on the same date, i.e., on October 25, the subsequent rate of sugar development was the same for sucrose and quite different for reducing sugar in the results of the two years. The progressive change in reducing sugar was the same in the two years only when different starting dates were employed (lines 1 and 4, columns 3 to 6, Table V).

IMPORTANCE OF THE DATE FOR STARTING STORAGE

In the previous year's test (7) it was noted that the late starting date, December 24, resulted in a slower rate of increase in reducing sugar, and finally in a higher amount of sucrose in the juice of the tubers. However, only two starting dates were employed, i.e., October 25 and December 24, and in the present tests this point was reinvestigated by the use of four

starting dates, August 25, October 25, December 1, and December 24. The tubers used for this test were from the varieties Irish Cobbler, Carman No. 3, Green Mountain, Bliss Triumph, and Katahdin.

The results for reducing sugar are shown in Figure 3 and for sucrose in Table VI. In preparing the reducing sugar graph, Figure 3, each total of the five varieties at each duration period was expressed as a percentage of the average of all the column totals at all duration periods. Figure 3 shows the unexpected result that the rate of change in reducing sugar content

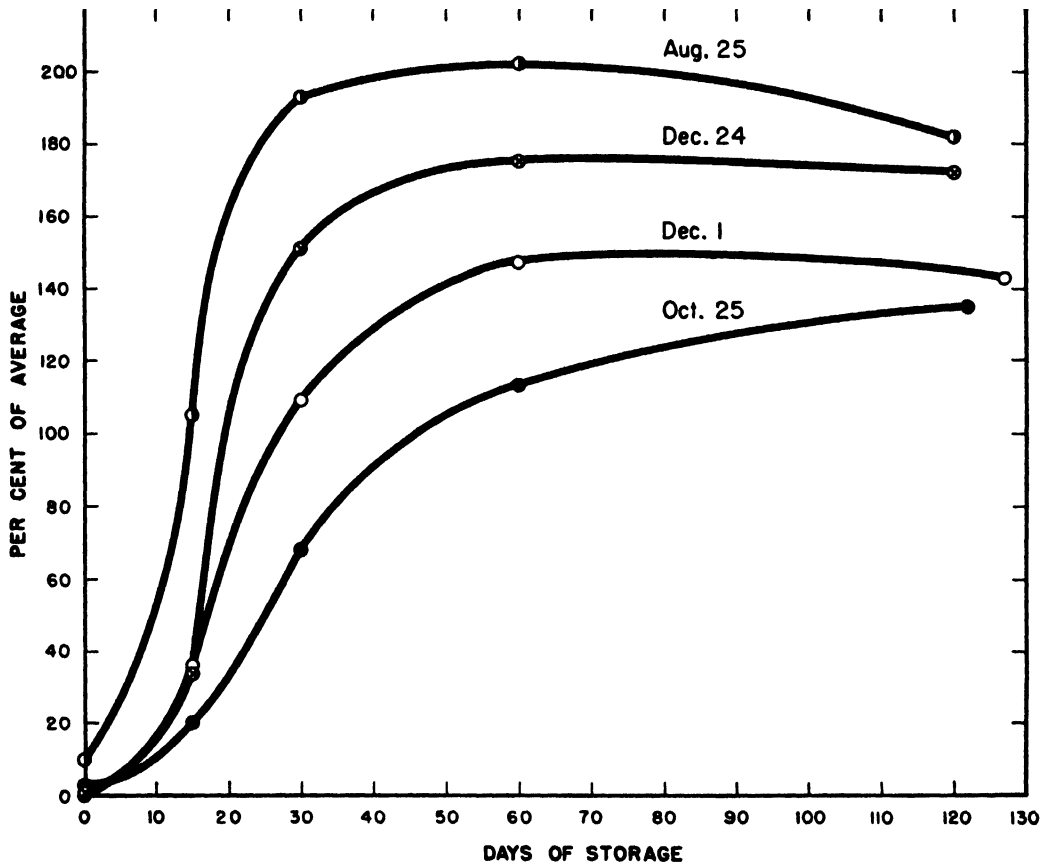


FIGURE 3. Changes in reducing sugar content of potato tubers during storage at 5° C. (41° F.) when the storage was started at different intervals after harvest (which was Aug. 12-15). Storage was at room temperature from harvest until dates shown.

was not progressive from the earliest to the latest date. The most rapid increase in reducing sugar, and the highest final amount, were shown by the lots with the earliest starting date, August 25. From that date onward the change was progressive but in the reverse order of dates, the slowest increase and lowest final amount of reducing sugar being shown by the lots with storage starting October 25; the December 1 and December 24 starting dates then followed in regular order with increasing rates and amounts.

The order for decreasing amounts of reducing sugar was, therefore, August 25, December 24, December 1, and October 25. The reasons for such a change in behavior with change in time at which cold storage was started are not apparent, but confidence is had in the reality of this response, not only on account of the uniformity shown by the curves in Figure 3, but also on account of the uniformity in the progressive changes in the sucrose values which were, of course, obtained from the same samples of juice that furnished the reducing sugar data. The sucrose results are shown in Table

TABLE VI
EFFECT OF STARTING DATE ON THE SUCROSE CHANGES DURING
STORAGE AT 5° C. (41° F.)

Date storage was started, 1941	Combined sucrose analytical values (mg. per cc. of juice) of 5 varieties (Irish Cobbler, Carman No. 3, Green Mountain, Bliss Triumph, and Katahdin)					
	Days of storage at 5° C. (41° F.)					
	0	15-16	30	59-60	120-122	Total
Aug. 25	15.2	43.8	46.3	30.9	20.4	156.6
Oct. 25	9.2	53.6	41.8	40.4	28.1	173.1
Dec. 1	7.6	61.9	55.0	62.3	50.2	237.0
Dec. 24	9.3	71.4	75.6	75.0	82.5	313.8

VI. The order for increasing amounts of sucrose was now in the exact order of dates: August 25, October 25, December 1, and December 24.

The results for the two years for which analyses on the effect of starting date were obtained agree in showing that the increase in reducing sugar was rapid when the tubers were placed in cold storage at an early date after harvest, and that the gain in sucrose was increasingly rapid at progressively later dates of starting storage. But the most favorable starting time for retarding reducing sugar development was not shown by these tests, a late date being indicated in the first year's test, and an intermediate date in the second.

Possibly the conditions for holding the tubers from the time of harvest until the commencement of cold storage influence the rate of reducing sugar development.

RANKING OF VARIETIES WITH RESPECT TO SUGAR

In the last two years of these tests, sugar analyses from 16 varieties have been obtained at two different starting dates in each year, and after varying periods of storage duration at 5° C. (41° F.). The four starting dates were October 25 and December 24 in 1940, and October 25 and December 1 in 1941. The amounts of sugar formed in the tubers have been different at different intervals and at the end in the four tests. These differences between starting dates have been small in some comparisons

and large in others. But whether the actual amounts of sugar have been relatively large or small in each test, the question is whether the varieties have kept pace with each other, and whether a given variety tended to reach about the same rank among the 16 varieties in each test.

Table VII shows the rank of each variety with regard to relative amounts of reducing sugar and sucrose in each of the four experiments. In summarizing the analyses for preparing the ranks, the weighted average for all of the data for all duration periods of 14 days or more was obtained for each variety at each starting date. The method of computing was illustrated previously (7, p. 221) and was the same in the present case except that the values at the start of storage (at 0 days) were not included. These weighted averages indicate the sugar level which a given variety could reach and maintain at that temperature.

Of the first six varieties in the list in Table VII all, except possibly Neverblight, are in the Rural group, and since in each series the varieties within the Rural group had sugar values differing from each other by a small amount, the number indicating the rank could vary considerably as the result of only a small change in sugar content. This explains the apparently considerable variation in the rank number with different samples in the first six lines of Table VII. The rank values for the reducing sugar of Chippewa and those for the sucrose of Katahdin showed variable values between samples, but in the rest of Table VII the rank numbers are surprisingly uniform.

A lack of correspondence between reducing sugar and sucrose amounts in the juice of a given variety is also shown in Table VII. For example, among these varieties Blue Mercer is perhaps the highest in reducing sugar, but it has only a medium amount of sucrose, while Neverblight, which is one of the three lowest in reducing sugar, is in a medium position with regard to sucrose. Warba is outstandingly high in reducing sugar but is only slightly below Neverblight in sucrose.

The answer to the question whether these lists in Table VII indicate a significant correlation between the ranks within the samples on different dates was first approached by determining the rank correlation coefficients according to the method described by Crist (5). The value of ρ was found and from this the value of r was computed by the formula given on page 595 of Crist's article. These correlation coefficients are shown in the summary at the bottom of Table VII in the first line of each of the cells which are above the diagonal of empty cells. It is seen that the coefficients vary from 0.9038 to 0.5947 for reducing sugar, and from 0.8526 to 0.4788 for sucrose. The t values (5, p. 595) were then found, and these are entered in the second line in each cell. Since there were 16 pairs of values in each comparison, the degrees of freedom are 14, and the t value corresponding to a probability of 0.05 is 2.145 (8, p. 248). This value is reached in all cases

except that for sucrose in the comparison October 25, 1940 vs. October 25, 1941, the value here being 2.039.

Since not only the ranks but also the actual sugar values which estab-

TABLE VII
CORRELATIONS WITHIN VARIETIES BETWEEN SAMPLES TAKEN
AT DIFFERENT TIMES

Variety	The rank of the varieties (No. 1 = lowest sugar)							
	Reducing sugar				Sucrose			
	Oct. 25 1940	Dec. 24 1940	Oct. 25 1941	Dec. 1 1941	Oct. 25 1940	Dec. 24 1940	Oct. 25 1941	Dec. 1 1941
Carman No. 3	4	7	1	2	6	5	2	1
Russet Rural	3	3	2	4	7	4	1	2
Neverblight	1	2	6	3	9	8	10	7
Number Nine	6	8	7	1	4	2	11	5
White Rural	5	6	3	5	1	3	4	4
Rural New Yorker	8	4	4	6	2	1	5	6
Chippewa	2	1	10	9	13	15	5	8
Katahdin	9	10	7	7	10	6	3	2
Blue Victor	6	5	9	12	3	10	8	10
Early Ohio	12	12	11	9	12	11	13	13
Irish Cobbler	10	9	12	12	15	13	9	14
Spaulding Rose	16	15	10	8	8	9	15	12
Green Mountain	14	11	13	14	16	14	16	15
Bliss Triumph	13	13	15	11	14	16	14	16
Warba	11	16	14	15	11	12	12	9
Blue Mercer	15	14	16	16	5	7	7	11

Summary of correlation coefficients and *t* values

As determined from the rank values given above

		Reducing sugar				Sucrose			
		Oct. 25 1940	Dec. 24 1940	Oct. 25 1941	Dec. 1 1941	Oct. 25 1940	Dec. 24 1940	Oct. 25 1941	Dec. 1 1941
As determined from the original data	Oct. 25 1940		.9038 7.913	.7384 4.097	.6862 3.530		.8526 4.849	.4788 2.039	.6047 2.841
	Dec. 24 1940	.7883 4.794		.6966 3.634	.5947 2.781	.8099 5.166		.5814 2.674	.7782 4.636
	Oct. 25 1941	.6431 3.142	.6690 3.368		.8720 6.667	.6860 3.530	.5310 2.345		.8278 5.520
	Dec. 1 1941	.6861 3.529	.6836 3.504	.6818 3.487		.7301 3.997	.8591 6.282	.7613 4.394	

Note: First value of each pair is the correlation coefficient and the second is the *t* value. Values of *t* greater than 2.145 indicate significance. Entries above the diagonals were computed from the rank values, and those below the diagonals from the analytical values for sugar.

lished the ranks were available, the sugar values themselves were used to determine the interclass correlation coefficients and corresponding *t* values

by the method described by Paterson (8, p. 125). The results are shown in the cells below the diagonals of empty cells in the summary at the bottom of Table VII. The correlation coefficients vary from 0.7883 to 0.6431 for reducing sugar, and from 0.8591 to 0.5310 for sucrose. The *t* values obtained in this way are all higher than the required value of 2.145.

In the previous report (7, p. 244) the 25 varieties were classified into groups on the basis of the amounts of reducing sugar and sucrose in the juice. In the present test 16 of the varieties were the same as those used in the previous year, and this made possible a grouping based on the combined data for the two years. The ranges of sugar values for establishing the three classes, high, medium, and low sugar, were changed from that used in the previous report, so as to indicate the actual amounts of sugar in milligrams per cc. of juice. The results are shown in Table VIII. The

TABLE VIII
GROUPING OF VARIETIES ON THE BASIS OF SUGAR CONTENTS OF JUICE

		Sucrose		
		High, over 10 mg./cc.	Medium, 7 to 10 mg./cc.	Low, less than 7 mg./cc.
Reducing sugar	High, over 10 mg./cc.	Green Mountain* Bliss Triumph* Irish Cobbler* Eureka Delaware Axtell's Bugless	Spaulding Rose* Warba* Blue Mercer* Russet Burbank Pride of Multnomah	
	Medium, 7-10 mg./cc.	Chippewa* Early Rose	Blue Victor* Early Ohio* Early Six Weeks	Katahdin* Sir Walter Raleigh
	Low, less than 7 mg./cc.		Neverblight*	Carman No. 3* Russet Rural* Number Nine* White Rural* Rural New Yorker* Heavyweight

* The positions of starred varieties are based on averages for two years' results; with other varieties for one year only; each year with samples at two different storage starting dates.

data for the nine varieties included in the previous year but not in the present test were re-computed on the basis of the sugar classes shown in Table VIII and were entered in their proper places. Differences in the position of any of these nine varieties in Table VIII as compared with the table for the previous year are therefore due merely to the differences set for the limits of sugar values.

A comparison of the grouping in Table VIII with that of the previous year (7, p. 244) shows that only four changes in the placement of the varie-

ties were required by the additional year's results. Irish Cobbler was transferred upward one space in the reducing sugar class, but the actual reducing sugar value was 10.1 which is barely beyond the margin between classes. Chippewa also had to be moved upward one space, and the actual value in this case was 7.1, again quite close to the dividing line. With Early Ohio a downward shift of one space was required, but here again the reducing sugar was 9.9, a border-line value. The largest change in position from last year's table is that for Blue Victor for which there is a shift of two spaces. But this is due to a difference in the limits set for the groups in the two years. If the previous year's data are computed upon the basis of that shown in Table VIII, the values for Blue Victor are 7.1 and 7.6 mg. per cc., respectively for reducing sugar and sucrose, and so last year's values would place Blue Victor in the cell in which it now stands in Table VIII.

DE-SUGARING TESTS

Tubers that had been stored at low temperatures for 60 to 150 days were transferred to a room maintained continuously at 27° C. (80.6° F.) in order to note the rate at which the sugar content would decrease, as shown by the analysis of samples removed from the de-sugaring room at intervals of 10, 20, and 40 days. The results are shown in Tables IX and X.

Reducing Sugar Changes in De-sugaring

De-sugaring from 7° C. Although 7° C. was a favorable temperature for continuous storage, some of the varieties formed reducing sugar in amounts somewhat in excess of that for obtaining potato chips of good color. A test was made of the rate of de-sugaring after continued storage at 7° C. The results are shown in columns 4 to 9, lines 1 to 12, in Table IX. On examining the values in the lines opposite 10 and 20 days, and considering that a reducing sugar value of 3.0 or less is indicative of good color of potato chips, it is seen that de-sugaring at least within 20 days of warm temperature storage was obtained with Irish Cobbler and Early Ohio. With Chippewa de-sugaring occurred satisfactorily when the tubers were placed in cold storage at the earliest date, August 26, but when the storage at 7° was delayed until the two later dates the reducing sugar loss was rapid if the previous duration at 7° was only 60 days, but was too slow if the duration was 150 days. The varieties Russet Rural and Rural New Yorker were included in this experiment but the values are not entered in Table IX, since with these varieties only small amounts of reducing sugar were formed at 7° after either 60 or 150 days of storage, and the small amount formed disappeared in all cases after a ten-day period of storage at 27° C. Tubers of Spaulding Rose were also tested (data not shown in Table IX) at the October 25 starting date at 7°, and, while the reducing sugar values were

too high after the 150-day storage period at 7° C., de-sugaring to a value below 3.0 occurred within ten days.

The results show that 7° C. (44.6° F.) was a favorable temperature for storage over extended periods, not only because the increase in reducing sugar was not excessive, but also because prompt loss of the reducing sugar

TABLE IX

DE-SUGARING RESULTS: SUGAR CHANGES IN TUBERS TRANSFERRED TO 27° C. (80.6° F.) AFTER HAVING BEEN STORED PREVIOUSLY AT 7° C. (44.6° F.) AND 5° C. (41° F.)

Temp.	Variety	No. days at 27° C.	Milligrams of sugar per cc. of juice											
			Reducing sugar						Sucrose					
			Storage at low temperature started on:											
			Aug. 26		Oct. 25		Dec. 24*		Aug. 26		Oct. 25		Dec. 24*	
			No. of days at low temperature before de-sugaring was started											
			60	150	60	150	60	150	60	150	60	150	60	150
7° C.	Irish Cobbler	0	6.8	6.1	3.7	4.0	3.1	4.2	3.3	2.9	5.0	4.2	6.6	8.1
		10	2.3	1.3	—	3.4	0.8	2.9	2.9	3.4	—	6.4	4.7	8.7
		20	0.9	0.0	0.0	2.4	0.0	1.3	3.0	3.1	3.6	7.4	5.1	7.6
		40	0.0	0.0	0.0	0.3	0.3	0.1	2.3	3.1	4.4	11.2	8.4	12.3
	Early Ohio	0	4.4	3.7	2.5	1.5	2.8	2.2	4.5	2.8	4.3	2.8	5.3	3.6
		10	1.2	0.0	—	0.0	0.0	0.0	3.4	3.6	—	4.8	4.6	4.6
		20	0.0	0.0	0.0	0.0	0.0	0.0	4.3	2.8	3.8	4.1	3.4	7.7
		40	0.0	0.0	0.0	0.0	0.0	0.0	3.6	2.0	2.3	7.0	2.1	9.6
	Chippewa	0	5.0	4.9	1.7	7.2	2.1	5.6	2.9	1.6	2.9	4.8	2.2	4.5
		10	2.4	1.7	—	6.0	2.3	4.8	2.3	2.9	—	6.5	4.7	9.0
		20	1.2	0.2	0.1	6.0	0.9	4.3	2.6	3.8	4.0	9.3	6.8	13.4
		40	0.0	0.0	0.0	4.5	0.2	1.4	1.8	5.9	4.1	16.1	13.4	19.3
5° C.	Irish Cobbler	0	14.0	11.8	7.3	9.3	6.8	10.4	4.6	4.6	9.4	4.9	19.3	19.6
		10	5.7	2.3	—	3.6	4.9	4.5	2.8	3.3	—	4.5	10.5	14.0
		20	2.7	1.3	4.2	2.7	2.4	2.4	3.6	3.4	4.3	6.1	7.6	12.9
		40	1.2	0.0	0.0	2.5	1.4	2.1	2.9	4.4	4.6	8.9	7.3	14.5
	Carman No. 3	0	4.9	2.3	5.2	0.0	8.2	1.5	4.2	2.3	5.1	3.3	9.0	4.6
		10	0.0	0.0	—	0.0	0.0	0.1	3.4	2.9	—	2.2	2.3	7.3
		20	0.0	0.0	0.0	0.0	0.0	0.0	3.0	2.4	2.3	2.6	2.0	7.3
		40	0.0	0.0	0.0	0.0	0.0	0.0	1.8	1.6	0.0	5.4	2.5	5.2
	Russet Rural	0	8.1	2.7	1.5	1.5	7.3	4.1	5.1	2.6	3.3	3.0	7.9	7.9
		10	1.2	0.1	—	0.0	0.0	1.1	3.9	4.2	—	6.1	4.3	11.7
		20	0.5	0.0	0.0	0.0	0.0	0.0	4.1	3.9	3.3	5.4	4.3	7.3
		40	0.0	0.0	0.0	0.0	0.0	0.0	2.6	2.9	1.6	9.7	6.5	8.4
	Green Mountain	0	16.5	14.6	9.9	11.8	11.4	14.2	6.8	6.1	13.7	5.0	15.1	12.2
		10	6.2	3.6	—	4.1	3.6	7.5	5.0	5.2	—	6.7	7.8	9.9
		20	3.8	2.9	1.9	4.9	2.8	4.8	4.4	4.6	4.9	7.4	7.5	13.2
		40	1.5	0.0	0.3	2.9	0.3	3.7	3.3	5.7	5.2	12.7	10.4	16.9
	Early Ohio	0	13.9	10.2	7.5	6.3	9.6	9.5	6.6	4.6	6.8	4.2	7.2	6.6
		10	3.1	1.4	—	0.0	2.3	1.8	3.1	4.0	—	5.1	4.7	5.7
		20	0.3	0.2	0.0	0.0	1.2	1.5	4.2	4.0	4.1	4.3	4.6	6.0
		40	0.0	0.0	0.0	0.0	0.0	0.0	3.4	2.9	2.6	5.1	4.7	8.6
	Chippewa	0	15.7	13.5	10.3	12.2	12.3	10.0	4.9	4.0	4.8	3.7	7.5	10.6
		10	7.1	5.6	—	9.8	6.1	9.3	2.1	2.6	—	6.9	7.0	10.1
		20	4.5	4.2	2.6	7.6	3.1	6.4	2.6	3.3	2.8	8.6	8.0	14.3
		40	1.4	1.9	1.1	4.3	2.6	3.6	1.8	4.9	3.2	12.7	8.8	21.0

* Dec. 1 for the lots at 7° C.

formed at this temperature ensued when the tubers were transferred from 7° to 27° C.

De-sugaring from 5° C. Although continuous storage at 5° C. (41° F.) results in the accumulation of too much reducing sugar for potato chips of good color, temperatures in this region are commonly used commer-

cially in order to inhibit sprouting, the tubers being removed from such low temperatures a few weeks before it is planned to use them, and being placed at a warm temperature for de-sugaring. However, managers of such plants have reported that occasionally a given lot of tubers may fail to de-sugar properly, and so the schedule of production is upset.

The present tests on de-sugaring from 5° C. (and also from 1° C.) were included to obtain information on the conditions of previous storage which might lead to a failure to de-sugar at a warm temperature. Six varieties were used, with three different dates for starting the cold storage, and with storage duration periods of two and five months. The results are shown in Table IX, lines 13 to 36, columns 4 to 9. Again examining the data for values of 3.0 or less, after 20 days of de-sugaring at 27° C., it is found that no failure in de-sugaring occurred with the varieties Irish Cobbler, Carman No. 3, Russet Rural, or Early Ohio at any starting date or duration of cold storage. Only the varieties Green Mountain and Chippewa showed reducing sugar values above 3.0 after 20 days at 27° C., and these failures were associated either with the early starting date, August 26, or with the 150-day duration periods at the low temperature with the other two starting dates. These results, therefore, while not furnishing a complete answer as to the cause of failure of certain lots of tubers to de-sugar, suggest that the time of starting storage, and particularly the variety of tubers, and the length of time at which the tubers were stored previously at the low temperature, are important factors.

De-sugaring from 1° C. Storage space was available for a test of three varieties at 1° C. (33.8° F.), and the duration at this low temperature before start of de-sugaring was varied from one to four months. The results for reducing sugar are shown in Table X, columns 4 to 6. Failure to de-sugar to 3.0 was obtained with the varieties Irish Cobbler and Green Mountain at both starting dates and at all duration periods of the previous cold storage, except with Irish Cobbler at the last starting date and shortest storage duration. The values in column 6 for White Rural show the considerable margin of superiority of this variety over the other two at this temperature. Although the condition is favorable for the accumulation of reducing sugar, White Rural not only formed much less sugar than the other varieties, but this sugar was lost rapidly at the temperature of 27° C., so that chips of good color were obtained within 20 days, sometimes within 10 days, after the tubers were placed under de-sugaring conditions.

Columns 4 and 5 in Table X may also contribute a suggestion as to one of the causes of occasional failure of a lot of tubers to lose sugar in storage at warm temperature. Perhaps the previous cold storage had been at too low a temperature for too long a period. Possibly even in a room with a certain average temperature, there could be portions at the edges and corners with an appreciably lower temperature.

TABLE X
DE-SUGARING RESULTS: SUGAR CHANGES IN TUBERS TRANSFERRED TO 27° C.
AFTER HAVING BEEN STORED PREVIOUSLY AT 1° C.

Storage started	Days		Milligrams of sugar per cc. of juice					
	At 1° C. (33.8° F.)	At 27° C. 80.6° F.)	Reducing sugar			Sucrose		
			Irish Cobbler	Green Moun- tain	White Rural	Irish Cobbler	Green Moun- tain	White Rural
Aug. 26	30	0	12.8	17.2	7.3	28.4	28.7	30.4
		10	6.7	9.3	0.9	16.9	9.9	12.4
		20	5.3	4.7	0.1	12.5	5.3	5.5
		40	3.0	1.9	0.0	5.2	3.1	3.4
	60	0	27.0	38.0	15.5	24.4	18.2	28.0
		10	14.1	9.9	4.7	16.4	5.9	18.3
		20	7.7	6.8	0.3	9.2	4.1	4.7
		40	4.1	1.3	0.0	4.2	3.2	2.8
	120	0	36.9	35.0	11.9	19.1	25.0	21.1
		10	—	—	—	—	—	—
		20	11.7	8.1	0.9	7.8	10.4	8.6
		40	3.1	2.6	0.1	4.1	4.4	2.5
Dec. 24	30	0	9.9	12.8	4.1	33.3	27.5	25.4
		10	4.5	6.6	0.0	25.6	19.8	12.1
		20	2.4	5.9	0.0	20.4	15.0	7.9
		40	0.0	2.7	0.0	13.4	11.8	4.9
	60	0	22.5	29.3	14.0	32.9	25.9	31.5
		10	14.9	14.6	5.9	21.9	20.1	18.3
		20	7.7	8.0	1.5	8.7	10.5	6.4
		40	3.5	6.4	0.0	9.7	10.6	5.1
	120	0	20.6	26.7	14.6	34.3	34.1	22.5
		10	17.2	13.6	5.5	29.0	17.9	18.6
		20	9.1	5.7	0.0	17.3	12.1	7.9
		40	3.9	4.7	0.0	10.3	14.3	7.4

Sucrose Changes in De-sugaring

Changes in sucrose after transfer from a low temperature to 27° C. (80.6° F.) were much less extensive than changes in reducing sugar, and in many cases "de-sugaring" of sucrose did not occur but instead there was a large increase of sucrose during storage at the warm temperature.

The values for the lots transferred to 27° C. after storage for 60 to 150 days at 7° C. (44.6° F.) are shown in Table IX, columns 10 to 15, lines 1 to 12. With the lots in which storage started on August 26, subsequent storage at 27° C. had little effect upon the sucrose content over the 40-day period; this was true also of the October 25 series when the warm temperature storage was started after 60 days at 7°. However, when the storage period was 150 days at 7°, a continuous increase in sucrose occurred in

storage at 27° C., the gains being from 50 to 300 per cent or more, over the 40-day interval. This increase in sucrose at 27° C. after 150 days' storage at 7° was obtained also with the Russet Rural and Rural New Yorker lots included in the test but the data of which are not shown in Table IX.

The sucrose analyses of the lots transferred to 27° C. after storage at 5° C. (41° F.) are shown in Table IX, lines 13 to 36, columns 10 to 15. Here, again, the sucrose content remained about the same over the 40-day period at 27° C. when the starting date was August 26. As for the values after the other starting dates, the response was quite different among the varieties. The increase in sucrose after the 150-day duration period when the previous temperature was 7° C. was not invariably found in the results obtained when the transfer to 27° C. was made from 5° C. The progressive increase in sucrose after the 150-day interval occurred among the lots in the October 25 series with all varieties except Early Ohio, column 13, lines 13 to 36; however, in the December 24 series, column 15, this increase in sucrose after the 150-day interval was obtained only with Chippewa. The 60-day lots in the October 25 series showed progressive decreases in sucrose with all varieties, column 11, lines 13 to 36; but this result was obtained with the 60-day lots of the December 24 series only with Irish Cobbler and Carman No. 3.

The sucrose changes at 27° C. after previous storage at 1° C. (33.8° F.) are shown in Table X, columns 7 to 9. Here we find a uniform response, a progressive decrease in sucrose after the tubers were placed at the warm temperature. In this case it is permissible to use the term de-sugaring. The difference between the two storage temperatures, 5° C. and 1° C., in their effect upon the sucrose changes after transference to 27° C. are striking, as is seen by comparing the complicated changes in values in Table IX, columns 10 to 15, with the quite uniform behavior found in Table X, columns 7 to 9.

POTATO CHIPS

With each of the samples in this experiment, slices from the tubers were obtained, and potato chips were prepared. The color of the chips was then compared with the reducing sugar value of the juice from the same sample of tubers. The results agree with those of the previous tests (6, 7) in showing a correlation between reducing sugar content and potato chip color. Reducing sugar values of 3.0 mg. per cc. of juice, or lower, were associated with light colored chips, and at values of 5.0 or over the chip colors were distinctly too brown. No correlation was found between the sucrose content and color of potato chips.

SUMMARY

Tubers of 19 varieties of potatoes (*Solanum tuberosum* L.) were harvested August 12 to 15, 1941 and were placed in storage at temperatures

of 5°, 7°, and 8° C. (41°, 44.6°, 46.4° F.); samples were removed at intervals varying from 16 to 221 days, the reducing sugar and sucrose contents of the juice were determined, and potato chips were prepared.

The color of the potato chips was correlated with the reducing sugar content, light colored chips being obtained when the reducing sugar value was 3.0 mg. per cc. of juice, or lower, and chips definitely too dark in color with a value of 5.0.

With all varieties tested continuous storage at 5° C. (41° F.) led to the accumulation of reducing sugar in amounts higher than 3.0, and with some varieties in amounts of 10 to more than 20 mg. per cc. The varieties of the Rural group were outstanding in the capacity to maintain low sugar values; these in most cases showed sugar values not far above the limit of 3.0 mg. per cc.

Much lower reducing sugar values were obtained with continuous storage at 7° C. (44.6° F.), some varieties being held to reducing sugar values of 3.0 or less for periods of three to six months.

A still further lowering of reducing sugar values was obtained by storage at 8° C. (46.4° F.), the values at 8° C. being only about one-half of those for the corresponding samples at 7° C. Of the seven varieties tested at 8° C., all but Green Mountain, and possibly Houma, maintained reducing sugar values at 3.0 mg. per cc., or below, for periods of storage up to four months, and up to seven months with the three varieties of the Rural group tested in this experiment.

Sprouting also began much earlier and was more extensive at 8° than at 7° C. Photographs are given of tubers of several varieties after a storage period of five to seven months at these two temperatures. None of the seven varieties tested at 7° C. showed an undesirable amount of sprout development, and of eight varieties tested at 8° the sprout condition was still satisfactory with five of them after seven months of storage.

After tubers had remained in storage at 7° C. (44.6° F.) for periods of 60 and 150 days they were transferred to 27° C. (80.6° F.) for de-sugaring. This occurred readily, so that reducing sugar values of 3.0, or less, were obtained after an interval of 10 to 20 days with all varieties in all tests, except with Chippewa tubers stored at 7° C. for 150 days before transference to de-sugaring conditions.

De-sugaring at 27° C. from a previous storage temperature of 5° C. (41° F.) was at a satisfactory rate with all varieties except Green Mountain and Chippewa.

When the storage temperature previous to placing under de-sugaring conditions was 1° C. (33.8° F.), the accumulation of sugar had been so extensive that a reducing sugar value of 3.0 was not obtained within 20 days at 27° C. with either Irish Cobbler or Green Mountain, but was obtained with White Rural.

Sucrose values for the lots undergoing de-sugaring at 27° C. were also obtained. The sucrose changes were much less extensive than those for reducing sugar under these conditions. When the transfer was made from either 7° or 5° C., many instances were observed in which the sucrose did not decrease at 27° C., but showed a progressive increase to high sucrose values at the end of the experiment. When the transfer was from 1° C. to 27° C., however, the decrease in sucrose was uniform and progressive.

When tubers were stored at 5° C. (41° F.), and samples were removed at intervals, the reducing sugar values increased to a maximum at a storage duration period of about 60 days, and then declined to a lower value at the end of the test, 122 to 127 days from the start. With sucrose this maximum was attained much earlier, i.e., at about 16 days from the start of storage, and thereafter the sucrose values decreased until the end of the test.

The results of the previous year's and the present tests show that the rate of change of sugar during storage at 5° C., and the highest amount reached during storage, depend upon the time after harvest at which the cold storage is started. The results of the two years agree in showing that reducing sugar values increase more rapidly, and reach a higher level, if the storage is started soon after harvest, but there is no agreement as to the proper starting time to obtain the lowest rate and amount of reducing sugar, this condition being obtained with a late starting date (Dec. 24) in the previous year's test, and with an intermediate starting date (Oct. 25) in the present experiment. The two years' results agree in showing that low sucrose values are obtained by the earliest, and high values with the latest starting date.

While the amounts of both forms of sugar, and the rates of change with time in storage at 5° C., have been different in the four different samples in the two years' tests (two in each year and 16 varieties in each test), the varieties have kept pace with each other in each of the tests, and show essentially the same behavior with both series in each of two years. The rank of the varieties for each of the four sets of analyses was listed, and the six separate comparisons available in this way were made by computing the correlation by ranks, and also the interclass correlations from the original sugar data. These showed good correlation between samples of a given variety taken at different times, and indicate that the varieties have behaved essentially uniformly in the different tests in the two years.

A table was made showing a grouping of the varieties, based on the analytical values for sugar, into groups formed by the classes: high, medium, and low amounts of each of the two forms of sugar, reducing sugar and sucrose.

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AN ANALYSIS OF FACTORS CAUSING VARIATION IN SPORE GERMINATION TESTS OF FUNGICIDES. IV. TIME AND TEMPERATURE¹

R. H. WELLMAN² AND S. E. A. MCCALLAN

The factors in the study of variation in spore germination tests of fungicides previously examined were: I. Methods of obtaining spores (14), II. Methods of spraying (15), and III. Slope of toxicity curves, replicate tests, and fungi (12). In a continuation of this study of variation the factors time and temperature have been examined with particular attention being paid to the relative importance of these variables compared to the variable concentration or dose of the fungicide. The investigation of the factor time may be considered a continuation and expansion of a previous paper (13) which has shown the effect of time of counting on the toxicity of copper sulphate and of sulphur.

The linear relation between reciprocal of elapsed time and germination is shown and attention has been given to the rôles of time and temperature in the germination of spores in distilled water containing 0.1 per cent orange juice and the value of the LD₅₀ time-concentration curve for comparing fungicides is shown. A presentation of the effect of temperature on LD₅₀ values of various compounds on one fungus, and of one compound on different fungi is also given.

METHODS

The spore germination technique of evaluating fungicides described in the third paper of this series (12) and in previous publications was used. Germination counts were made after various time intervals; in earlier tests these time intervals varied in a logarithmic manner but after the linearity of the reciprocal function of time and probit germination had been established, the time interval varied by equal reciprocal units. Five temperatures were chosen from the facilities available to cover the range from 10° C. to 35° C. at intervals of from 5° to 8°.

The fungi used were: *Sclerotinia fructicola* (Wint.) Rehm., *Glomerella cingulata* (St.) Sp. & von S., *Alternaria solani* (Ell. & Mart.) Jones & Grout, Delaware strain (12, p. 50, footnote 5), and *Macrosporium sarcinaeforme* (Cav.). These fungi were grown under the conditions and the spores were obtained by the methods and used at the concentrations described previously (12, 14). In all spore germination tests, 0.1 per cent ultra-filtered

¹ A preliminary report of this paper was presented before the American Phytopathological Society, Philadelphia, Pa., December, 1940 (17).

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orange juice (12) was used to insure a high percentage of germination that would be uniform from day to day.

Compounds were selected to be representative of the general types that might be investigated as fungistatic agents (11). For the work with the variable time, two soluble metallic salts, copper sulphate and zinc chloride, two synthetic organic chemicals, and two insoluble copper fungicides, "Standard Laboratory Bordeaux" (1) and red cuprous oxide, were examined on each of the four test fungi. The examination of time was continued in the temperature experiments. The introduction of the variable temperature with the resultant five-fold increase in labor of counting made necessary the examination of only four chemicals ("Standard Laboratory Bordeaux," red cuprous oxide, copper zeolite, and a synthetic organic chemical) on two fungi (*Sclerotinia fructicola* and *Alternaria solani*).

Following the recommendations set forth previously (12), only two different dose ratios were employed. For the soluble metallic salts and the organic compounds in the examination of time, a dose ratio of 1.414, i.e., $\sqrt{2}$, was used in the test tube method (12). All other compounds were used at dose ratios of 2 and applied by the settling tower technique (15). For each investigation, the entire series of compounds and fungi were tested on the same day and each test was replicated on three different days. These experiments (14), or replicate tests (12), thus involved obtaining data for and construction of 1140 toxicity curves as a basis for the interpretations drawn regarding time and temperature.

LD values have been obtained by the use of logarithmic-probability paper (18) which was adopted from the system of probits devised by Bliss (2).

The use of the term "time" or "elapsed time" in the present paper is confined to the time lapse between the moment when the spores are placed in contact with the chemical and the moment when counts were made.

It should be mentioned that certain species of fungi, for example *Glomerella cingulata*, produce secondary spores that tend to confuse the results obtained in counts made after 24 hours. This production of secondary spores is sometimes great enough so that a smaller percentage of germinated spores will be recorded at the longer time intervals.

EFFECT OF TIME ON SPORE GERMINATION IN WATER

Before the effect of time on toxicity of a fungistatic agent could be determined, it was necessary to investigate the relation between time and germination of spores in distilled water plus 0.1 per cent orange juice. It will be remembered that at the start of such a test no spores are germinated, as time elapses more and more spores germinate. As germination is the measure of viability of spores, those spores which are not potentially viable can be determined only at the end of the experiment. When the population of spores concerned are not all potentially viable, a correction

must be made at the end of the experiment for the non-viable spores before a linear relation can be obtained. If germination reaches 98 per cent or more after 50 hours' elapsed time, a sufficiently accurate correction may be made by subtraction of the percentage non-viable spores at the end of the experiment from the observed percentage non-viable spores at any time of counting. A more accurate correction in this case, that holds for all percentage germination, may be obtained by the formula: per cent corrected germination = $100 (\text{per cent observed germination}) / \text{per cent germination after 50 hours}$.

The value of recording results in such a manner that a linear relationship can be obtained between the variables under consideration has been pointed out by Clark (6), Bliss (2), and others (7, 18). It is obvious that, when linear relations are possible, graphical extrapolation or interpolation from the data obtained is easier and more precise from a straight line than from any other type of curve. The mathematical analyses of results where linear relations are established form a standard statistical procedure and provide an accurate method of handling such data.

Bliss (3) has given a very adequate description of the time-mortality curve in which he states that such curves will often be linear when logarithm of elapsed time is plotted against probability units and less frequently when reciprocal (rate) of elapsed time is plotted against probability units. The time-germination curve may be considered to have an inverse relation to the time-mortality curve, since in the former case as time elapses there is indication of life on the part of more and more individuals as measured by germination while in the latter case there is indication of death on the part of more and more individuals.

The relation between probit germination and various functions of time at 21° C. appears in Figure 1. It may be seen that of these simple functions of elapsed time only the reciprocal function gives a linear relation, within limits of experimental error, when plotted against germination in probability units. Thus it may be said that the germination of spores is normally distributed against reciprocal units of time. However, Clark (6) points out that in biological work the data in many instances may be fitted within limits of experimental error by some simple curve when the appropriate function is chosen, though there is often no theoretical justification for compelling it to do so. It is sufficient for the purposes of the present paper that such a relation exists and that it can be usefully employed in fungistatic tests.

These germination curves in distilled water are of particular interest since they indicate that the rate of germination of various fungi differ, as shown by the difference in slopes of curves in Figure 1 C, and also the time at which germination starts is not the same for the different fungi, as shown by the position of the curves in Figure 1 C.

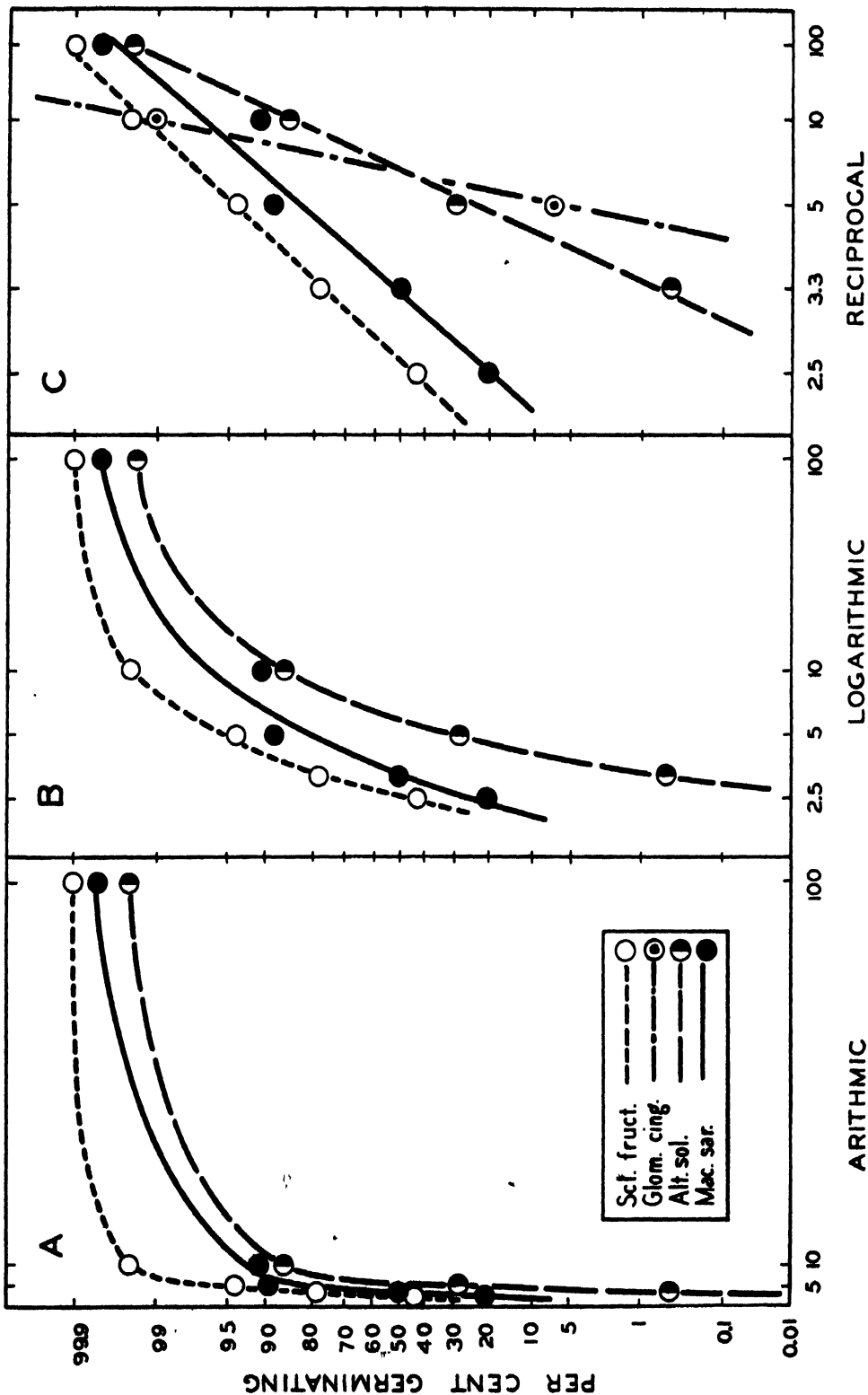


FIGURE 1. Germination of spores in distilled water showing the relation of germination on a probit scale to various functions of time, in hours. Spores of *Sclerotinia fructicola*, *Glomerella cingulata*, *Alternaria solani*, and *Macrosporium sarcinaeforme*.

EFFECT OF TEMPERATURE ON SPORE GERMINATION IN WATER

If the factor time is considered, a three component system (germination, time, and temperature) exists in the study of effect of temperature on germination in distilled water. If the factor temperature is held constant, time-germination curves of the type explained in the previous section are formed. These are shown in Figure 2 A, in which time-germination curves at different temperatures are shown for *Sclerotinia fructicola*. The results in Figure 2 A show that temperature has a marked effect on the time of germination; compare the curve for 10° C. where eight hours are required for 50 per cent of the spores to germinate with that for 27° C. where 50 per cent germination is reached in less than two hours. At all temperatures used, 98 per cent or higher germination was reached within 50 hours, except in the case of *Sclerotinia fructicola* and *Glomerella cingulata* at 35° C. where the germination was about 90 per cent. Thus the effect of the temperatures used, other than at the optimum, was a delay of germination rather than a permanent inhibition.

It is well known that spores of different fungus species have different optimum temperatures for germination. The results obtained after 4.35 hours' elapsed time in Figure 2 B show that the four fungi examined in the present experiments differed in this respect: *Alternaria solani* probably has an optimum of germination between 27° C. and 35° C., while that for *Sclerotinia fructicola* and *Macrosporium sarcinaeforme* is between 21° C. and 27° C. The optimum for *Glomerella cingulata* appears to be near 27° C. The optimum temperature ranges for *Alternaria solani* and *Sclerotinia fructicola* are in agreement with the data recently presented by McClellan (16) and with early results by McCallan for *S. (americana) fructicola* (10). It is impossible with the present data to fix the optimum within several degrees. It would not, in any case, seem to be either necessary or feasible to fix the optimum at a given temperature because of the inadequacy of temperature control equipment and because of biological variation. At best the optimum temperature cannot be determined within a smaller range than the temperature intervals used in such a determination and then only when the results obtained differ significantly.

ELAPSED TIME AS A FACTOR IN THE ESTIMATION OF THE POTENCY OF A FUNGISTATIC MATERIAL

One purpose of this investigation was to determine after what period of elapsed time the estimation of fungistatic potency might give the least day-to-day variation and be measured most precisely. In order to answer this question, analyses were conducted on the LD₅₀ values of each time of counting, and the results obtained are presented in Table I. As these analyses were based on logarithms of LD₅₀ values and as the total sums of squares did not vary greatly in this related series of analyses, it seems justi-

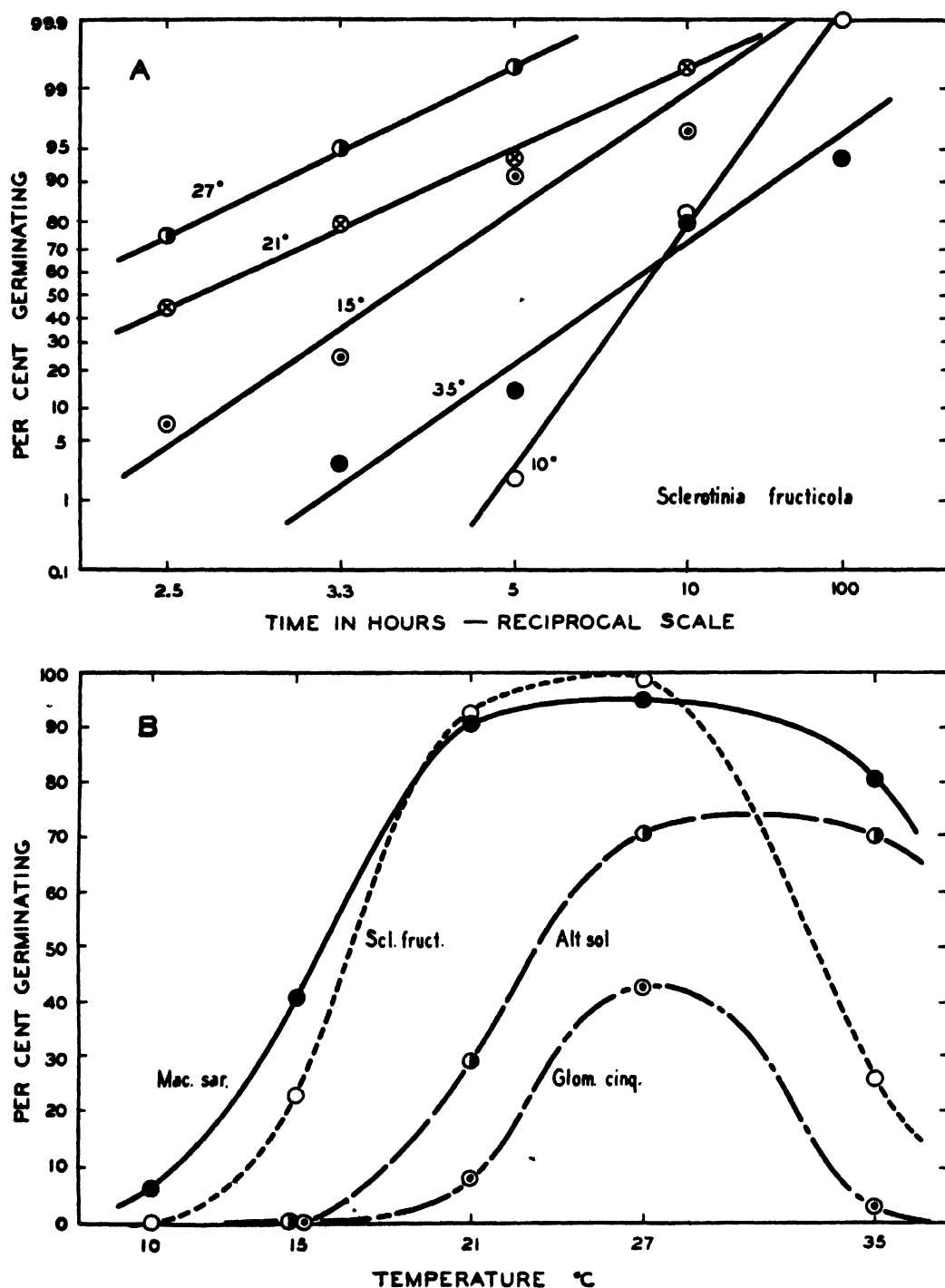


FIGURE 2. Effect of temperature on germination of spores in water. A. Time-germination curves for *Sclerotinia fructicola* spores in distilled water at various Centigrade temperatures. B. Germination of spores of *Macrosporium sarcinaeforme*, *Sclerotinia fructicola*, *Alternaria solani*, and *Glomerella cingulata* at various temperatures, after 4.35 hours' elapsed time. Average values for 3 experiments.

fiable to make direct comparisons between analyses at different times of counting. It will be seen from Table I that Experiments (day-to-day variation) were not significant at any time of counting and it is also apparent that the variance value for Experiments does not differ markedly or in a consistent manner at the various times of counting. From this it may be concluded that no greater day-to-day variation may be expected at one time of counting than at another. Further, the triple interaction (Compounds $\times$ Fungi $\times$ Experiments), which is the basic error term, does not differ significantly nor consistently at the various times of counting; thus counts may be made with equal precision at any time of counting.

TABLE I
COMPARISON OF PRECISION OF LD₅₀ VALUES ATTAINED AT DIFFERENT
TIMES OF COUNTING

Variance due to	D.F.	Variance				
		6 hrs.	12 hrs.	24 hrs.	48 hrs.	96 hrs.
Compounds	5	1.9554 High**/CF	1.8341 Sign./CF	1.5191 Sign./CF	1.3635 No/CF	1.2753 No/CF
Fungi	3	.5420 No†/CF	.9800 No/CF	1.1705 No/CF	1.2554 No/CF	1.1375 No/CF
Experiments	2	.0614 No/CE	.0582 No/CE	.0410 No/CE	.0974 No/CE	.1495 No/CE
Comp. $\times$ Fungi	15	.4004 High	.4070 High	.4905 High	.4982 High	.5375 High
Comp. $\times$ Exp.	10	.0134 No	.0145 No	.0214 Sign.	.0537 High	.0602 High
Fungi $\times$ Exp.	6	.0285 Sign.*	.0082 No	.0051 No	.0171 No	.0157 No
Comp. $\times$ Fungi $\times$ Exp.	30	.0113	.0070	.0089	.0178	.0104

† Not significant (odds less than 20:1).

* Significant (odds between 20:1 and 100:1).

** Highly significant (odds greater than 100:1).

Error terms: CF = Compounds $\times$ Fungi, CE = Compounds $\times$ Experiments, Unspecified = Compounds $\times$ Fungi $\times$ Experiments.

Since it has been shown that there is a linear relation between the reciprocal of elapsed time and percentage of spores germinated when spores are germinated in water, it is to be expected that the same relation should hold when a time-germination curve is constructed for spores germinated in a given concentration of a fungistatic agent. From the examples presented in Figure 3 it may be seen that such data may be fitted satisfactorily by a straight line. Contrary to the usual action of a toxicant over a period of time, in this instance it takes higher concentrations of the chemical to give an LD₅₀ as time elapses. This response is dependent on the

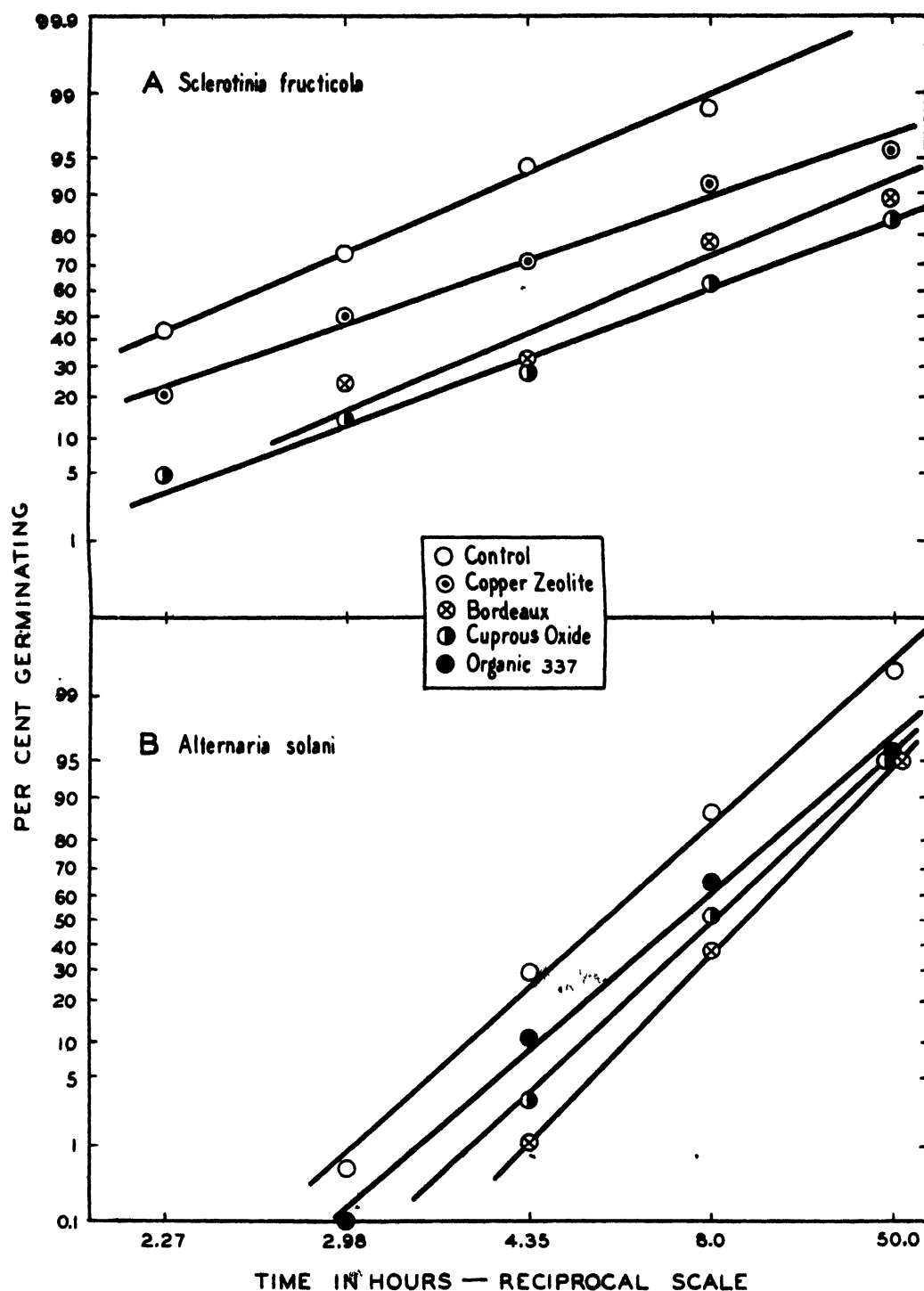


FIGURE 3. Time-germination curves of a given concentration of several fungicides. A. *Sclerotinia fructicola*. Dose in micrograms copper per sq. cm.: copper zeolite 0.75, Bordeaux 0.30, and red cuprous oxide 3.00. B. *Alternaria solani*: red cuprous oxide 1.50, Bordeaux 0.15, and Organic 337 total deposit 0.16 micrograms per sq. cm. Control, distilled water. 21° C.

method of determining toxicity in spore germination tests, for by this method spores cannot be proved dead but can be shown to possess life if they germinate. Thus in a given concentration of the chemical some spores may be temporarily prevented from germinating but may possess the ability to germinate later. Also, as may be seen from Figure 3, the germination of spores in distilled water has continued throughout the period under investigation. Limited data indicate that the slopes of time-germination curves (when reciprocal time units are plotted against probit germination) are a function principally of the fungus and seem largely independent of the chemical used. Thus in Figure 3 A the slopes of the toxicity curves probably do not differ significantly and the fungus used in all cases was *Sclerotinia fructicola*. In Figure 3 B the slopes again do not differ significantly on *Alternaria solani*. It will be noted that steeper toxicity curves are obtained with *Alternaria solani* (Fig. 3 B) than with *Sclerotinia fructicola* (Fig. 3 A).

The time-germination curve at a constant concentration is usually of less interest in fungistatic tests than is the concentration-time curve at a constant germination. Bliss (4) has given a complete treatment of a similar curve (the exposure time-concentration curve for a given mortality) when the function of time necessary for linearity is arithmetic or logarithmic; so that, in the present instance, it will be necessary only to show that linearity may be achieved using the reciprocal function of time. Since germination is normally distributed against logarithm of concentration and against reciprocal of elapsed time, it follows that for a given LD value (such as LD₅₀) when logarithm of concentration is plotted against reciprocal of elapsed time, a linear relation should result. The examples presented in Figure 4 show graphically that this relation holds within limits of experimental error. A statistical method for comparing regression lines has been developed by Gaddum (8) and adapted by Bliss and Marks (5), by means of which single and double curvature may be tested. Table II shows such an analysis of the data obtained for *Sclerotinia fructicola* at 21° C. and includes the data for red cuprous oxide presented graphically in Figure 4 A. It will be seen from Table II that as the quadratic term, which is a measure of single curvature, is not significant over error (from the regular analysis) for any of the four compounds the data may be fitted satisfactorily by a straight line. In all cases the cubic term, which is a measure of double curvature, is negligible. It is obvious that at the time interval necessary for 50 per cent germination in the check this linear relation ceases to hold because at this time all concentrations of the chemical having no toxicity will give an LD₅₀.

The LD₅₀ values change most rapidly during the first few hours and after 50 hours very little change is to be expected unless the slope of the time-concentration curve is extremely steep. An error of a few minutes in the time of counting after three hours will cause a greater change in the

LD₅₀ values than an error of hours in counting after 50 hours. In comparing fungicides it has been customary to count the germinated spores after 20 to 24 hours (12). It will be seen from Figure 4 that the value obtained at this time will approximate sufficiently the LD₅₀ value to be expected at infinity unless the slope of the concentration-time curve is steep. (For example of steep concentration-time curve see Organic 212 in Figure 4 B.) However, in the field, conditions favorable for the germination of spores may exist for long or short periods of time. It is necessary, therefore, to know whether compounds differ in fungistatic rating after various time intervals.

TABLE II

TESTS FOR LINEARITY OF LINE FOR TIME-CONCENTRATION CURVES AT LD₅₀ VALUES FOR FOUR COMPOUNDS ON *SCLEROTINIA FRUCTICOLA* AT 21° C.

Term	Polynomial coefficients (X) for dose				Di- visor NS (X ²)	Com- pound*	Sum of prod- ucts S(XYp)	Variance S ² (XYp) NS(X ²)	Var. ratio	Signifi- cance
	1	2	3	4						
Linear	-3	-1	+1	+3	60	A	3.69	.227	41.3	High
						B	13.16	2.87	41.0	High
						C	2.80	.131	21.4	High
						D	2.14	.076	47.8	High
Quadratic	+1	-1	-1	+1	12	A	-.47	.018	3.22	No
						B	.94	.074	1.02	No
						C	-.36	.011	1.80	No
						D	-.17	.002	1.44	No
Cubic	-1	+3	-3	+1	60	A	.21	.0007	.012	No
						B	.52	.0045	.006	No
						C	.38	.0024	.394	No
						D	.09	.0001	.075	No
Error (obtained from analyses of variance conducted separately and is the interaction of Experiments and Times of count- ing)						A		.0055		
						B		.0735		
						C		.0061		
						D		.0016		

* A = Copper zeolite; B = Red cuprous oxide; C = Bordeaux mixture; D = Organic 337.

Three determinations for each curve. Single degree of freedom for linear, quadratic, and cubic terms, six degrees of freedom for error.

It has been shown that a toxicity curve, which is a single straight line, cannot be completely characterized by an LD₅₀ value (12, 7), but must be characterized either by two separate LD values or by one LD value and the slope. It has also been shown that toxicity curves differ from each other in slope (12, 7). If the time-concentration curves for the LD₅₀ values differ for various compounds or fungi, it is apparent that they cannot be adequately compared at one time. LD₅₀ time-concentration curves for various compounds on one fungus are presented in Figure 4 B and for one compound on various fungi in Figure 4 C.

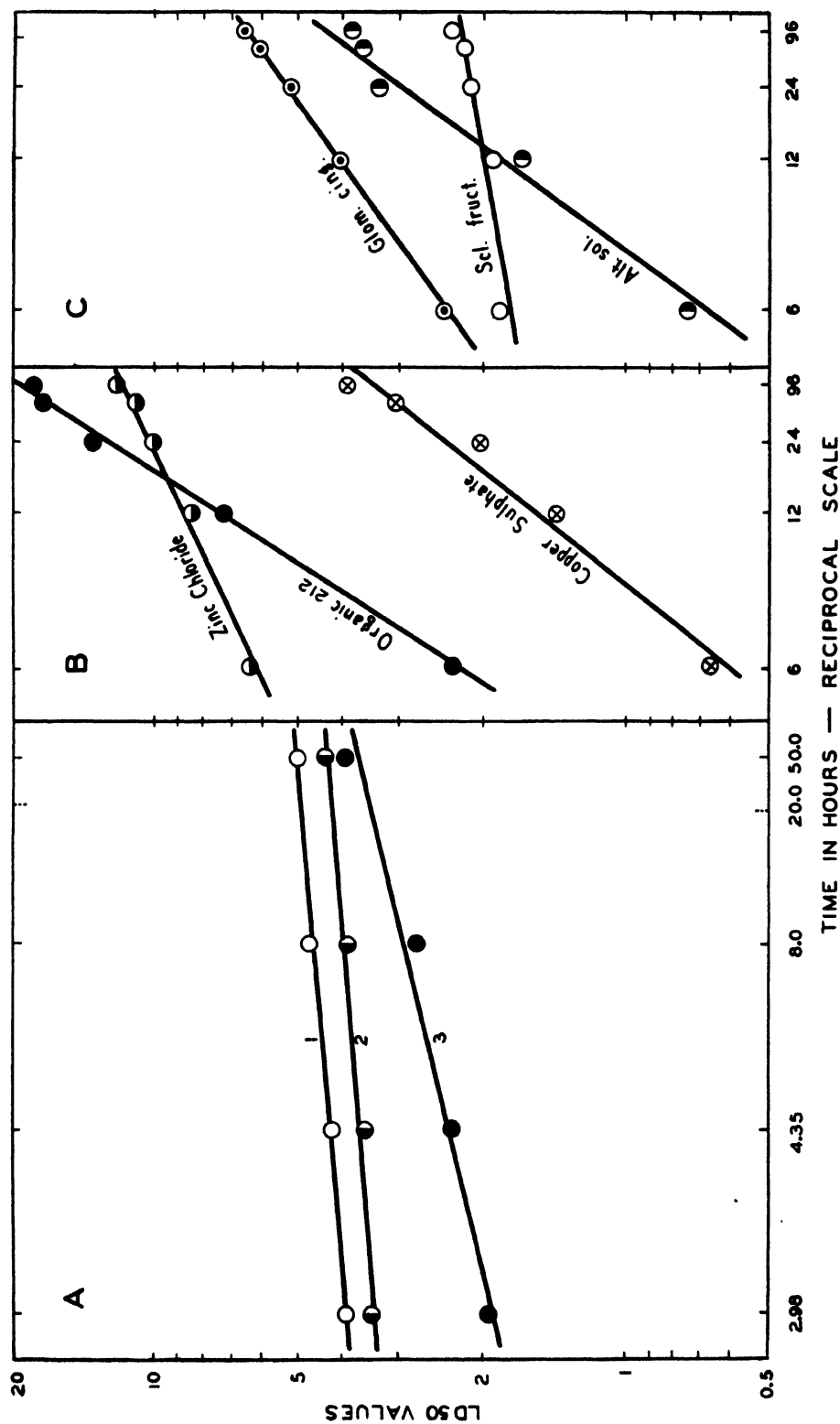


Figure 4 B shows that the slopes of the LD₅₀-time curves differ on the same organism (*Alternaria solani*) for different chemicals. For example, Organic 212 is twice as toxic as zinc chloride as measured by the LD₅₀ obtained after six hours' elapsed time but only about half as toxic as measured by the LD₅₀ obtained after 96 hours' elapsed time.

Figure 4 C shows that the relative resistance of fungi to a given compound may depend on the time at which the count is made. After six hours, *Sclerotinia fructicola* was three times as resistant to Bordeaux mixture as was *Alternaria solani*, yet after 96 hours *Alternaria solani* was twice as resistant as *Sclerotinia fructicola*.

The conclusions drawn from the graphical examples presented in Figure 4 B and C are confirmed by the results of an analysis of variance conducted on the data as a whole and shown in Table III. Since the interaction Compounds×Times is highly significant, compounds are not rated alike at

TABLE III
ANALYSIS OF VARIANCE FOR TIME EXPERIMENTS, CONDUCTED ON
LOGARITHMS OF LD₅₀ VALUES

Variance due to	Degrees of freedom	Sums of squares	Variance	Significance
Compounds	5	37.6281	7.5256	No/CF
Fungi	3	11.8343	3.9448	No/CF
Experiments	2	.7111	.3556	No/CE
Times of counting	4	18.7730	4.6932	High/FT
Comp.×Fungi	15	34.5204	2.3014	High/CFE
Comp.×Exp.	10	1.0447	.1045	High/CFE
Comp.×Times	20	2.1174	.1059	High/CFT
Fungi×Exp.	6	.3313	.0552	No/CFE
Fungi×Times	12	2.7285	.2274	High/CFT
Exp.×Times	8	.1039	.0131	No/CET
Comp.×Fungi×Exp.	30	.7001	.0233	High
Comp.×Fungi×Times	60	.8781	.0146	High
Comp.×Exp.×Times	40	.5543	.0139	High
Fungi×Exp.×Times	24	.1154	.0048	No
Comp.×Fungi×Exp.×Times	120	.9427	.0078	
Total:	359	112.9833		

Error Terms: CF = Compounds×Fungi, CE = Compounds×Experiments, FT = Fungi×Times, CFT = Compounds×Fungi×Times, CFE = Compounds×Fungi×Experiments, CET = Compounds×Experiments×Times, Unspecified = Compounds×Fungi×Experiments×Times.

different times. The interaction Fungi×Times is also highly significant so that fungi are not rated alike at different times.

It can be said in general that the dosage-response curves, uncorrected for control germination, obtained with *Macrosporium sarcinaeforme* at three hours and with *Alternaria solani* at three hours and six hours, were flatter than those obtained at longer time intervals while those obtained with *Sclerotinia fructicola* did not differ in slope between three hours and

96 hours. Figure 1 C shows the germination of *Sclerotinia* spores in water is 70 per cent after three hours while that of *Macrosporium* spores is less than 40 per cent and *Alternaria* spores do not reach 50 per cent germination until six hours' elapsed time. If the dosage-response curves were corrected for the germination of spores in distilled water by the formula: $\text{corrected germination} = \text{observed germination} \times 100 / \text{germination of control}$, to give the true effect of the chemical, slopes could no longer be shown to differ at any time for any fungus. Dimond *et al.* (7) present data to show that the slope of the dosage-response curve is flatter at the shorter periods of incubation time. A correction for control germination may tend to obviate the lack of parallelism they observed.

TEMPERATURE AS A FACTOR IN THE ESTIMATION OF THE POTENCY OF A FUNGISTATIC MATERIAL

The effect of temperature on potency is most marked at the shorter time periods. This follows from the discussion on the effect of temperature on germination of spores in water since it was shown that at the shorter time periods temperature most markedly affects germination in water. McClellan (16) has recently shown in spore germination tests of fungicides, that chemicals were least effective at the optimum temperature and that both above and below this optimum temperature these materials were more toxic. In essence the use of any temperature but the optimum is the use of a toxic agent whose concentration increases as temperature departs farther from the optimum. A comparison of the data presented for temperature in Figure 2 A and for a chemical toxicant in Figure 3 will show the similarity of action of these two types of toxic agents. Thus it becomes clear that any study at the various times of counting of the effect on chemical toxicity of temperature becomes a study of the combined action of two toxicants. The relations involved in such a study may well be important in determining the effectiveness of a compound under all conditions; however, it is important first to determine the relationships involved in the simpler case where time has been held constant. For this reason the effect of temperature at various times will not be considered in the present paper and attention will be devoted instead to the effect of temperature on toxicity when chemical toxicity has approached an equilibrium with respect to time as is the case when 50 hours elapse before counts are made.

The results presented in Figure 5 A show the effect of temperature on the action of four chemicals on *Sclerotinia fructicola* after 50 hours' elapsed time. Figure 5 A shows that in general, within limits of experimental error, temperatures between 15° and 27° C. had no significant effect on LD₅₀ values after 50 hours. However, the extreme temperatures of 10° C. and of 35° C. frequently affect the LD₅₀ values; and further, compounds behaved differently at these two temperatures. This graphic interpretation

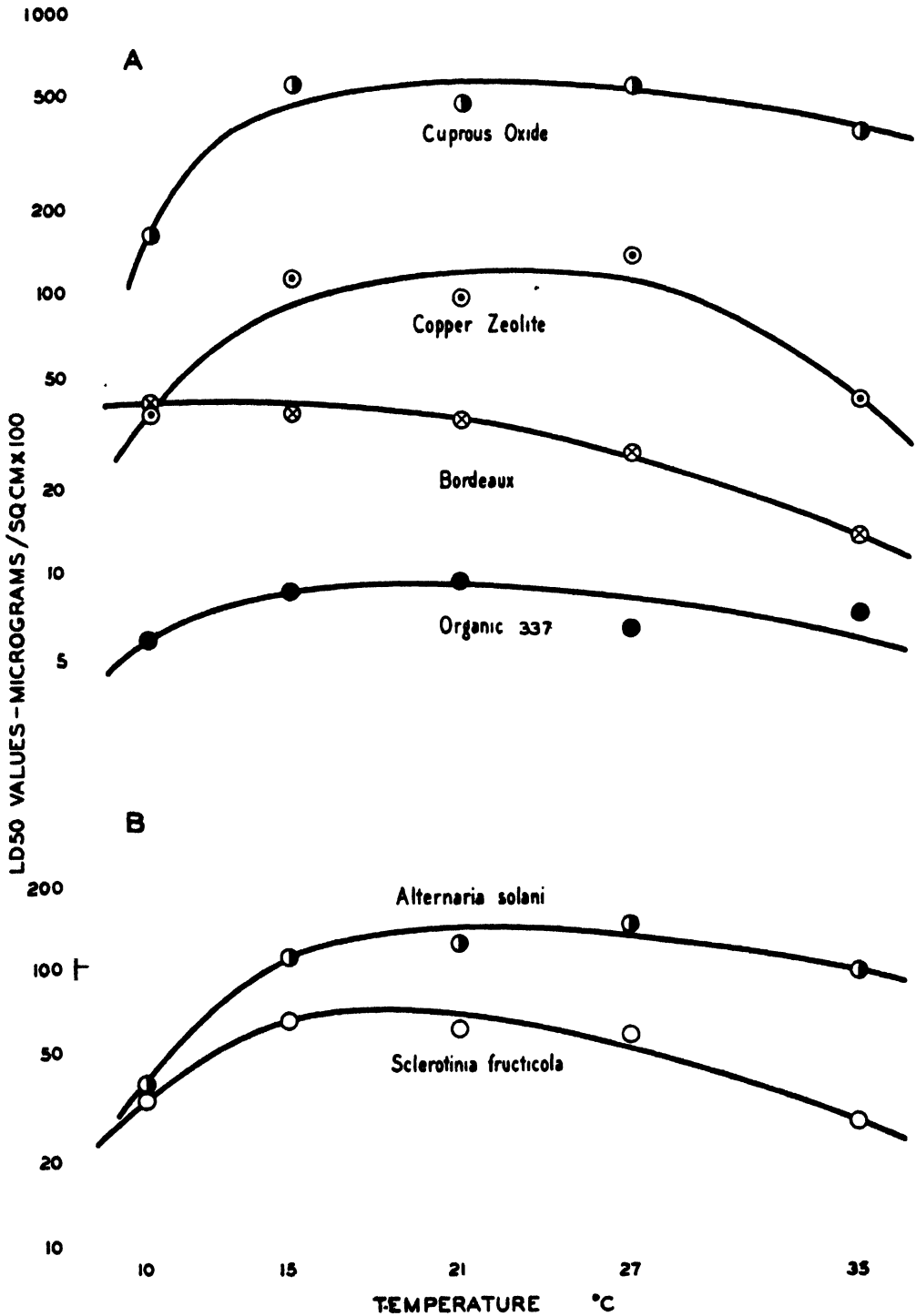


FIGURE 5. LD₅₀ values at various temperatures. Time of counting, 50 hours. A. Several compounds on *Sclerotinia fructicola* showing differing behaviors at 10° and 35° C. Average of 3 experiments. B. Comparison of the average result for the four compounds on *Sclerotinia fructicola* and *Alternaria solani* for 3 experiments showing increasing divergence in LD₅₀ values from low to high temperatures.

is confirmed by the analysis presented in Table IV A which shows that there was a significant temperature effect (due to the effect at 10° and 35° C. as shown in Table IV B where these two temperatures were removed), and a highly significant Compounds $\times$ Temperature interaction which means that compounds behaved differently at the various temperatures (again due to their behavior at 10° and 35° C. as shown in Table IV B). Table IV A also shows that the two fungi examined behaved differently at the various temperatures since the interaction Fungi $\times$ Temperature was highly significant. As is shown graphically in Figure 5 B where the results with the various compounds have been summed so only the overall difference between fungi appears, this difference is not associated only

TABLE IV

ANALYSIS OF VARIANCE. TIME OF COUNTING—50 HOURS. CONDUCTED
ON LOG. 100 (LD₅₀ IN MICROGRAMS/SQ. CM.)

A. Including all temperatures examined, i.e. 10°, 15°, 21°, 27°, and 35° C.

Variance due to	Degrees of freedom	Sums of squares	Variance	Significance
Compounds	3	31.2523	10.4174	Sign./CF
Fungi	1	2.6049	2.6049	No/CF
Experiments	2	.2361	.1080	No/CE
Temperature	4	2.9888	.7672	Sign./CT
Comp. $\times$ Fungi	3	3.2092	1.0697	High/Residue
Comp. $\times$ Exp.	6	.2258	.0376	Sign./Residue
Comp. $\times$ Temp.	12	1.8336	.1528	High/Residue
Fungi $\times$ Exp.	2	.0276	.0128	No/Residue
Fungi $\times$ Temp.	4	.7586	.1897	High/Residue
Exp. $\times$ Temp.	8	.1422	.0178	No/Residue
Residue	74	1.2107	.0164	

B. Including temperatures 15°, 21°, and 27° C.

Compounds	3	21.3312	7.1104	Sign./CF
Fungi	1	1.6780	1.6780	No/CF
Experiments	2	.1385	.0693	No/CE
Temperature	2	.0134	.0067	No/CT
Comp. $\times$ Fungi	3	1.5064	.5021	High/Residue
Comp. $\times$ Exp.	6	.1114	.0186	No/Residue
Comp. $\times$ Temp.	6	.1534	.0256	No/Residue
Fungi $\times$ Exp.	2	.0275	.0138	No/Residue
Fungi $\times$ Temp.	2	.0860	.0430	No/Residue
Exp. $\times$ Temp.	4	.0342	.0085	No/Residue
Residue	40	.5445	.0136	

Error terms: CF = Compounds $\times$ Fungi, CE = Compounds $\times$ Experiments, CT = Compounds $\times$ Temperature.

with the 10° and 35° C. temperatures, though they contribute the major part of the variance as shown in Table IV B, where these two temperatures have been eliminated from consideration; the interaction Fungi $\times$ Temperature is not significant yet it approaches the borderline of significance. It will be remembered from the discussion on Figure 2 A that the

optimum for germination for *Alternaria solani* lay between 27° and 35° C., while that for *Sclerotinia fructicola* was between 21° and 27° C. At temperatures differing from their respective optimums, two toxicants, temperature and the chemical, are operating together and as the divergence from the optimum temperature increases, less chemical is required for an LD₅₀ since temperature toxicity plays a greater rôle. It is logical to assume, therefore, that it would take proportionately more chemical to inhibit *Alternaria* spores at the higher temperatures and *Sclerotinia* spores at the lower temperatures. It will be seen from Figure 5 B that this is the case, since the divergence between *Sclerotinia* and *Alternaria* values becomes progressively greater from 10° to 35° C.

DISCUSSION

The difference in ratings of compounds in the field may derive in part from the interaction of compounds and temperature at the higher temperatures, since field temperatures of 27° C. or below may well be encountered during one test while 35° C. or more may be encountered with another test with the same disease. Taken together with the difference in ratings of compounds after various times of counting, corresponding to various periods favorable for infection in the field, the factors time and temperature may offer a partial explanation why investigators in different areas or the same investigator in different seasons have been unable to rate a series of compounds in the same order. If one compound is to be more toxic than another in spore germination tests it must be shown to be more toxic at all times of counting and at all temperatures as well as all LD values (7, 12). As stated previously (13) one toxicity surface must lie wholly within the other. Therefore, if one compound is always to give better control than another in the field, after tenacity (9) is taken into account, it must be better for all periods favorable for infection, for all temperatures, for all degrees of spore load (12), and probably for other factors not yet demonstrated.

Since LD₅₀ values, obtained after 50 hours' elapsed time, do not differ significantly between 15° and 27° C. for either *Sclerotinia fructicola* or *Alternaria solani*, it follows that for counts taken after such times, i.e. 20 hours or longer, temperatures need be controlled only within 4° or 5° C. of the optimum. Thus, as regards the temperature factor alone, the results of spore germination tests for *Sclerotinia fructicola* and *Alternaria solani* in any laboratory room, which did not go below 15° C. or above 27° C., that is approximately 60° to 80° F., during the tests, should be as precise as tests conducted in rooms or chambers in which the temperature is accurately controlled. This may be stated in a more general fashion by saying that in fungistatic tests where 20 hours or more elapse before counting, temperatures varying by 4° or 5° C. on either side of the optimum

for germination have little effect on the toxicity of a compound, while temperatures that differ by more than 10° from the optimum for germination may have a marked effect. It should be obvious that where temperature controlled facilities are available it is desirable to use them, as wide fluctuations in laboratory temperatures may contribute markedly to the variation of results obtained.

In a general way, time of counting and extreme temperatures are as important sources of variation in fungistatic tests as is the difference in susceptibility of fungi. In view of the demonstrable differences in slopes of time-concentration at constant germination, it is recommended that the LD values be determined after at least two different time intervals (for example, 6 and 24 hours) in comparison of compounds that differ only slightly in toxicity, or compounds in which there is a particular interest. If more than two times of counting are used, the time should vary in equal reciprocal units. If more than one temperature is used in the determination of toxicity in spore germination tests, a larger amount of information may be secured by choosing the optimum temperature and temperatures that vary from it by ten degrees Centigrade and by counting after at least 20 hours' elapsed time, than by choosing temperatures which are closer together.

SUMMARY

1. In determining the effect of time in the spore germination methods of evaluation of fungicides, toxicity curves were established for copper sulphate, zinc chloride, "Standard Laboratory Bordeaux," red cuprous oxide, and two synthetic organic chemicals on *Sclerotinia fructicola*, *Alternaria solani*, *Glomerella cingulata*, and *Macrosporium sarcinaeforme* after various periods of elapsed time. The variable temperature was studied at 10° , 15° , 21° , 27° , and 35° C., toxicity curves being determined for four chemicals on the first two fungi.

2. Fungus spores of different species do not start germinating at the same time nor once started do they germinate at the same rate.

3. A linear relation, within limits of experimental error, between reciprocal of elapsed time and germination expressed in probits is shown for *Sclerotinia fructicola*, *Macrosporium sarcinaeforme*, *Glomerella cingulata*, and *Alternaria solani* spores germinating in water at temperatures of 10° , 15° , 21° , 27° , and 35° C.

4. Temperature has a marked effect on the rate of germination of spores of these four species in water but all fungi reached 98 per cent or better germination by 50 hours at all temperatures with the exception of *Sclerotinia fructicola* and *Glomerella cingulata* at 35° C. where the germination was about 90 per cent.

5. The optimum temperature for germination of *Alternaria solani*

spores is between 27° and 35° C., while that for *Sclerotinia fructicola* and *Macrosporium sarcinaeforme* is between 21° and 27° C., and the optimum for *Glomerella cingulata* appears to be near 27° C.

6. No significant difference in precision could be shown between counts made at 6, 12, 24, 48, or 96 hours.

7. A linear relation between reciprocal of elapsed time and germination expressed in probits exists when spores are germinated in a given concentration of a chemical, provided the concentration permits germination.

8. The action of elapsed time in fungistatic tests is the reverse of the usual action of time in toxicity tests at a given concentration. In fungistatic tests the inhibition of germination becomes less as time goes on since the spores continue to germinate and thus give indication of viability.

9. A linear relation is shown for LD₅₀ values when logarithm of concentration is plotted against reciprocal of elapsed time. This curve is important in the estimation of the potency of a fungistatic agent, since compounds are rated differently at various times on the same fungus; also, fungi may differ in their relative susceptibility to a single compound, depending on the elapsed time before counts are made.

10. Slopes of the dosage-response curves did not differ significantly for the various times of counting, provided an appropriate correction was made for control germination.

11. Determining the effect of temperatures, other than the optimum, on the fungistatic action of chemicals involves a system where two toxicants, i.e. chemical and temperature, are acting simultaneously. Interpretation of results from such a system is simplified if the variable time is held constant. This may be done by determining LD values of the chemical after 20 hours or more of elapsed time, when the chemical effect has approached an equilibrium with respect to time.

12. No significant difference in LD₅₀ values could be demonstrated at 15°, 21°, or 27° C. but there was a temperature effect at 10° and 35° C. Compounds were not rated in the same order at 10° and 35° C. but in general it took less chemical to give an LD₅₀ value at these extreme temperatures. It took proportionately more chemical to give an LD₅₀ on *Alternaria solani* at the higher temperatures and on *Sclerotinia fructicola* at the lower temperatures.

13. In spore germination tests where 20 hours or more elapse before counting, temperature need be controlled only within approximately 5° C. above or below the optimum temperature for spore germination. In a general way, time of counting and extreme temperatures are as important sources of variation in fungistatic tests as is the difference in susceptibility of fungi.

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FUNGICIDAL VERSUS FUNGISTATIC

S. E. A. MCCALLAN AND R. H. WELLMAN¹

A fungicide may be defined as an agent that kills or inhibits the development of fungus spores or mycelium (1). In the literature dealing with fungicides the expression "fungicidal action" or "fungicidal property" with few exceptions (3, 4, 8) has been used in the general sense as above, no separation being made between killing and inhibition. On the other hand, in bacteriological literature (9) usually there has been a clear differentiation between these two properties of killing and of inhibiting, the former being called bactericidal and the latter bacteristatic. It is the purpose of this paper to demonstrate the distinction between fungicidal in the restricted sense, namely the property of killing fungi, and fungistatic, the property of inhibiting the development of fungus mycelium or spore germination.

In actual operation these two actions frequently cannot be separated. Protective fungicides can function in their initial action fungistatically though in time the effect may be lethal or fungicidal. Only when the fungus, or fungus spores, are removed from actual contact with the fungicide and placed in a favorable environment can the distinction be made between fungicidal and fungistatic (4). If the organism develops the action is fungistatic, if not it is assumed to be fungicidal. In practice the majority of fungicides can function satisfactorily merely in a fungistatic manner provided contact is maintained with the fungus or surface to be protected, that is, the fungicide is not removed by weathering or other means. One of the few practical examples of true fungicidal action is fruit dips for controlling storage decays of citrus and apple (2, 5). Here the fruit is dipped for a few minutes in a fungicide which must kill all surface contaminating spores. Subsequently the fruit is rinsed in water and hence any prolonged protective or fungistatic action is prevented.

METHODS

Compounds. Fifteen chemicals representing a fairly wide range of inorganic and organic compounds were selected for this study, the majority being chosen because of their use or interest as fungicides. In order to simplify the method of testing, the selection was limited to water-soluble compounds. The compounds were: copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), silver nitrate (AgNO_3), mercuric chloride (HgCl_2), potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$), uranyl acetate ($\text{UO}_2(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$), sodium arsenate (Na_2 -

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$\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$), sodium hypochlorite (NaOCl), iodine-potassium iodide, formaldehyde (HCHO), acetic acid (CH_3COOH), phenol ($\text{C}_6\text{H}_5\text{OH}$), malachite green ($[(\text{C}_6\text{H}_5)[(\text{CH}_3)_2\text{N}-\text{C}_6\text{H}_4]\text{C}=\text{C}_6\text{H}_4\text{N}(\text{CH}_3)_2\text{Cl}]$), crystal violet ($[(\text{CH}_3)_2\text{NC}_6\text{H}_4]_2\text{C}=\text{C}_6\text{H}_4\text{N}(\text{CH}_3)_2\text{Cl}$), berberine sulphate ($\text{C}_{21}\text{H}_{19}\text{NO}_5 \cdot \text{H}_2\text{SO}_4$), and 8-hydroxyquinoline sulphate ($(\text{C}_9\text{H}_7\text{ON})_2 \cdot \text{H}_2\text{SO}_4$). These chemicals were prepared in a series of aqueous solutions, the dose ratio for the fungistatic tests being 2, while for the fungicidal, the dose ratio was 4, 10 or $\sqrt{10}$, i.e. 3.16. In all the fungicidal tests, because of the limited capacity of the centrifuge, only four doses were used.

Fungi. The fungi selected were: *Sclerotinia fructicola* Wint. (Rehm.), *Alternaria solani* (Ell. & Mart.) Jones & Grout, Delaware strain (6, footnote 5), *Penicillium expansum* Link, and *Rhizopus nigricans* Ehr., + strain. The spores of all four fungi were obtained by the rubbing and centrifuging technique (7) from 7-day-old cultures growing on potato dextrose agar at $21.5 \pm 0.5^\circ \text{C}$. The ultimate spore germination tests were performed in ultrafiltered orange juice at a concentration of 0.1 per cent for the two former species and 0.316 per cent for the two latter.

Fungistatic technique. The standard slide-moist chamber technique was employed in testing fungistatic action (6, 7). The test tube method (6, 7) was used for suspending the spores in the chemical solution and at the customary concentration of 50,000 spores per cc. Counts on percentage spore germination were taken after 20 to 24 hours at 21.5°C . and again at 48 to 50 hours.

Fungicidal technique. The fungicidal technique differs essentially from the fungistatic in that the spores were allowed to remain in contact with the chemical only a limited time, and were then placed in distilled water containing orange juice. Nine cc. of each dose of each chemical were placed in a clinical centrifuge tube and to this was added 1 cc. of spore suspension. The spores were obtained as described above. Due to subsequent losses in washing it was necessary to add from 1,000,000 to 3,000,000 spores to each tube, i.e. 10 cc., in order to obtain in the final preparation a concentration of approximately 50,000 spores per cc. The time was recorded at the moment of adding the spores to the solutions and they were allowed to remain in contact for periods of (a) 10 minutes, (b) 20 hours, or (c) 2.5, 10, 40, 160, 640, and 2560 minutes. For the longer time periods the spores were held at 21.5°C .; the short periods were at room temperature. The fungicide was removed from the spores by centrifuging for 20 seconds at 1666 r.p.m. and decanting. Approximately 13 cc. of distilled water were then added to the spores in the tube. In part, this procedure resembles that of Hwang and Klotz (5). The time of "fungicidal action" was considered to terminate at the instant of adding the first wash water. The spores were again centrifuged and decanted, this process of washing with water taking place three times. The water was applied with force from a wash bottle

in order to stir up the spores. Finally the spores were taken up in 10 cc. of orange juice of the required strength and spore suspensions pipetted onto glass slides for the regular spore germination tests.

RESULTS

FUNGICIDAL AND FUNGISTATIC COMPARISONS

All comparisons of fungicidal or fungistatic activity are based on LD₅₀ values which were determined graphically on logarithmic-probability paper in accordance with previously described methods (6, 11). It is to be noted that fungicidal or fungistatic activity increases as the LD₅₀ values decrease, hence a chemical possessing high fungicidal or fungistatic activity will have a low LD₅₀ value. In view of the large number of variables involved it was not possible to run all tests on all materials with the same lot of spores; hence day-to-day variation (6) has been used as error. However, in many cases the fungicidal and fungistatic activity of a particular fungicide have been determined with the same lot of spores. All LD₅₀ values reported in Table I and Figure 1 are based on the logarithmic mean of at least two determinations made at different times. In the case of copper sulphate, silver nitrate, and sodium hypochlorite, three or four determinations were made. The Standard Error for the mean of the replicate tests was determined in logarithmic form respectively for the fungicidal and fungistatic activities. These Standard Errors were: fungicidal 10 minutes 0.177, fungicidal 20 hours 0.165, fungistatic 0.126. The difference between the first and third is significant.

The LD₅₀ values in p.p.m. for the mean of the replicate tests are shown in Table I for fungicidal action after 10 minutes and after 20 hours, and for fungistatic after 20 hours, for all four fungi.

In Figure 1 the graphic correlation is shown between fungistatic and fungicidal action after 20 hours' exposure. It will be seen that for many of the compounds the correlation is of a high order. However, in certain other cases, materials which possess marked fungistatic efficacy, show practically no fungicidal activity after 10 minutes and but little more after 20 hours. Compounds that are outstanding in their lack of correlation between fungistatic and fungicidal action for all four fungi are sodium arsenate, potassium dichromate, uranyl acetate, and 8-hydroxyquinoline sulphate. In addition at 20 hours the correlation is very poor for copper sulphate on *Penicillium expansum* and for malachite green and crystal violet on *Rhizopus nigricans*, and only fair for malachite green on *Sclerotinia fruticola* and *Penicillium expansum*.

It follows from the definition and from the method of testing that theoretically the fungicidal activity of a compound can not exceed its fungistatic activity. Hence, compounds may be classified into three groups: A. Fungistatic and fungicidal activity high, B. Fungistatic and fungicidal

TABLE I
LD50 VALUES IN P.P.M. FOR FUNGICIDAL ACTIVITY AFTER 10 MINUTES AND AFTER 20 HOURS, AND FUNGISTATIC ACTIVITY AFTER 20 HOURS.
SPORES OF SCLEROTINIA FRUCTICOLA, ALTERNARIA SOLANI, PENICILLIUM EXPANSUM, AND RHIZOPUS NIGRICANS.
GEOMETRIC MEAN FOR 2 TO 4 REPLICATE TESTS

Compound	Symbol (see Fig. 1)	<i>Sclerotinia fructicola</i>			<i>Alternaria solani</i>		
		Fungicidal		Fungistatic	Fungicidal		Fungistatic
		10 mins.	20 hrs.		10 mins.	20 hrs.	
Copper sulphate	Cu	31.6	0.71	11.5	77.7	4.79	9.33
Silver nitrate	Ag	0.15	0.11	0.11	0.10	0.12	0.10
Mercuric chloride	Hg	23.4	0.68	0.98	8.13	2.57	1.48
Potassium dichromate	Cr			0.42		31.6	0.20
Uranium acetate	U	> 100,000	21,500	20.4	13,500		6.76
Sodium arsenate	As	> 100,000	25,700		871	27.6	
Sodium hypochlorite	Cl	42,700	11,000	0.23	6,930	5,500	1.15
Iodine—KI	I	2.29	1.66	4.79		3.24	2.95
Formaldehyde	F	2.89	2.51	4.68	4.07	7.94	7.94
Acetic acid	A	1,860	224	224	1,380	219	339
Phenol	P	3,710	1,860	537	2,340	447	129
Malachite green	MG	6,310	2,400	6,310	4,900	2,090	3,020
Crystal violet	CV	64.5	4.68	0.25	89.1	1.26	1.12
Berberine sulphate	BS	1.70	0.29	0.58	490	4.58	2.63
8-Hydroxyquinoline SO ₄	HQ	832	182	174	7,080	162	67.6
		> 100,000	3,980	0.70	> 100,000	589	2.45

TABLE 1 (Cont'd.)

Compound	<i>Penicillium expansum</i>			<i>Rhizopus nigricans</i>		
	Fungicidal		Fungistatic	Fungicidal		Fungistatic
	10 mins.	20 hrs.		10 mins.	20 hrs.	
Copper sulphate	> 100,000	> 100,000	12.0	28.8	2.00	6.31
Silver nitrate	6.99	0.22	0.04	0.03	0.15	0.05
Mercuric chloride	41.7	1.91	0.87	19.1	2.46	0.83
Potassium dichromate	> 100,000	30,200	0.18	7,770	66.1	0.14
Uranium acetate	> 100,000	> 10,000	3.89	> 100,000	1,030	30.2
Sodium arsenate	> 100,000	3,720	1.45	52,500	8,710	0.28
Sodium hypochlorite	2.51	2.88	8.32	3.55	1.45	8.13
Iodine—KI	1.78	4.16	6.17	2.19	4.17	6.92
Formaldehyde	479	174	158	1,740	115	151
Acetic acid	19,500	4,070	933	18,200	777	759
Phenol	17,800	3,550	3,160	7,770	3,800	4,680
Malachite green	2,510	11.20	0.45	2,090	1,590	0.76
Crystal violet	447	0.80	0.40	6,030	7,770	1.35
Berberine sulphate	13,800	1,200	742	> 100,000	1,120	447
8-Hydroxyquinoline SO ₄	> 100,000	> 100,000	61.8	> 100,000	> 100,000	1.23

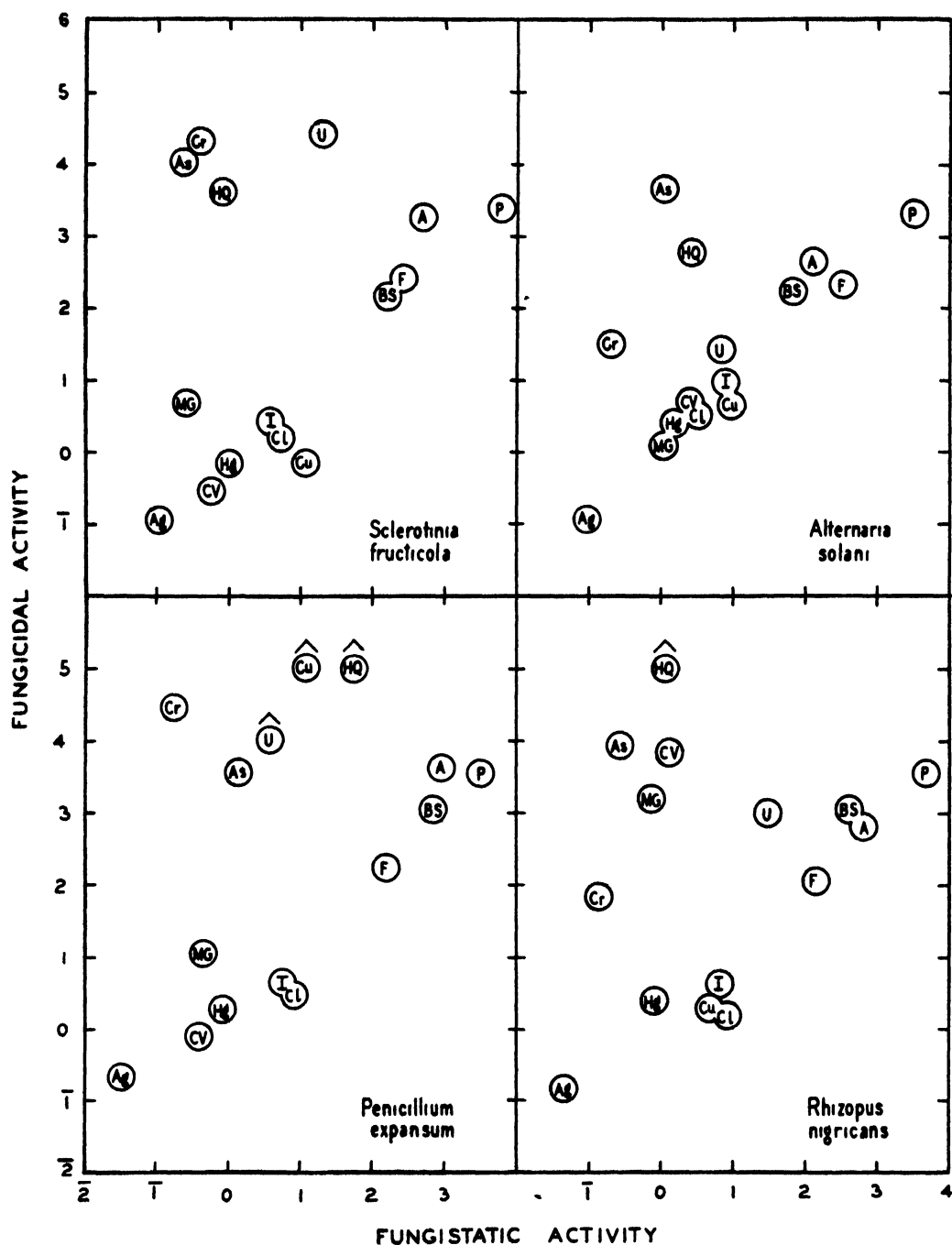


FIGURE 1. Correlation of fungicidal activity after 20 hours' exposure, with fungistatic activity. Units—logs. LD₅₀ in p.p.m. For compound symbols see Table I. ^ indicates greater than.

activity low, and C. Fungistatic activity high and fungicidal activity low. The results of these tests indicate that, against all four fungi, the compounds may be grouped as follows:

A. Fungistatic and Fungicidal Activity High

Silver nitrate

Mercuric chloride

Copper sulphate (except *Penicillium expansum*)

Sodium hypochlorite

Iodine

Crystal violet (except *Rhizopus nigricans*)Malachite green (except *P. expansum* and *R. nigricans*)

B. Fungistatic and Fungicidal Activity Low

Formaldehyde

Acetic acid

Phenol

Berberine sulphate

C. Fungistatic Activity High, Fungicidal Activity Low

Potassium dichromate

Uranyl acetate

Sodium arsenate

8-Hydroxyquinoline sulphate

Copper sulphate (*Penicillium expansum* only)Crystal violet (*Rhizopus nigricans* only)Malachite green (*P. expansum* and *R. nigricans* only)

In the case of the A and B classes the correlation is necessarily high, though predictions as to the effectiveness of the one response from the other can be made only in two cases. If the fungicidal activity is high, the fungistatic will be high and if the fungistatic is low the fungicidal will be low. The C class compounds are not correlated nor can they be predicted. Out of the 15 compounds, 7 fell in this class. At the present time information is not available to correlate fungicidal or fungistatic effectiveness in the laboratory with field performance. Hence for the present it is desirable to base preliminary laboratory testing on fungistatic methods in order not to overlook C type compounds which may give a good field performance.

Effect of time of counting. All the results reported are based on counts of spore germination made after 20 to 24 hours. As stated above, counts were also made after 48 to 50 hours, but no particular changes were noted other than the minor difference due to the prolonged time (10). It was noted, however, that in the case of malachite green on *Penicillium expansum* the germination continued to some extent.

Effect of orange juice. It was shown above that the standard errors for the mean values of Table I expressed as logarithms varied from 0.126 to 0.177. Hence in order for two arithmetic values to differ significantly approximately a four-fold difference would be required, i.e. $2 \times \sqrt{2} \times$ the antilogarithm of 0.126 or 0.177. It is to be expected that the LD₅₀ values

of Table I would decrease progressively from fungicidal 10 minutes to fungicidal 20 hours to fungistatic. In all cases it will be seen that the fungicidal LD₅₀ values at 20 hours are less than those at 10 minutes, or at least do not differ significantly. Likewise, in most cases, the fungistatic LD₅₀ values are less than the fungicidal at 20 hours, and in some there is no significant difference. But with a few, e.g. copper sulphate on *S. fructicola*, the fungistatic LD₅₀ value is significantly higher than the fungicidal at 20 hours. In the fungistatic test the spores are exposed to the action of the chemical while in the presence of the small amount of ultrafiltered orange juice, but with the fungicidal exposures the orange juice is not present. It was thought that this absence of orange juice might explain the apparent discrepancy in the LD₅₀ values.

Accordingly a series of tests was performed on all fungi and chemicals in which the spores were exposed to the fungicidal action of the chemicals for 10 minutes or for 20 hours while in the presence of orange juice of the same concentration as employed in the fungistatic tests. That is, 0.1 per cent orange juice for *S. fructicola* and *A. solani*, and 0.316 per cent for *P. expansum* and *R. nigricans*. In all cases, with but four exceptions, the addition of orange juice did not significantly increase the LD₅₀ value. The four exceptions were: copper sulphate on *S. fructicola* in which the fungicidal LD₅₀ value at 10 minutes was increased 20-fold and at 20 hours 170-fold, and copper sulphate on *R. nigricans*, silver nitrate on *S. fructicola*, and sodium hypochlorite on *R. nigricans* where the increase was approximately 3- to 10-fold. Two tests were performed with copper sulphate on *S. fructicola*, and gave excellent agreement; the results of one are shown graphically in Figure 2. The effect of orange juice in increasing the resistance of the spores, or partially inactivating the chemical while restricted to very limited cases will explain the apparent discrepancies of Table I.

SLOPE OF FUNGICIDAL DOSAGE-RESPONSE CURVES

In earlier papers, studies have been made of the slope of the dosage-response curve for fungistatic action after 20 hours (6, 11) and for the response resulting from fungistatic action for a given dose after varying periods of time (10). The former showed that when percentage inhibition of germination is plotted as probits against the logarithm of the dose, straight lines usually result; exceptions were, however, noted. In the latter case it was found that a straight line relation resulted when probits were plotted against reciprocal time. Hence it was considered desirable to obtain some information relative to the nature of the fungicidal curves under these two circumstances.

The dosage-response curves obtained after 10 minutes' or 20 hours' exposure, when plotted on logarithmic-probability paper generally gave straight lines as illustrated in Figure 3. It was noted that in practically all

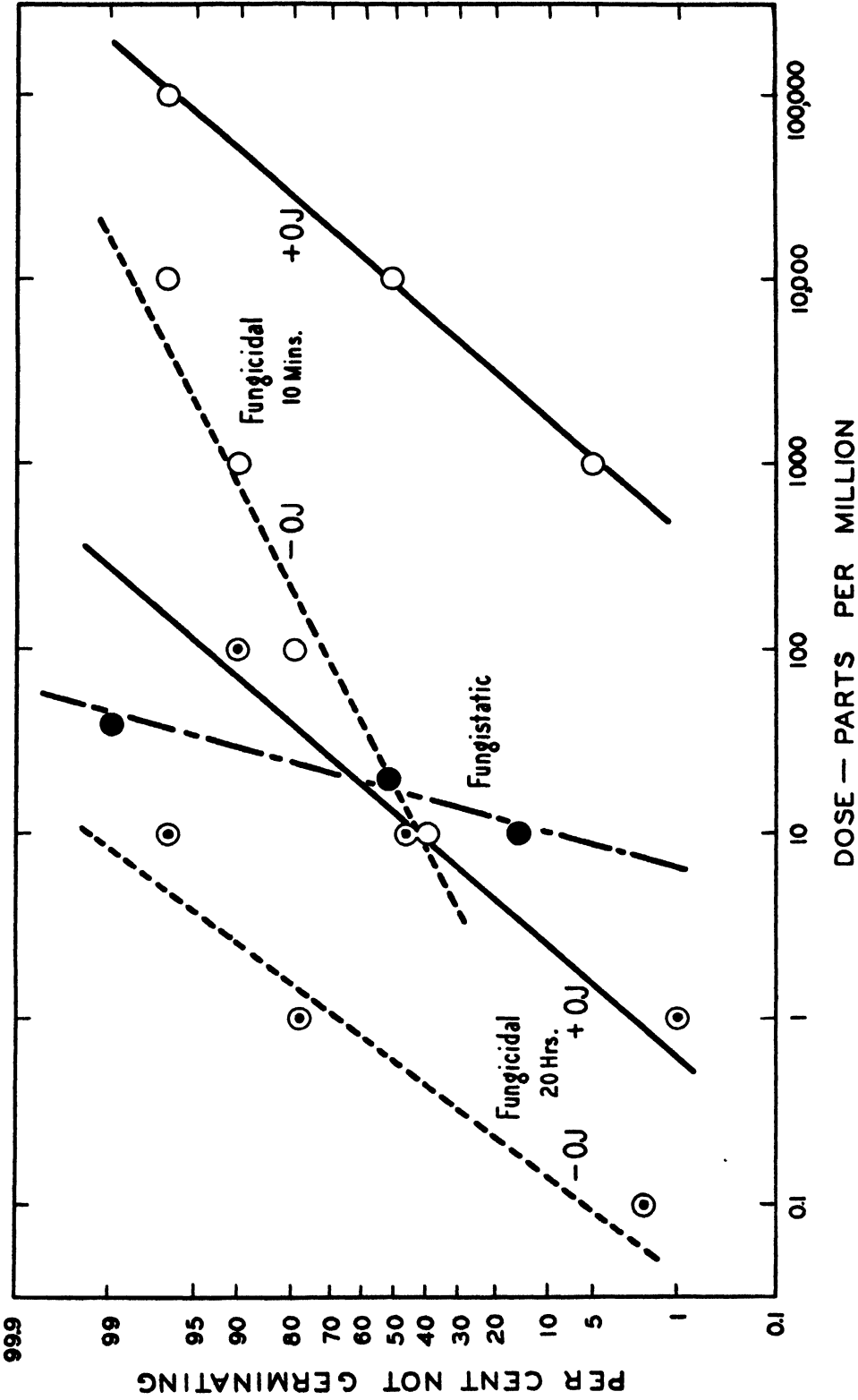


FIGURE 2. Effect of orange juice on the response of spores of *Sclerotinia fruticola* to the fungicidal action of copper sulphate after 10 minutes' and after 20 hours' exposure. Fungistatic curve also included.

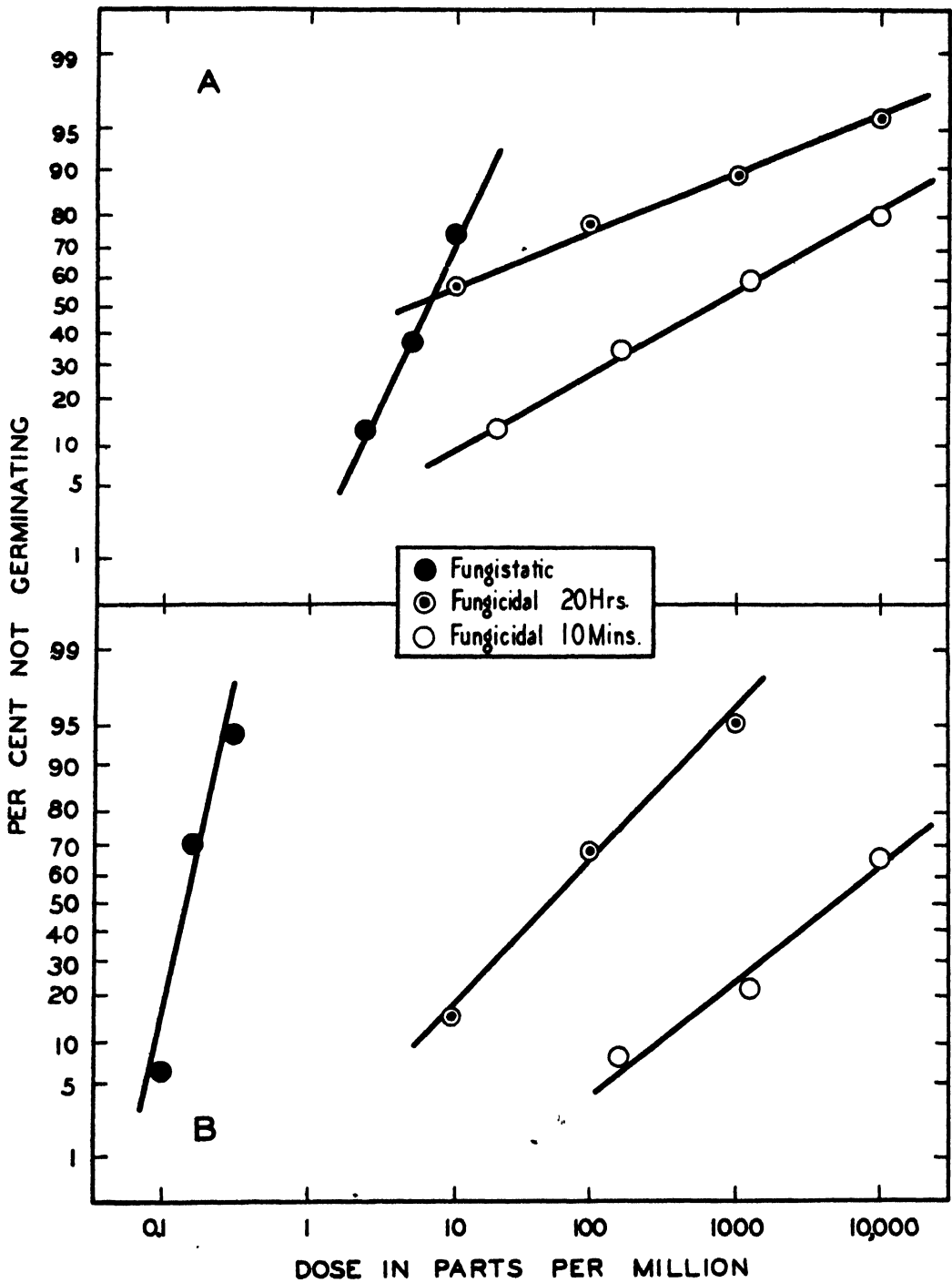


FIGURE 3. Examples of steep fungistatic dosage-response curve and flat fungicidal curves. Each curve result of one test, 100 spores per point. A. Uranium acetate on *Allernaria solani*. B. Potassium dichromate on *Rhizopus nigricans*.

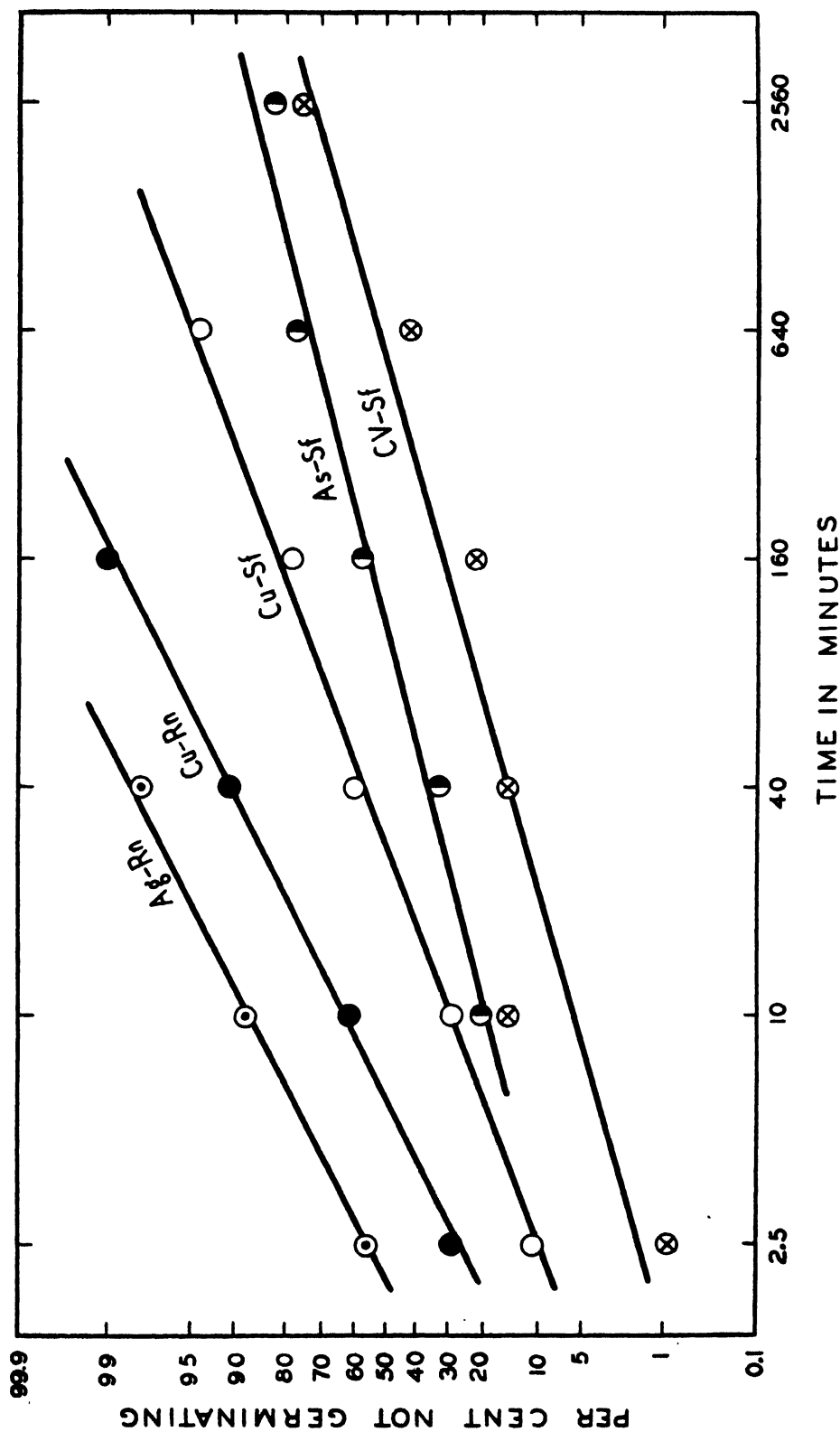


FIGURE 4. Time-response curves for fungicidal action respectively of: silver nitrate at 0.1 p.p.m. on *Rhizopus nigricans*, copper sulphate at 10 on *R. nigricans*, copper sulphate at 10 on *Sclerotinia fructicola*, sodium arsenate at 10,000 on *S. fructicola*, and crystal violet at 0.316 on *S. fructicola*.

cases the fungistatic curves had steep slopes, that is arithmetic λ values of less than 2, whereas, the fungicidal curves frequently had decidedly flatter slopes. This was the case particularly with the compounds copper sulphate, potassium dichromate, uranyl acetate, sodium arsenate, malachite green, berberine sulphate, and 8-hydroxyquinoline sulphate. The effect may also be noted in Figure 2. No explanation is offered to account for this difference in slope; however, if the theory is accepted that the straight line toxicity curve results from the normal distribution of individual lethal doses (11), then it follows in these cases that the individual sensitivity of spores to fungicidal agents is less homogenous than to fungistatic agents.

Time-response curves. In order to examine further the effect of fungicidal action after varying times of exposures, a study was made of *Sclerotinia fruticola* and *Rhizopus nigricans* with exposure times of 2.5, 10, 40, 160, 640, and 2560 minutes. The compounds selected were copper sulphate, silver nitrate, sodium arsenate, sodium hypochlorite, and crystal violet. These chemicals are representative of the A and C types listed on page 457 as well as of those having steep and flat fungicidal-dosage-response curves. These limited tests indicate, as illustrated in Figure 4, that the percentage of viable spores is normally distributed against the logarithm of the time of exposure to a given concentration of fungicidal agent. This response is distinct from that of a fungistatic agent in which it was previously shown (10) that the distribution was normal against reciprocal time.

SUMMARY

1. The fungicidal property of a material may be defined in general terms as the ability to kill or inhibit the development of fungus spores or mycelium. In the restricted sense fungicidal is the property of killing fungi, and fungistatic, the property of inhibiting.

2. Fifteen water-soluble chemicals were compared as to their fungicidal (restricted) and fungistatic action against spores of *Sclerotinia fruticola*, *Alternaria solani*, *Penicillium expansum*, and *Rhizopus nigricans*. The slide-moist chamber method was employed for the fungistatic tests; here the spores remain in contact with the chemical under conditions otherwise favorable for germination. In the fungicidal tests the spores were suspended in the chemical solutions for periods of time ranging from 2.5 minutes up to 2560 minutes. The chemical was removed by centrifuging and decanting, the spores washed and then allowed to germinate on slides in moist chambers.

3. Fungicidal activity cannot exceed fungistatic activity, hence the correlation between the two is high when (A) both values are high or (B) both values are low, but when (C) fungistatic activity is high and fungicidal activity is low, the correlation is poor. For all four fungi the results were: A. silver nitrate, mercuric chloride, sodium hypochlorite, iodine,

copper sulphate (except *Penicillium expansum*), crystal violet (except *Rhizopus nigricans*), and malachite green (except *P. expansum* and *R. nigricans*); B. formaldehyde, acetic acid, phenol, and berberine sulphate; C. potassium dichromate, uranyl acetate, sodium arsenate, 8-hydroxyquinoline sulphate, copper sulphate (*P. expansum* only), crystal violet (*R. nigricans* only), and malachite green (*P. expansum* and *R. nigricans* only).

4. In a few cases, especially copper sulphate on *Sclerotinia fructicola*, the addition of a small quantity of ultrafiltered orange juice markedly lowered the fungicidal action.

5. The fungicidal dosage-response curves gave straight lines when plotted on logarithmic-probability paper. In many cases the fungicidal curves were decidedly flatter than the corresponding fungistatic curves. Outstanding examples are copper sulphate, potassium dichromate, uranyl acetate, sodium arsenate, malachite green, berberine sulphate, and 8-hydroxyquinoline sulphate.

6. Limited data indicate that the fungicidal time-response curves give straight lines when plotted on logarithmic-probability paper.

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SYNTHESIS OF β -2-TRICHLOROETHYL-D-GLUCOSIDE AND ITS ISOLATION FROM CORN AND DANDELION PLANTS TREATED WITH CHLORAL HYDRATE

LAWRENCE P. MILLER

Previous experiments have shown that when chloral hydrate is absorbed by various species of plants, glycosides of trichloroethyl alcohol are formed. In the species thus far studied the glycoside isolated has been either β -2-trichloroethyl-D-glucoside or β -2-trichloroethylgentiobioside or a mixture (unpublished results with some solanaceous species) of these two (3, 4). In these experiments the glycosides have been obtained as the acetates and have been characterized through their identity with the corresponding synthetic glycoside acetates. In further studies reported in the present paper synthetic β -2-trichloroethyl-D-glucoside has been prepared for the first time by the deacetylation of synthetic β -2-trichloroethyl-D-glucoside tetraacetate and this glucoside has also been obtained by the deacetylation of the glucoside acetate isolated from corn (*Zea mays* L.) plants grown in a medium containing chloral hydrate and by direct isolation from dandelion (*Taraxacum officinale* Weber) tops by a procedure to be described. Dandelion tops have also been found to form from absorbed chloral hydrate a new and as yet unidentified glycoside which has been isolated in crystalline form as the acetate and which seems to be a glycoside of trichloroethyl alcohol and a C_{11} disaccharide.

EXPERIMENTAL

Synthetic β -2-Trichloroethyl-D-Glucoside

Synthetic β -2-trichloroethyl-D-glucoside tetraacetate (4) (1.09 g.) was dissolved in 20 cc. of anhydrous methanol and the solution cooled in an ice-salt mixture. The equivalent of 3 cc. N barium methylate in methyl alcohol was added and the mixture kept at 5° C. overnight (2). The solution was then neutralized exactly with N sulphuric acid and filtered through Celite. The filtrate was evaporated to dryness under reduced pressure and the residue dissolved in a mixture of absolute alcohol and ethyl acetate. The product crystallized after the addition of petroleum ether. Yield of crude product melting at about 150° was 0.65 g. or 91 per cent. Several recrystallizations from ethyl acetate gave 0.28 g. of the pure substance, melting point, 152.5° to 153.5° (corr.). Specific rotation was found to be $[\alpha]_D^{25} = -39.7^\circ$ (concn., 2.44 g. in 100 cc., H_2O).

Analysis: Calcd. for $C_8H_{13}O_6Cl_3$: Cl, 34.14. Found: Cl, 34.21.

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EXPERIMENTS WITH CORN PLANTS

Three seeds of Golden Bantam sweet corn were planted on July 8, 1941 in each of 60 six-inch clay pots filled with clean quartz sand and supplied with saucers. After the seed had germinated, 150 cc. of double strength nutrient solution based on the solution described by Shive and Robbins (5) and previously used for drip cultures (4) were added twice weekly. After the plants had shown some development, the cultures were thinned to two plants per pot. Since the use of saucers allowed unabsorbed salts to accumulate, the cultures were flushed through thoroughly on August 11, after which nutrient additions were again made as formerly. Beginning on August 13 when the plants averaged 32 inches in height, 0.6 millimol of chloral hydrate dissolved in 50 cc. of water was added to each of 50 cultures six times weekly. The quantity of chloral hydrate added was increased to one millimol for each application on August 18, and by August 23, when the addition of chemical was discontinued, each culture had received 8.4 millimols. By this time some evidence of injury was apparent as shown by yellowing and browning of some of the leaves. The untreated controls had well colored leaves and were also somewhat taller. The plants were sampled two days after the last application and the expressed juice and additional aqueous extracts obtained as described in previous papers (3). Analyses (Table I) of the expressed juices for non-ionic chlorine showed that appreciable quantities of chloral hydrate had been absorbed and that only a small quantity of non-ionic chlorine was recoverable on distillation; increased recovery of a compound containing non-ionic chlorine after hydrolysis by emulsion gave qualitative evidence of the presence of a β -glycoside involving the added chemical.

Isolation of β -2-trichloroethyl-D-glucoside as the tetraacetate from the tops and roots. The purification procedure followed to obtain preparations of the glycoside suitable for acetylation was the same as that used in the case of tomato plants treated with chloral hydrate (3). Three separate acetylations were carried out on acetone extracts of the concentrated lead precipitated and de-leaded tops juice. The acetylations were carried out separately in order to be able to obtain more readily separation of more than one product in case more than one glycoside was present. The solubility of different glycosides in the extractions with aqueous acetone would not be expected to be the same and the proportions found in the various acetone extracts would vary. Only one product was isolated, however. Preparations containing, totally, non-ionic chlorine equivalent to 22.2 millimols of a trichloro-compound, yielded on acetylation 5.77 g. of crude crystalline product. Several recrystallizations from absolute alcohol gave pure β -2-trichloroethyl-D-glucoside tetraacetate, melting point and mixed melting point, 144.5° to 145.5°; $[\alpha]_D^{26} = -29.3^\circ$ (concn., 4.165 g. in 100 cc., CHCl_3). Synthetic β -2-trichloroethyl-D-glucoside tetraacetate (4)

has a melting point of 144.5° to 145.5° and a specific rotation of $[\alpha]_D^{25} = -29.0^{\circ}$.

Analysis: Calcd. for β -2-trichloroethyl-D-glucoside tetraacetate, $C_{16}H_{21}O_{10}Cl_3$: Cl, 22.17. Found: Cl, 22.13.

The concentrate of tops juice remaining after five extractions with aqueous acetone still contained a considerable quantity of non-ionic chlorine. Since previous experience had shown that some glycosides which are removed with difficulty by the acetone procedure are separated by continuous extraction with ethyl acetate, such an ethyl acetate extraction was carried out on this material to determine if possibly another glycoside might be present in addition to the β -glucoside. Acetylation of the material extracted by ethyl acetate over a period of several days, however, yielded only the acetate of β -trichloroethyl-D-glucoside (2.48 g.).

Acetylation of a purified preparation of the glycoside from the roots, containing non-ionic chlorine equivalent to 3 millimols of a trichloro-compound gave 0.77 g. of crystalline acetate. After several recrystallizations from absolute alcohol the melting point was 144.5° to 145.5° and no depression was observed in a mixed melting point with synthetic β -2-trichloroethyl-D-glucoside tetraacetate. The specific rotation was $[\alpha]_D^{25} = -28.8^{\circ}$ (concn., 3.97 g., $CHCl_3$).

Deacetylation of the acetate from the tops to form β -2-trichloroethyl-D-glucoside. β -2-Trichloroethyl-D-glucoside tetraacetate (1.75 g.) from corn tops was dissolved in 25 cc. of absolute methanol and deacetylated with 5 cc. N barium methylate (2) as described for the synthetic compound. Yield of crude product, 1.00 g. or 91 per cent. Several recrystallizations from ethyl acetate gave pure β -2-trichloroethyl-D-glucoside with melting point and mixed melting point of 152.5° to 153.5° and a specific rotation of $[\alpha]_D^{25} = -40.5^{\circ}$ (concn., 3.36 g., H_2O).

Analysis: Calcd. for $C_8H_{13}O_6Cl_3$: Cl, 34.14. Found: Cl, 34.26.

EXPERIMENTS WITH DANDELION PLANTS

First Series

Dandelion root cuttings about 0.75 inch in length, collected in Lehigh County, Pennsylvania, were placed in moist chambers until small shoots had developed. They were then planted in sand cultures in 24 five-inch clay pots and the cultures handled as described above for corn. The plants grew rapidly and after about one month the older leaves were eight to ten inches long. The addition of chloral hydrate was begun at this stage at the rate of 0.7 millimol six times weekly until each culture had received 7.7 millimols. Two days after the last application the plants were sampled. Analytical data, based on quantitative determinations of the non-ionic chlorine content of the expressed juice from the tops and roots, are given in Table I.

TABLE I

FORMATION OF β -GLYCOSIDES IN CORN AND DANDELION PLANTS GROWN IN A NUTRIENT MEDIUM CONTAINING CHLORAL HYDRATE

Species	Chloral hydrate added, millimols per culture	Amt. absorbed, millimols per 100 cc. juice		Recovery on distillation			
				Tops		Roots	
		Tops	Roots	Emulsion hydrolysis	Without emulsion	Emulsion hydrolysis	Without emulsion
<i>Zea mays</i>	8.4	0.78	0.39	0.25	0.07	0.13	0.07
<i>Taraxacum officinale</i>	7.7	2.30	2.17	0.35	0.07	0.53	0.03

Isolation of two chlorine-containing glycosides as the acetates from the tops. The expressed juice and aqueous extracts obtained from the tops when carried through the regular purification procedure failed to give, on acetylation of the purified extracts, a product which crystallized from absolute alcohol. When the acetylated material, dissolved in a small amount of ethyl alcohol, was stirred into a large quantity of boiling water, several fractions based on the rate at which the material separated out from the water were obtained and these on further solution in alcohol and water gave crystalline acetates. In this way preparations which contained non-ionic chlorine equivalent to 6.4 millimols of a trichloro-compound gave a total of 1.79 g. of crude product which melted from 123° to 135° . On fractional crystallization from absolute alcohol, two substances were separated, the one being β -2-trichloroethyl-D-glucoside tetraacetate with a melting point of 144.5° to 145.5° and which showed no depression in a mixed melting point determination with the synthetic compound, and the other being a new glycoside not previously obtained. About 75 per cent of the crystalline product consisted of this new glycoside acetate. In melting point determinations this substance will melt either partially or completely (depending upon the particular specimen) at 158° to 159° . If only partially melted, or if completely liquid and allowed to crystallize again in the tube, further heating will give a sharp melting point at 170° to 171° . Specific rotation was $[\alpha]_D^{25} = -47.2^{\circ}$ (concn., 2.87, CHCl_3). The chlorine content corresponded to that required for the hexaacetate of a trichloroethyl glycoside involving a C_{11} disaccharide. A positive test for pentose was obtained with Bial's solution (1, p. 770). Further work is now being carried out which it is hoped will lead to the characterization of this glycoside through synthesis.

Analysis: Calcd. for the hexaacetate of a trichloroethyl glycoside of a C_{11} disaccharide, $\text{C}_{28}\text{H}_{33}\text{O}_{16}\text{Cl}_3$: Cl, 15.29. Found: Cl, 15.39.

Isolation of β -2-trichloroethyl-D-glucoside as the tetraacetate from the roots. Acetylation of purified preparations from the roots, containing non-ionic chlorine equivalent to 5.2 millimols of a trichloro-compound, follow-

ing the usual procedure, gave 1.59 of crude product. Recrystallization from absolute alcohol yielded pure β -2-trichloroethyl-D-glucoside, melting point and mixed melting point, 144.5° to 145.5° ; $[\alpha]_D^{25} = -28.7^{\circ}$ (concn., 5.05, CHCl_3). None of the new glycoside obtained from the tops was isolated from the root sample.

Analysis: Calcd. for $\text{C}_{16}\text{H}_{21}\text{O}_{10}\text{Cl}_3$: Cl, 22.17. Found: Cl, 22.12.

Second Series

For the second experiment with the dandelion, young plants were collected in the vicinity of Yonkers, New York, and transferred to sand cultures. When the older leaves averaged six inches in length, the addition of chloral hydrate was started at the rate of 0.7 millimol six times weekly to each of 78 cultures. Each culture received a total of 8.4 millimols and the leaves were sampled one week after the last application. The leaves (1126 g.) were ground through a food chopper and the expressed juice obtained; two further aqueous extracts were also made following the procedure described previously (3).

Direct isolation of β -2-trichloroethyl-D-glucoside. The combined extracts were treated with an excess of lead acetate, filtered, and the lead removed from the filtrate by H_2S . The lead sulphide was filtered off and a stream of N passed through the filtrate to remove H_2S . The filtrate was then concentrated under reduced pressure to about 350 cc. and placed in a continuous extractor in which it was extracted with ethyl ether. On spontaneous evaporation of the ether solution resulting from several hours of extraction, crystals separated out. These were filtered off, washed with ether, and were found to have a melting point within a few degrees of synthetic β -2-trichloroethyl-D-glucoside. Extraction with ether was continued for several days, until no further separation of crystalline material could be made. To obtain the crystalline glucoside from the ether solutions it was found most convenient to evaporate off the ether under reduced pressure, drive off the water with absolute alcohol, and finally dissolve the residue in a minimum of absolute alcohol and precipitate the glucoside by addition of petroleum ether. In this way a total of 5.88 g. of product was obtained. Furthermore, acetylation of the mother liquors which remained from the crystallizations gave an additional 3.38 g. of β -2-trichloroethyl-D-glucoside tetraacetate, equivalent to 2.2 g. of the glucoside. Extraction with ether did not seem to remove any of the second chlorine-containing glycoside present in the dandelion leaves. In order to characterize definitely the crystals obtained from the ether extractions, a 1.28 g. portion melting at 151.5° to 152.5° was recrystallized twice from ethyl acetate to give 0.57 g. melting at 152.5° to 153.5° . No depression in melting point was observed in a mixed melting point determination with synthetic β -2-trichloroethyl-D-glucoside. Specific rotation was $[\alpha]_D^{25} = -39.6$ (concn., 3.49, H_2O).

The concentrated dandelion extract which yielded no further material

on extraction with ether was then extracted with several portions of ethyl acetate over a period of a number of days. The material extracted by the ethyl acetate, acetylated in four separate portions, gave a total of 2.45 g. of crystalline acetates. Of this amount only about 0.1 g. was the tetraacetate of β -2-trichloroethyl-D-glucoside, while the remainder consisted of the new glycoside acetate obtained also in the first series. One recrystallization gave the pure substance identical in melting point with that previously obtained.

The new procedure is thus definitely superior in that not only does it give crystalline β -2-trichloroethyl-D-glucoside directly, but also gives a separation of this glucoside from the second glycoside formed in the leaves from absorbed chloral hydrate.

It will be noted that the total quantity of glycosides isolated from the leaves was equivalent to nearly 1 per cent of the fresh weight. Since considerable losses no doubt occurred in the many manipulations involved, it is clear that the leaves probably contained well over 1 per cent of glycosides on a *fresh* weight basis.

SUMMARY

Deacetylation of synthetic β -2-trichloroethyl-D-glucoside tetraacetate gave crystalline β -2-trichloroethyl-D-glucoside, m.p. 152.5° to 153.5° and $[\alpha]_D^{25} = -39.7^{\circ}$ (H_2O).

Corn plants grown in a medium containing chloral hydrate were shown to form β -2-trichloroethyl-D-glucoside in both tops and roots through the isolation of the glucoside as the crystalline acetate. Deacetylation of some of the acetate from the tops yielded β -2-trichloroethyl-D-glucoside.

β -2-Trichloroethyl-D-glucoside was also obtained as the tetraacetate from both tops and roots of dandelion plants which had been treated with chloral hydrate. This glucoside was also obtained in crystalline form directly from the leaves by ether extraction of aqueous extracts which had been subjected to lead precipitation. In addition, a new glycoside, not yet identified, was isolated as the crystalline acetate from the leaves. This appears to be a trichloroethyl glycoside involving a C_{11} disaccharide.

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ADULT LIFE SPAN ANIMAL FEEDING EXPERIMENTS WITH THIOUREA (THIOCARBAMIDE)

ALBERT HARTZELL

The use of thiourea, NH_2CSNH_2 , also called thiocarbamide, to prevent the browning of the cut surface of fruits and vegetables (3) has necessitated a study of the toxic effect in order to determine whether the use of this compound in food would be injurious to health.

Preliminary experiments conducted by the present writer (7) have indicated that on the basis of body weight rats can ingest without harmful effects 570 times and guinea pigs 523 times the amount of thiourea consumed daily by a 70-kg. man in eating 200 g. of fruit treated with 0.05 per cent thiourea solution.

Gast (5), in an extensive study of the origin and significance of thiosulphate in the animal organism, found that thiourea produced no extra thiosulphate excretion.

Blood (1) found that thiourea administered either orally or subcutaneously produced a decrease in urinary sulphate but no change in ethereal sulphate and concluded that the action of thiourea is not due to toxicity.¹

Feeding experiments with dogs and mice by Hans Molitor (7, p. 259) also indicate that thiourea is a non-toxic substance.

Flinn and Geary (4) find no unfavorable effects on rats and rabbits with 300 to 700 times the amount of thiourea ingested by a man eating 200 g. of treated fruit.

Experience has emphasized, nevertheless, the necessity of studying from the public health standpoint the possible chronic effects of continual intake of small amounts of substances ingested with food. As no prolonged feeding experiments with thiourea have been reported in the literature, the writer undertook to study the possible chronic effects of continued feeding of thiourea to herds of rats and mice throughout the adult life of the animals. These tests are still in progress but as the experiment with mice has run for a period of approximately 2 years, covering the entire adult life span of these mice, it was thought that a brief summary of the results might be of interest. The feeding experiment with rats here reported covers only 53 weeks, the duration of the experiment to date. A full report on the rats will be made when their life span is completed. Because of the difficulty of feeding large numbers of animals by means of a pipette or

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¹ Thiourea is definitely known to be toxic to cold-blooded animals such as insects. According to Minaeff and Wright (9), thiourea has mothproofing properties. Recently Hoskins, Bloxham, and Van Ess (8) have reported that thiourea is toxic to the larva of the fleshfly (*Lucilia sericata*).

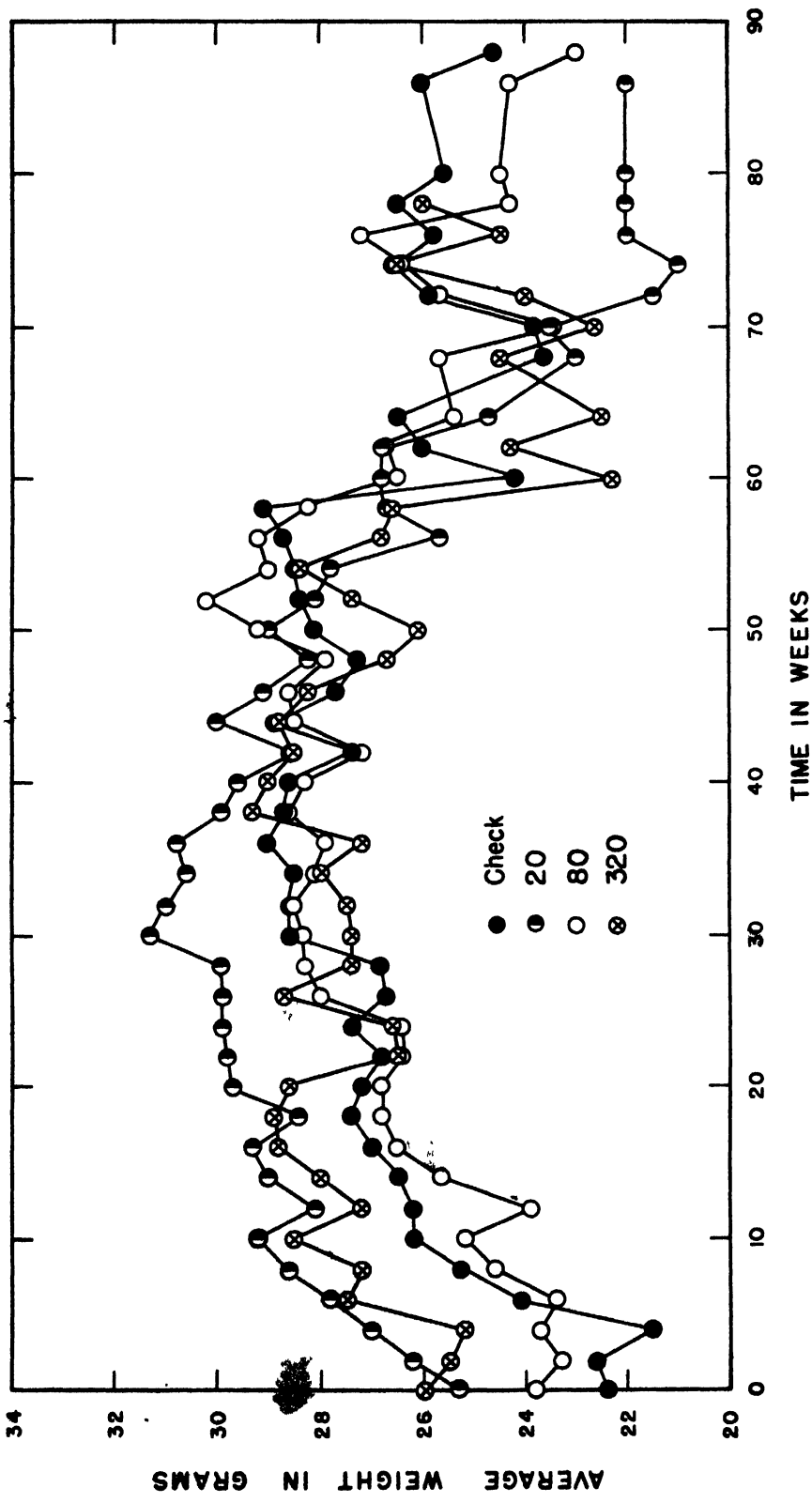


FIGURE 1. Curves for average weight of mice fed thiourea in the following amounts in mg. per kg. of body weight: 1.72, or 20 times man's dose; 6.88, or 80 times man's dose; and 27.5, or 320 times man's dose.

stomach tube, thiourea was dissolved in tap water and fed in the drinking water.

FEEDING TESTS WITH MICE

Albino mice² were selected for the purpose of studying the possible chronic effects of continuous feeding of thiourea. Previous tests had indicated that the mouse would drink approximately 5 cc. per day under normal conditions of temperature. The weight of the mice at the beginning of the experiment averaged 25 g. One hundred and twenty mice, approximately two months old and segregated by sex, ten to a cage, were used in these tests as follows:

(a) 20 mice fed 1.72 mg. per kg. of body weight daily, or 20 times man's dose.³

(b) 20 mice fed 6.88 mg. per kg. of body weight daily, or 80 times man's dose.

(c) 20 mice fed 27.5 mg. per kg. of body weight daily, or 320 times man's dose.

(d) 60 control mice.

The ration consisted of Beacon dog pellets.⁴ This was supplemented by a weekly ration of cabbage and carrots. During the winter months cod liver oil at the rate of 0.25 cc. per mouse was fed weekly on the cabbage leaves.

The mice were weighed by cage groups at intervals of two weeks and the dose of thiourea (C.P.) adjusted to the average gain or loss in weight of each group treated.

The effects of these treatments on the average weight of the mice are shown graphically in Figure 1. It will be observed in Figure 1 that the mice in both the treated and the check showed normal weight curves. There were no significant differences due to treatment. The general appearance of the mice was normal throughout the experiment in both the controls and treated, as shown in Figures 2 and 3. The percentage survival at the end of seven months was 80 per cent. This compares with 83.6 per cent survival for a normal herd of mice reported by Greenwood and others (6) for the same period. Unfortunately there are no figures available on the survival of a normal herd of mice until time of death of the last animal.

The number of mice surviving at the end of one year was 28 in the checks and 24 in the treated. If a comparison is made of total deaths in any treated cage with the check lots, it will be found that there are no definite differences between treated and check lots. The mortality of the mice

² Obtained from Breeder Co., 446 East 104th Street, New York, N. Y.

³ Man's dose equals 0.086 mg. per kg. of body weight (7).

⁴ Manufactured by the Beacon Milling Co., Inc., Cayuga, N. Y. The guarantee, as stated on the container, follows: protein, not less than 24%; fat, not less than 3%; fiber, not more than 5%; carbohydrates, not less than 50%.

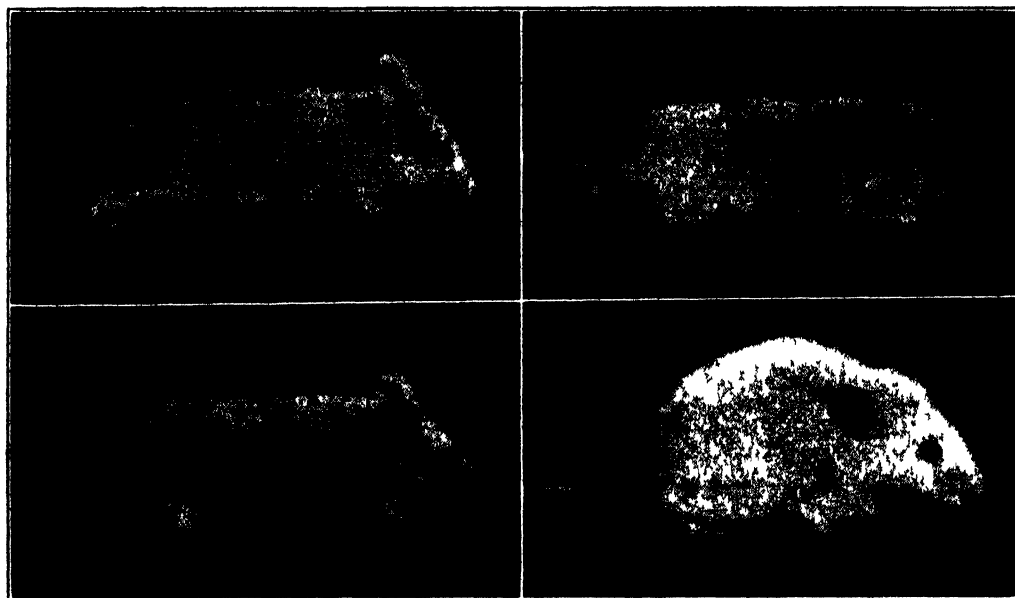


FIGURE 2. Mice used in thiourea feeding experiment photographed at the end of one-year period. A. Control. B. Mouse fed 1.72 mg. per kg. of body weight, or 20 times man's dose. C. Mouse fed 6.88 mg. per kg. of body weight, or 80 times man's dose. D. Mouse fed 27.5 mg. per kg. of body weight, or 320 times man's dose.

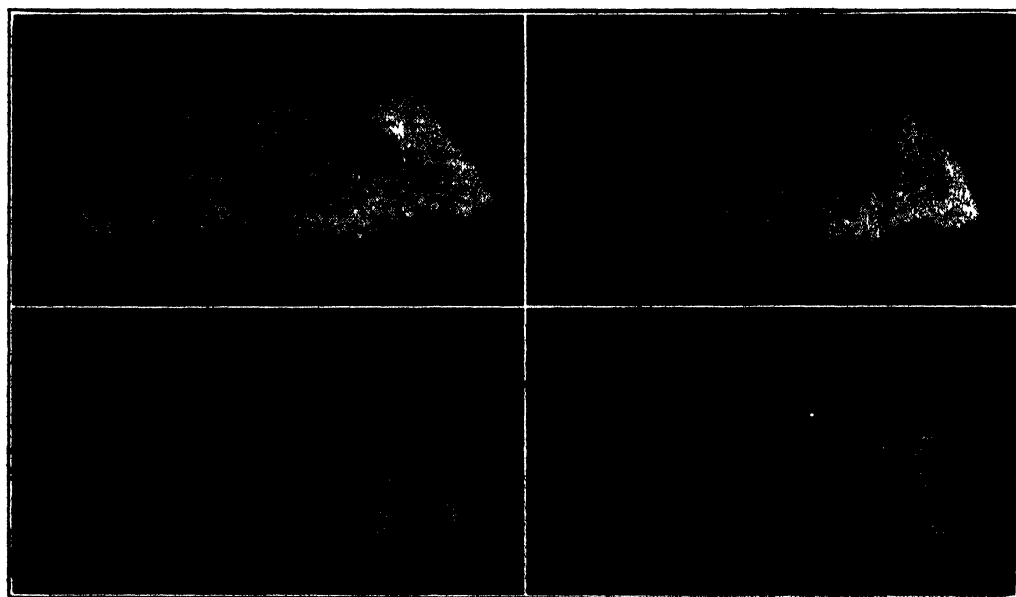


FIGURE 3. Mice used in thiourea feeding experiment photographed at the end of one year and six months. A. Control. B. Mouse fed 1.72 mg. per kg. of body weight, or 20 times man's dose. C. Mouse fed 6.88 mg. per kg. of body weight, or 80 times man's dose. D. Mouse fed 27.5 mg. per kg. of body weight, or 320 times man's dose.

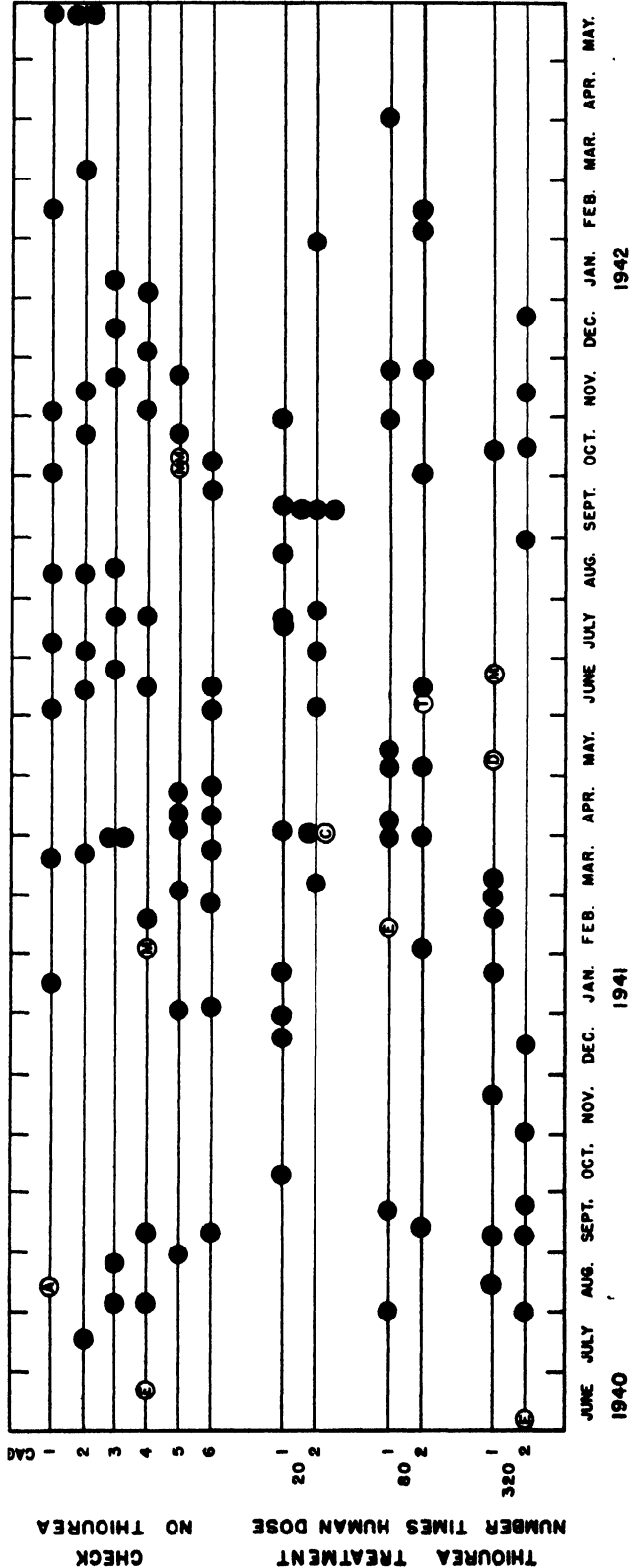


FIGURE 4. Mortality chart of mice in thiourea feeding experiment: solid circle = normal; (A) = accidental; (C) = cannibalism; (D) = middle ear disease; (E) = escaped; (M) = missing; (T) = tumor.

throughout the experiment is shown graphically in Figure 4. An examination of the curve (Fig. 5) shows that the points for both treated and check fall on a characteristic mortality curve. The ratio of males to females at the beginning of the experiment was 2 to 1. At the end of the first year the

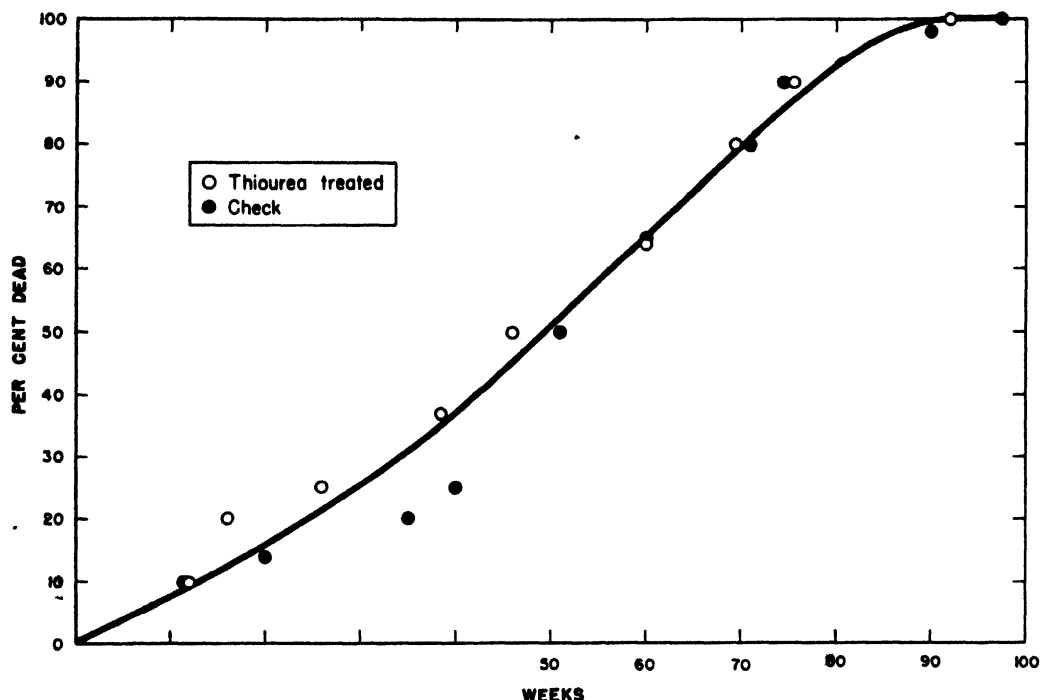


FIGURE 5. Mortality curve of mice in thiourea feeding experiment.

ratio was 2.1 to 1 and remained fairly constant throughout the course of the experiment.

Autopsies⁵ and bacteriological examinations made of mice that died showed no evidence of epidemic disease throughout the course of the experiment nor any pathologic change that could be attributed to thiourea (Table I).

PRELIMINARY FEEDING TESTS WITH RATS

Albino rats⁶ were selected for the purpose of studying the possible chronic effects of continuous feeding of thiourea. Previous tests had indicated that the rat would drink approximately 29.2 cc. per day under normal conditions of temperature. The weight of the rats at the beginning of the experiment averaged 161.9 g. One hundred and nineteen female rats, approximately three months old, five to a cage, were used in these

⁵ The writer is indebted to Dr. Ward H. Cook and Dr. Kendrick McCullough of the Bureau of Laboratories, Yonkers, N. Y., for autopsies of animals.

⁶ Sherman strain obtained from Columbia University, New York, N. Y.

TABLE I

TYPICAL GROSS FINDING AT AUTOPSY OF RATS* AND MICE** WHICH DIED IN THE THIOUREA-FEEDING EXPERIMENT

Organs	Mice				Rats	
	Concentration of thiourea times man's dose†					
	0 (Check)	20 (1.72 mg./kg.)	80 (6.88 mg./kg.)	320 (27.5 mg./kg.)	0 (Check)	20 (1.72 mg./kg.)
Brain	Congested	Congested, occasionally necrotic	Normal	Congested	Diffuse encephalitis	Normal
Lungs	Moderately distended, congested and hemorrhagic. Bronchopneumonia	Congested, cellular infiltration, pneumonic areas	Moderately distended and hemorrhagic	Congested and hemorrhagic	Bronchopneumonia and acute fibrinous pleuritis	Peribronchial foci of pneumonia, with mononuclear infiltration
Heart	Normal	Normal	Occasionally distended	Normal	Normal	Normal
Spleen	Congested	Normal	Normal	Congested	Normal	Normal
Liver	Congested. Occasionally pale, shrunken, coarsely granular, soft, acute necrotizing hepatitis	Congested	Occasionally fatty change	Congested	Normal	Normal
Gall-bladder	Normal	Normal	Occasionally distended	Normal	††	††
Stomach	Occasionally distended	Normal	Normal	Normal	Normal	Normal
Intestines	Congested	Normal	Congested	Congested	Normal	Normal
Kidneys	Congested. Few small interstitial foci of mononuclear cells	Congested	Normal	Congested	Normal	Normal

* Based on 4 animals that died during the first 53 weeks of the experiment. Three rats that died of middle ear disease not autopsied.

** Based on 36 animals that died during the course of the experiment.

† Man's dose equals 0.086 mg. per kg. of body weight.

†† Gall bladder absent in the rat.

tests as follows:

(a) 30 rats fed 1.72 mg. per kg. of body weight daily, or 20 times man's dose.

(b) 29 rats fed 6.88 mg. per kg. of body weight daily, or 80 times man's dose.

(c) 30 rats fed 27.5 mg. per kg. of body weight daily, or 320 times man's dose.

(d) 30 control rats.

The ration consisted of complete Rockland rat diet pellets.⁷

The rats were weighed by cage groups at intervals of one week until full grown and the dose of thiourea (recrystallized three times) adjusted to the average gain or loss in weight of each group treated. After the rats were full grown, weighings were made every two weeks and doses adjusted to the average gain or loss in weight.

⁷ Manufactured by the Arcady Farms Milling Co., Chicago, Ill. The manufacturer's guarantee is as follows: protein, 24%; fat, 4%; fiber, 4%; carbohydrates, 49.83%.

Studies by Buchbinder *et al.* (2) have shown that many rodents are chronic excretors of paratyphoid organisms and that it is possible for some rats to become chronic carriers for the duration of their natural lives. All rats in this experiment, therefore, were tested bimonthly for the presence

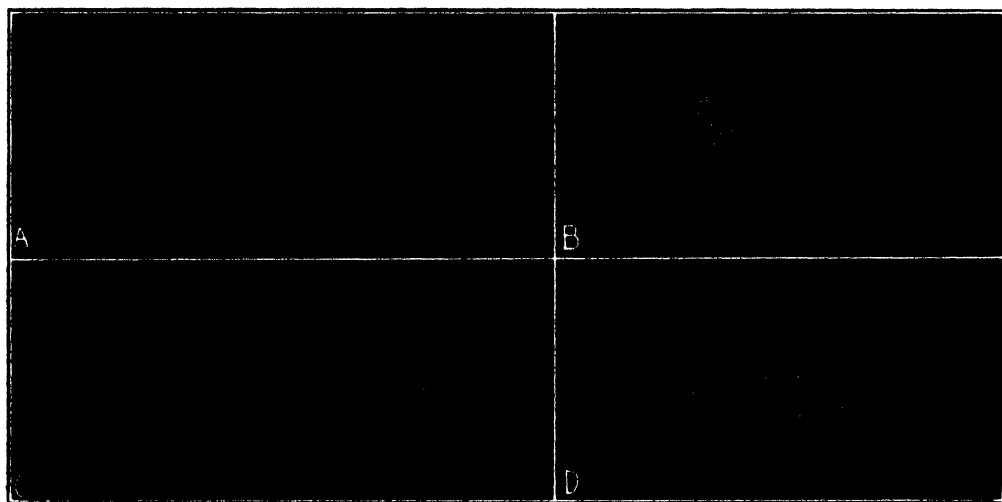


FIGURE 6. Rats used in thiourea feeding experiments photographed at the end of one year. A. Control. B. Rat fed 1.72 mg. per kg. of body weight, or 20 times man's dose. C. Rat fed 6.88 mg. per kg. of body weight, or 80 times man's dose. D. Rat fed 27.5 mg. per kg. of body weight, or 320 times man's dose.

of paratyphoid bacilli in the feces by the brilliant green agar test as described by the above named authors. We are indebted to Dr. C. A. Slanetz of Columbia University, New York, N. Y., for the paratyphoid tests, the results of which fortunately have all been negative.

The effects of thiourea treatments on the average weight of the rats are shown in Figure 7. It will be observed in Figure 7 that the rats in both the treated and check showed normal weight curves. There were no significant differences due to treatment and the general appearance of the rats is normal in both the controls and treated groups (Fig. 6).

Only seven rats died during the 53-week period, as follows: two in the control, four in the lowest concentration of thiourea (1.72 mg. per kg. of body weight), and one in the medium concentration (6.88 mg. per kg. of body weight).

Autopsies made of rats that died showed no evidence of epidemic disease, nor pathologic change that could be attributed to thiourea (Table I).

SUMMARY

Mice fed thiourea (NH_2CSNH_2 , also called thiocarbamide) throughout their adult life span for a period of approximately 24 months in the drink-

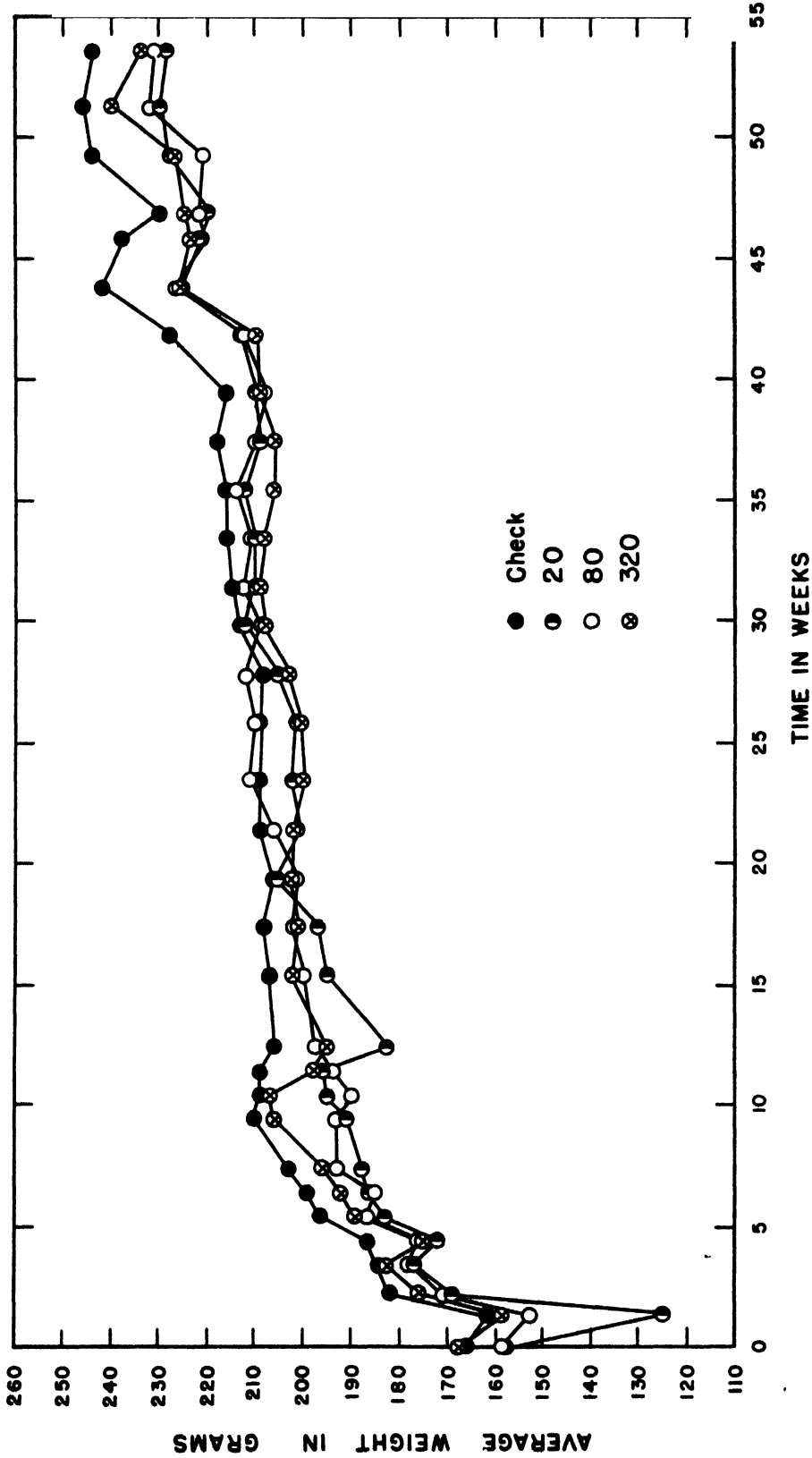


FIGURE 7. Curves for average weight of rats fed thiourea in the following amounts in mg. per kg. of body weight: 1.72, or 20 times man's dose: 6.88, or 80 times man's dose: and 27.5, or 320 times man's dose.

ing water at the rate of 1.72 mg., 6.88 mg., and 27.5 mg. per kg. of body weight daily showed normal growth.

The general appearance of the treated mice was normal when compared with the checks.

There was no evidence of an accumulative effect upon the survival rate of mice even with the highest amount of thiourea used or that thiourea either increased or diminished the mortality rate.

Rats fed thiourea for a portion of their life span for a period of 53 weeks in the drinking water at the rate of 1.72 mg., 6.88 mg., and 27.5 mg. per kg. of body weight daily also showed normal growth. Treated and control rats were normal in appearance at the end of this period.

Only seven rats of 119 have died during the 53-week period, two in the check, four in the low, one in the medium, and none in the group receiving high concentrations of thiourea.

Autopsies of mice and rats revealed no pathologic change that could be attributed to thiourea.

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VEGETATIVE PROPAGATION OF RED SQUILL

ALBERT HARTZELL

Powders made of the dried bulbs of red squill (*Urginea maritima* [L.] Baker) have been used extensively as raticides (2). *Urginea maritima* (Fig. 1 D) is a liliaceous plant that grows wild on the coast of southern Italy, Sicily, Sardinia, southern France, Greece, North Africa, and elsewhere along the Mediterranean Sea (2, 5). The bulbs produced by the plants are very large (7.5 cm. to 15 cm. in diameter), pear-shaped, and weigh from 300 g. to 2 kg. The bulb is composed of overlapping fleshy scales. The outer scales are brittle and reddish-brown in color while the inner scales vary from light yellow to mahogany. The scales of the central core are usually white.

In several respects red squill fulfills the requirements of an ideal raticide. The rat poisons commonly used contain as the effective ingredients either arsenic, barium, phosphorus, thallium, or strychnine, all of which severely menace the safety of man and other animals. Animals other than rats and mice usually refuse to eat red squill in concentrations used in raticides (2). As red squill is a potent emetic (4, p. 252) there is little likelihood of animals other than rodents retaining enough of the ingested material in the stomach for a sufficient length of time to cause fatal poisoning.

The toxic principle of red squill was found by Stoll and Renz (3) to be a glucoside named scilliroside. The median lethal dose for rats was determined to be approximately 0.070 mg. per kg. of body weight.

Unfortunately the war has cut off the supply of red squill from the western hemisphere. As the number of bulbs available in this country is very limited, and the life history of the plant is relatively long, a rapid method of propagation that will permit the securing of the greatest number of plants from a single bulb is desired. Little appears to have been published on the life history and methods of propagation of red squill. There is no agreement, for instance, on the length of time between blooming periods (1).

The red squill plants in the present experiments were grown from bulbs obtained in June, 1933, through the courtesy of Mr. F. M. Connable, of the K-R-O Company, Springfield, Ohio. They were grown in 12-inch pots in the greenhouse. The plants bloomed in August, 1933 (Fig. 1 A), and again in August, 1941 (Fig. 1 B and C). The flower stalk is steel blue in color, over a yard in height, and terminates in a raceme of pale yellow and white flowers. The color is more intense in the center of the segments. The flowers failed to set seed. The leaves appear after the flower stalks.

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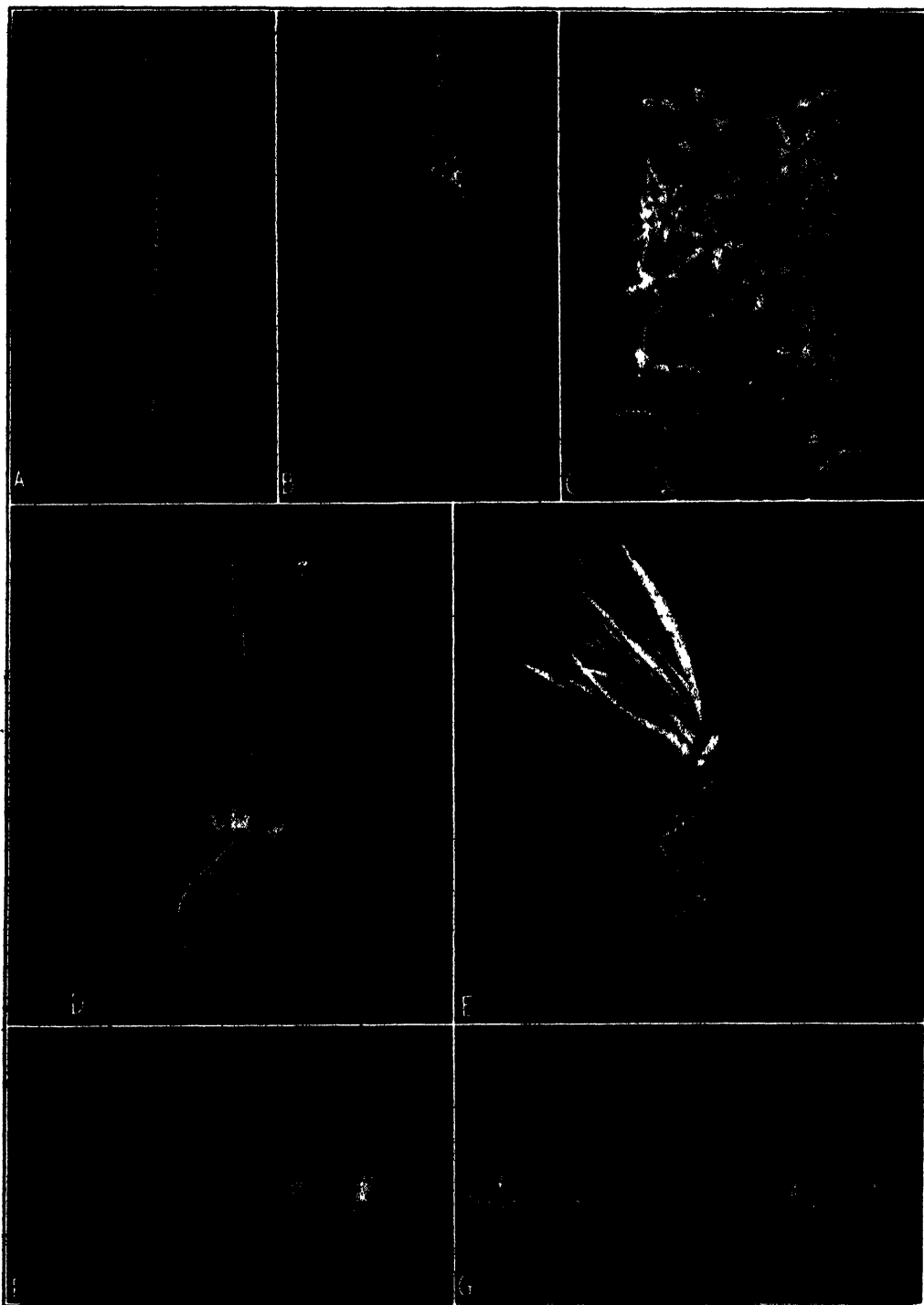


FIGURE 1. A. Red squill plant in bloom. Rule at right graduated in inches for comparison. B. Raceme of red squill. C. Enlargement of raceme showing individual florets. D. Red squill plant showing leaves, bulb, and roots. E. Plants grown from a segment of bulb with the stem portion attached. F. Scales showing bulblets and roots. G. Young plants grown from scales.

In March, 1942, several bulbs were sliced longitudinally. The scales¹ were carefully separated and removed from the base of the bulb and planted to a depth of one and one-half inches in moist sand in a greenhouse bench. The house was kept at approximately 70° F. at night. A large proportion of the scales produced bulblets and roots (Fig. 1 F). When the bulblets had rooted they were planted in soil in 4-inch pots and kept in the greenhouse. Representative plants (Fig. 1 G) were photographed August 12, 1942. In some cases segments of the bulbs with the stem portion attached produced several vigorous plants (Fig. 1 E) under greenhouse conditions. In many respects the regeneration of red squill from storage scales resembles that of the Easter lily (*Lilium longiflorum* Thunb.).

From the results obtained in greenhouses it appears logical to assume that red squill could be propagated readily and grown in the southern states of this country. In the northern states greenhouse culture would be possible but perhaps not profitable.

In order to determine whether the particular strain of red squill used in the present vegetative propagation experiments was toxic to rats, a bulb taken from the same lot was used to prepare red squill powder. Thin slices of this bulb were dried in an oven at 80° C. for 24 hours. The dried slices were ground into a powder. A bait was made by mixing 1 g. of this powder with 15 g. of powdered milk. Two caged female albino rats² one year old weighing 212 g. and 249 g., respectively, were fed the above named bait. Both were dead in 12 hours. Eight control rats remained normal.

SUMMARY

Red squill (*Urginea maritima*) was propagated vegetatively by scales from bulbs under greenhouse conditions. Powder made from bulbs of the same strain of red squill as used for propagation was found to be toxic to rats.

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¹ Both the viscous mucilaginous juice and the dry scales are irritating to the skin. The stinging effect on the skin and the acrid prickly taste are attributed to calcium oxalate raphides (2).

² Sherman strain obtained from Columbia University, New York, N. Y.

EFFECT OF THE ADDITION OF NITROGEN UPON GERMINATION OF SEEDS OF SYMPHORICARPOS RACEMOSUS

FLORENCE FLEMION

In order to induce germination in seeds of snowberry (*Symphoricarpos racemosus* Michx.) the seed coats must be partially destroyed prior to the required period at low temperature (1). When the seeds are kept in moist peat moss at 25° C. for three to four months the coats disintegrate sufficiently so that germination can subsequently occur. In the experiments described below, nitrogen as potassium nitrate (KNO_3), ammonium nitrate (NH_4NO_3), and ammonium sulphate ($[\text{NH}_4]_2\text{SO}_4$) was added to the medium at various intervals during the period in which the seeds were held at 25° C. This addition of nitrogen resulted in higher germination percentages.

MATERIALS AND METHODS

The berries were collected in the vicinity of the Institute at Yonkers, New York. They were freed of pulp, washed, laid out in thin layers to dry, and then stored at room temperature.

Duplicate lots of 200 seeds each were mixed in moist, granulated peat moss (purchased from Atkins & Durbrow Inc., New York, N. Y.), placed in bottles, and kept at 25° C. On every fifth or sixth day 5 cc. aliquots of solutions containing KNO_3 , NH_4NO_3 , and $(\text{NH}_4)_2\text{SO}_4$ were thoroughly mixed in the peat. The solutions of the three salts were equivalent in nitrogen content. The total amount of nitrogen mixed in with the seeds during the period at 25° C. varied from 0 to 82.8 mg.

After the various periods at 25° C. (during which nitrogen had been added at regular intervals) the seeds were removed by washing the peat through a sieve. The seeds were then mixed in untreated, damp peat moss and kept at the required after-ripening temperature of 5° C. for six months during which germination occurred. The effect of adding nitrogen to the media while the seeds were held at 25° C. is shown by the percentage germination obtained as compared with controls receiving no added nitrogen.

RESULTS

In a preliminary experiment two concentrations each of potassium nitrate and ammonium sulphate were tested. In one set, seeds were held in moist peat moss at 25° C. for 7, 10, and 13 weeks during which nitrogen was added, while to another set held at 25° C. only water was added during the first three weeks and nitrogen added in the subsequent 4, 7, and 10 weeks. After these various treatments at 25° C. the seeds were transferred

to 5° C. for subsequent germination. The results, as seen in Table I, when compared with the controls to which only water had been added, showed higher germination percentage in many of the treated lots.

TABLE I

EFFECT OF ADDITION OF NITROGEN COMPOUNDS TO PEAT MOSS DURING VARIOUS PERIODS AT 25° C. UPON SUBSEQUENT GERMINATION OF SNOWBERRY SEEDS*

Total weeks at 25° C.	Lots treated during entire period at 25° C.		Nitrogen addition started after 3 weeks at 25° C.	
	Total mg. of N** added	% Germ. after 6 mos. at 5° C.	Total mg. of N** added	% Germ. after 6 mos. at 5° C.
7	8.48	11	5.30	11
	5.54	20	3.47	31
	1.69	32	1.06	17
	1.11	39	0.69	20
	0	23	0	22
10	11.66	43	8.48	39
	7.62	48	5.54	67
	2.33	54	1.69	62
	1.52	69	1.11	76
	0	43	0	46
13	14.84	45	11.66	50
	9.70	56	7.62	60
	2.90	67	2.33	79
	1.95	76	1.52	81
	0	68	0	74

* Experiment started February, 1934.

** Source of nitrogen as follows: 1st and 3rd figures in each column, $(\text{NH}_4)_2\text{SO}_4$, while 2nd and 4th figures KNO_3 .

Another experiment was conducted using a greater variation of amounts of nitrogen added as well as an additional nitrogen compound. During the period at 25° C., 5 cc. aliquots of the various solutions (distilled water used for controls) were mixed at definite intervals with the medium of the various lots. After 7, 10, and 13 weeks the seeds were freed of the peat used while at 25° C. and were then mixed in untreated, damp peat moss and held at 5° C. The percentage germination obtained during the six months at this low temperature is shown in Table II which also gives the total amount of nitrogen mixed in each series as well as the time in weeks at 25° C. prior to the period at 5° C. The addition of nitrogen produced an increase in percentage germination. The effect of all three salts was much the same. The various amounts of nitrogen produced approximately the same results in each of the three time periods except for the lowest amount in each set which was in most cases intermediate between the controls and those receiving larger amounts of nitrogen.

Another experiment was conducted in which the shortest time period

TABLE II

EFFECT OF THE ADDITION OF NITROGEN COMPOUNDS TO THE MEDIUM DURING VARIOUS PERIODS AT 25° C. UPON SUBSEQUENT GERMINATION OF SNOWBERRY SEEDS AT 5°C.*

Treatment at 25° C.		% Germ. during 6 months at 5° C.		
Weeks	Total mg. N added	KNO ₃	(NH ₄) ₂ SO ₄	NH ₄ NO ₃
7	5.30	61	39	56
	2.65	57	47	48
	1.33	40	57	50
	0.66	38	57	46
	0.33	33	41	37
	0	34	33	44
10	7.42	84	57	74
	3.71	71	68	70
	1.86	77	71	71
	0.93	71	68	69
	0.46	60	57	71
	0	44	44	52
13	9.54	86	68	86
	4.77	87	88	86
	2.39	84	85	83
	1.19	87	82	87
	0.60	71	77	76
	0	61	56	61

* Experiment started January, 1935.

of 7 weeks was duplicated but a much greater range of quantity of nitrogen was added. The data presented in Table III show no great increase in percentage germination as the quantity of nitrogen was increased.

Time is an important factor as illustrated in Tables I and II. When

TABLE III

EFFECT OF A WIDER RANGE OF THE AMOUNT OF NITROGEN ADDED TO THE MEDIUM DURING A PERIOD OF 7 WEEKS AT 25° C. UPON SUBSEQUENT GERMINATION*

Total mg. N added to peat while at 25° C.	Percentage germination during 6 months at 5° C		
	KNO ₃	(NH ₄) ₂ SO ₄	NH ₄ NO ₃
82.8	9	—	—
42.4	23	22	27
21.2	33	31	27
10.6	32	28	39
5.3	27	28	21
2.65	35	31	27
1.33	33	25	29
0.66	31	16	26
0.33	26	17	23
0 (Distilled H ₂ O)	17	17	17
0 (Tap water)	22	22	22

Experiment started August, 1935.

FLOWERING HABIT AND CORRELATION OF ORGANS MODIFIED BY TRIODOBENZOIC ACID

P. W. ZIMMERMAN AND A. E. HITCHCOCK

Recent reports (1, 2, 3, 4) have been concerned with formative influences exhibited by β -naphthoxy compounds and substituted phenoxy and benzoic substances. It was shown that under the influence of these hormone-like substances the size and pattern of fruit, leaves, etc., became considerably modified. One of the substituted compounds, triiodobenzoic acid, first attracted attention because it induced abnormal color of leaves and growth habit, though it did not cause epinasty of leaves or curvature of coleoptiles. It differed in the latter respect from the substituted phenoxy compounds which were active for both types of growth.

The present report concerns correlation phenomena and the flowering habit of tomato plants as modified by the influence of 2,3,5-triiodobenzoic acid.

MATERIALS AND METHODS

Triiodobenzoic acid, purchased from Eastman Kodak Company, was used in water solution and as lanolin preparations. When sprayed on plants with an atomizer the concentration in solution ranged from 25 mg./l. to 500 mg./l. Soil treatments were made by applying from 1 to 10 mg. of the acid in 50 cc. of water to a four-inch pot. Lanolin preparations (concn. 1 to 20 mg./g.) were applied around the stem of potted plants. Plants were exposed to vapors of the acid under bell jars where the chemical in a watch glass was warmed on a hot inverted crucible. Best results were obtained with young plants treated when four to six inches in height. The substance was effective for various species but the results reported herewith concern principally tomatoes, *Lycopersicon esculentum* Mill.

EXPERIMENTAL RESULTS

Under normal conditions flower clusters arise from tomato plants along the stem some distance above or below the leaf (Fig. 1 A). They seldom occur directly opposite the leaves. Occasionally the cluster may include both flower buds and leaves. Frequently also the flower cluster has a terminal growing point which produces stem and leaves. The main shoot of the plant, under normal conditions, has a terminal growing point which continues to provide for a stem with leaves and flower clusters at intervals.

The natural growth and flowering habit of plants were greatly modified under the influence of triiodobenzoic acid. Correlation of organs involving location of flowers, direction of growth of axillary shoots, and flower clusters was disturbed by the chemical. Affected plants grew odd-shaped leaves with translucent veins with pronounced pubescence.

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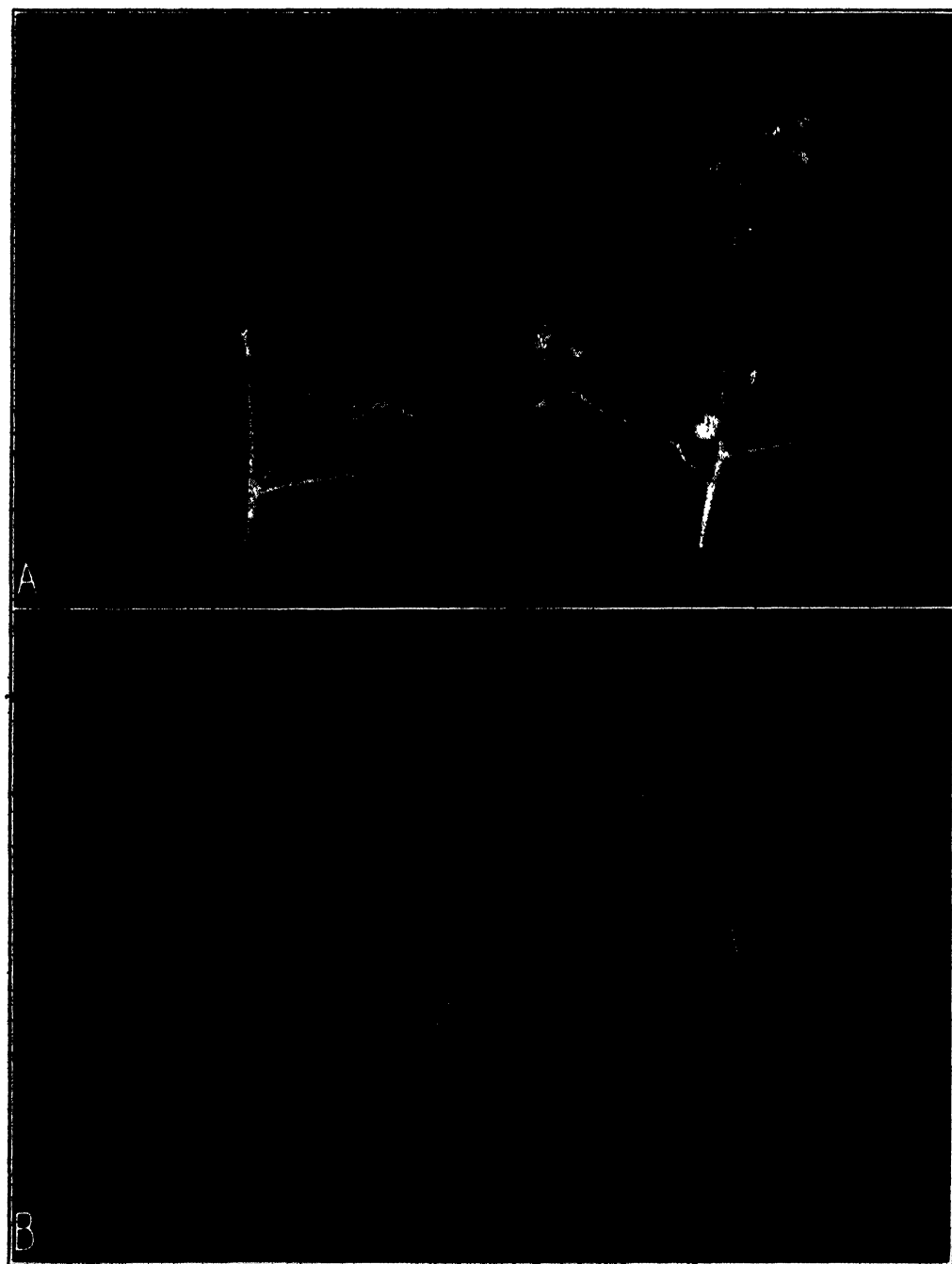


FIGURE 1. Tomato shoots and plants to show the effect of triiodobenzoic acid on configuration, flowering habit, and correlation of organs. A. Left, control; right, upper portion of main shoot of plant 51 days after 1.25 mg. of the substance had been added to the soil. Note that the terminal shoot ended in a flower cluster with an abnormal pedicel. Arrow points to node-like structure. B. Left, normal plant; right, plant showing response to the substance 21 days after 5 mg. had been added to the soil. The plant was approximately 6 inches in height when treated. Note curvature of stem at nodes and modification of new leaves which were formed after treatment.

Triiodobenzoic acid was effective when applied to the soil, sprayed on the plant as a water solution, used as a vapor, or as a lanolin preparation. Though this substance did not cause epinasty of leaves as induced by the substituted phenoxy compounds (4) the stems showed odd curvatures as the plants grew after being treated. Figure 1 B shows how the stem bends at the nodes after 5 mg. of triiodobenzoic acid in 50 cc. of water had been added to the soil. Similar effects were produced by spraying the substance on the growing point of the plant or treating around the stem with a lanolin preparation. Figure 1 B also shows how the leaves which grew after the treatment are modified. Leaves or parts of leaves present at the time the chemical was applied were not modified. The influence is, therefore, of a formative nature effective on growing organs. In this respect, triiodobenzoic acid resembles substituted phenoxyacetic acid and β -naphthoxyacetic acid (3, 4) but it differs from indole and naphthalene growth substances.

There was a tendency for plants treated with triiodobenzoic acid to produce more axillary growth than occurred in controls. The data in Table I show more total growth of axillary stems as well as a larger number of shoots than controls. The early inhibition of the terminal bud by the chemical might influence the growth of axillary buds. However, other influences might play a part since low concentrations also induced more than normal axillary growth.

TABLE I

EFFECT OF SOIL TREATMENT WITH TRIIODOBENZOIC ACID ON NUMBER AND LENGTH OF AXILLARY SHOOTS OF TOMATO

Amt. of chemical per 4-inch pot	Total no. of axillary shoots on 5 plants	Total length of shoots, in inches	Av. total length of shoots per plant
0 (Control)	34	116.5	23.3
0.5 mg.	38	148	29.6
1.25 mg.	40	277.5	55.5
2.5 mg.	44	328.5	65.7
5 mg.	43	168.5	33.7

As the treated plant approached the flowering stage, correlation phenomena were greatly disturbed, affecting both axillary leafy shoots and the location of flower clusters. Axillary shoots were sometimes but not always displaced by flower clusters (Fig. 2 A and B). There were often some leafy shoots and some axillary flower clusters on the same plant. Also some axillary growths were a combination of flower buds and leaves. The upper part of the plant illustrated in Figure 2 A has three flower clusters in a row along the stem—one from an axillary bud, one in a normal internodal position, and one (upper) possibly assuming the position of a terminal bud. No terminal shoot buds remained on the stem.



FIGURE 2. Tomato plant photographed with transmitted light to show location of flower clusters and modification of leaves with translucent veins induced with triiodobenzoic acid. **A.** Upper portion of a plant which grew after the soil was treated with 2.5 mg. of the substance. Note 3 flower clusters along one side of the stem, the lower one representing growth from an axillary bud and the upper one taking the position of the terminal bud. The simple leaf between the flower cluster and the compound leaf appears to have grown from an axillary bud. No actively-growing terminal buds remained on the plant. The middle cluster appears to be in normal position. (Compare with control of Figure 1A.) **B.** Axillary buds produced flower clusters and some combination leaf and flower clusters after the growing point of the plant had been sprayed with a solution containing 50 mg. of the substance per liter of water. Note clearing of the veins of modified leaves.

Both axillary shoots and the main stem were induced to terminate in flower clusters by treating the soil with the chemical (Fig. 3 B). By spraying a solution of the substance on the growing tip, plants were induced to grow terminally flower clusters with stem-like pedicels having nodes but no leaves (Fig. 3 A). The picture also shows that axillary shoots (tops were removed) produced nodes without associated leaves. The pedicel of the

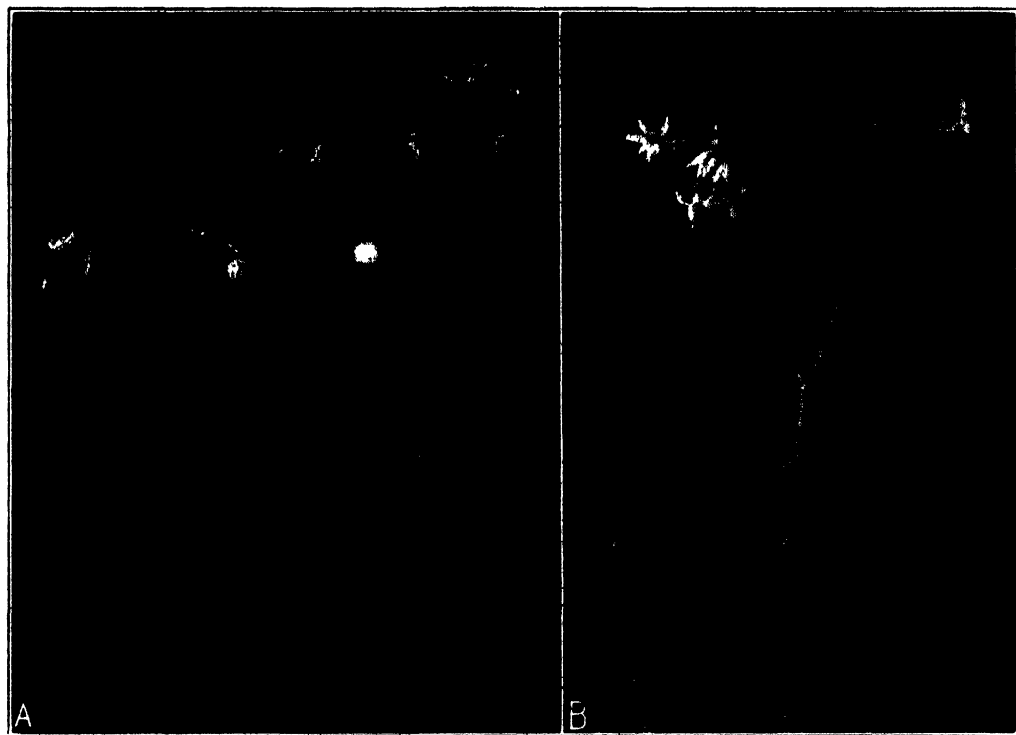


FIGURE 3. Upper portions of 2 tomato plants to show how main stems and axillary shoots terminate in flower clusters with abnormal pedicels after soil treatment with triiodobenzoic acid. A. Response of plant to 1.25 mg. of the substance applied to the soil. Note the abnormal swellings along the pedicel of flower shoots as well as the lower portions of the stems which were removed. These were leafless node-like structures. No active terminal-growing buds remained excepting on axillary shoots. The pedicels appear to be a combination of leafless stem and flower pedicel. B. Upper portion of a plant 63 days after the soil had been treated with 5 mg. of triiodobenzoic acid. Note that axillary shoots as well as the main stem terminated with flower clusters.

flower clusters resembled the stems of the axillary leafy shoots on the same plant. Axillary shoots often grew long stems without nodes in contrast with those shown in Figure 3 A. These shoots usually appeared after the main stem terminated in flower clusters. Where two flower clusters appeared at the tip of a stem (Fig. 3 A) it was difficult to determine which represented the terminal bud. In some cases the axillary bud near the tip appeared to produce a flower cluster while in other cases the axillary bud was entirely lost (Fig. 1 A).

DISCUSSION

From results described it appears that triiodobenzoic acid has a morphogenetic influence on plants, affecting flowering, growth habits, and correlation of organs. This substance does not induce rapid cell elongation as do many of the well-known growth substances of the auxin group. The old definition of auxins would exclude triiodobenzoic acid. However, any

substance which can so definitely regulate the growth of plants must be considered as having hormone-like characteristics. We do not have a good definition to characterize all of the physiologically active synthetic substances which induce hormone-like responses. They should not be called "plant hormones" because, with few exceptions, they are not known to exist in green tissue, nor are they chemically identical with naturally occurring substances. The term "synthetic hormone" has a popular appeal like "synthetic rubber" though most people know the latter is not identical with natural rubber. It would be fortunate if plant physiologists could agree upon terms which would separate the chemical groups according to the type of response induced. At the present time the meaning of the term "auxin" varies with different authors. Some extend its meaning to include natural hormones while others restrict it to substances which cause curvatures of *Avena* coleoptiles. Neither extreme is acceptable. The chemist objects to the former because synthetic substances involved are not chemically identical with natural substances. The physiologist objects to the latter because many physiologically active substances do not cause curvatures of *Avena* coleoptiles. New proposals so far have met with many objections. However, now that we have a new group of substances which is characterized by formative influences on plants there is need for a term which sets these substances apart from the auxin group. Due to the nature of the responses induced, involving shape, size, pattern, etc., of organs, the term "formagen" is proposed.

SUMMARY

Triiodobenzoic acid has been shown to regulate the growth of plants, affecting flowering, growth habit, and the correlation of organs. Solutions of the substance were applied to the soil or sprayed on the plants with similar results. Under the influence of this hormone-like substance, axillary buds which normally produced leafy shoots were induced to grow flower clusters. The main shoot of the plant also lost the shoot-producing bud and terminated in flower clusters. It was concluded that though triiodobenzoic acid does not cause immediate (within a few hours) cell elongation of test objects, the ultimate results more nearly resemble those of true hormones than the well known indole and naphthalene substances commonly referred to as auxins.

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ROOT-INDUCING ACTIVITY OF PHENOXY COMPOUNDS IN RELATION TO THEIR STRUCTURE

A. E. HITCHCOCK AND P. W. ZIMMERMAN

The importance of substituted phenoxy and benzoic acid compounds in the field of plant hormone research and some of the possible practical applications for this entirely new group of compounds were discussed in a recent report (2). The present report deals mainly with the root-inducing properties of the phenoxy compounds. It is of especial interest that the relatively inactive phenoxyacetic acid should be rendered highly active for inducing roots (also other responses) merely by the introduction of one or more substitutions in the ring, particularly the halogens, chlorine and bromine. In addition, the root-inducing activity of the dihalogen derivative of the two higher homologs was considerably greater than for the acetic acid homolog. Thus in some cases α (2,4-dichlorophenoxy) propionic acid and α (2,4-dichlorophenoxy) butyric acid were about 30 times more effective than 2,4-dichlorophenoxyacetic acid.

Comparative tests with β -indolebutyric acid, α -naphthaleneacetic acid, and certain phenoxy compounds showed that particularly the dichloro- and dibromophenoxyacetic acids and their propionic and butyric homologs were, in general, more effective root-inducing substances than indolebutyric acid or naphthaleneacetic acid. Although the greater effectiveness of the phenoxy compounds ranged from two to ten times that of indolebutyric acid or naphthaleneacetic acid, in some cases this difference was nearly 100 times. Mixtures of the phenoxy compounds with indolebutyric acid or naphthaleneacetic acid or both, gave synergistic effects even more pronounced than those previously reported for other substances (2). Considering the relatively high activity of the phenoxy compounds for inducing roots on cuttings of the species and varieties thus far tested, the dihalogen substituted phenoxy compounds in particular should prove of considerable practical importance when used alone or in mixtures with other root-inducing substances or other substances which function as activators.

MATERIALS AND METHODS

Cuttings of the following plants were used: *Ardisia japonica* Thunb., azalea (*Azalea* mixed Kurume varieties and *Rhododendron arborescens* Torr., *R. calendulaceum* Torr., *R. canescens* Sweet., *R. molle* Ct. Don., *R. vaseyi* Gray, *R. viscosum* Torr., *R. yedoense* Maxim. var. *poukhanense* Nakai), bittersweet (*Celastrus scandens* L.), *Cotoneaster apiculata* Rehd. & Wils., *Euonymus radicans* Sieb., *E. radicans* var. *vegeta* Rehd., ivy

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(*Hedera helix* L.), privet (*Ligustrum ovalifolium* Hassk.), rose (*Rosa* vars. Brownell Hybrid Creeper [salmon-pink], Charles K. Douglas, Duchess of Wellington, Excelsa [crimson rambler], Mary Wallace, and Paul's Scarlet), and tomato (*Lycopersicon esculentum* M ll.).

The source of the phenoxy compounds was the same as mentioned previously (2). Stock solutions were made up at a concentration of 20 mg./cc. in 95 per cent ethyl alcohol for solution treatments. These concentrates were then diluted with distilled water for use on cuttings. Powder preparations were made by adding to finely ground talc the required amounts of the compounds in alcoholic solution, then evaporating the alcohol and drying the preparation by means of an air blower.

In preparation for treatment with powders the cuttings were first prepared and sorted into lots and the basal ends were immersed in tap water. The cuttings were removed from the tap water, the excess water shaken off, and the basal ends of each lot were dipped into the powder to a depth of about one-half inch and stirred one turn. The excess powder was tapped off on the edge of the container and the cuttings were planted. This procedure of treating cuttings was performed on the propagating bench.

Cuttings were planted in a light colored builder's sand or in a mixture of 75 per cent (by volume) sand and 25 per cent German granulated peat moss. The sand was exceptionally coarse, containing a considerable amount of small gravel and very little fine sediment. When used alone or with peat moss, this type of coarse sand has proved superior to any other previously used in this laboratory. Regardless of the frequency of watering and the quantity of water applied, the medium (sand or mixture) has not become water-logged. These two media are particularly favorable for callus growth on buried stems.

RESULTS

2,4-DICHLOROPHENOXYACETIC ACID

Solutions. 2,4-Dichlorophenoxyacetic acid was equally or more effective than indolebutyric acid or naphthaleneacetic acid for inducing roots on cuttings of most of the species and varieties of plants thus far tested. In the case of 18- to 24-hour treatments, 2,4-dichlorophenoxyacetic acid was as effective at a concentration of 0.1 mg./l. on *Euonymus* cuttings as indolebutyric acid was at 3.2 mg./l. For privet cuttings taken in August, 2,4-dichlorophenoxyacetic acid was slightly more effective than indolebutyric acid but less effective than naphthaleneacetic acid (Table I). 2,4-Dichlorophenoxyacetic acid and indolebutyric acid were about equally effective at a concentration of 40 mg./l. on hardwood cuttings of *Celastrus scandens* taken in February (Fig. 1 A). In contrast to the above results, 2,4-dichlorophenoxyacetic acid was not effective up to 40 mg./l. on cuttings

of *Cotoneaster*, whereas indolebutyric acid was increasingly effective over the range 5 to 40 mg./l.

Powders. When 2,4-dichlorophenoxyacetic acid was incorporated in finely ground talc, concentrations of from 0.1 to 0.25 mg./g. were optimum

TABLE I
COMPARATIVE ACTIVITY OF CHLORINE SUBSTITUTED PHENOXY COMPOUNDS FOR
ROOTING CUTTINGS OF CALIFORNIA PRIVET. AVERAGE NUMBER
OF ROOTS PER CUTTING

Substance*	0	0.1	0.32	1.0	3.2	10	32 mg./l.
Cl ₂ POA	0	0	4	2	6	2	14
Cl ₂ POP	1	3	4	23	42	46	9
Cl ₂ POB	1	2	3	21	42	52	25
<i>p</i> -ClPOA	1	0	1	0	0	1	4
IB	2	0	0	0	0	0	9
NA	4	—	—	7	3	18	40

* Cl₂POA = 2,4-dichlorophenoxyacetic acid, Cl₂POP = 2,4-dichlorophenoxypropionic acid, Cl₂POB = 2,4-dichlorophenoxybutyric acid, *p*-ClPOA = *p*-chlorophenoxyacetic acid, IB = indolebutyric acid, NA = naphthalene acetic acid.

for rooting *Euonymus radicans* and the common ivy (*Hedera helix*), whereas concentrations 10 to 30 times these were optimum for indolebutyric acid and naphthaleneacetic acid. These differences are similar to those mentioned for solution treatments. On rose cuttings the optimum concentration of 2,4-dichlorophenoxyacetic acid was usually between 0.1 and 0.75 mg./g., but the rooting was generally not so good as that induced by indolebutyric acid at a concentration of 1 mg./g. Thus for rose cuttings the margin of safety was narrower for 2,4-dichlorophenoxyacetic acid than for indolebutyric acid.

Results with rose cuttings varied considerably according to the variety, time of year, and the type of cutting. For example, Paul's Scarlet and Excelsa varieties taken in late summer were most tolerant to concentrations of 2,4-dichlorophenoxyacetic acid in the range 0.5 to 1.0 mg./g. and the Duchess of Wellington cuttings were least tolerant. Cuttings of Mary Wallace, Charles K. Douglas, and one of the Brownell hybrid creepers (salmon-pink) were of intermediate tolerance. Short side shoots 2 to 4 inches long were more tolerant than cuttings made from normal flowering canes one foot or more in length. In general, indolebutyric acid induced a more desirable type of root system on rose cuttings at concentrations of 1 to 3 mg./g. than 2,4-dichlorophenoxyacetic acid at any concentration. Over-treatment or undesirable effects produced by relatively high concentrations of 2,4-dichlorophenoxyacetic acid (0.5 to 1.0 mg./g.) were characterized by some roots of large diameter, noticeably fasciated roots, and an initial delay in root growth. Fasciation appears to be a type of modification on roots which corresponds to modifications induced by phenoxy and

benzoic acid compounds on above-ground parts (1). The fasciated root response appears to be characteristic of phenoxy compounds used in concentrations which are near optimum for rooting, as well as at higher concentrations.

Cuttings of mixed Kurume varieties of azalea taken June 4 responded about equally well to the same concentrations of 2,4-dichlorophenoxyacetic acid, indolebutyric acid, and naphthaleneacetic acid. Branched cuttings

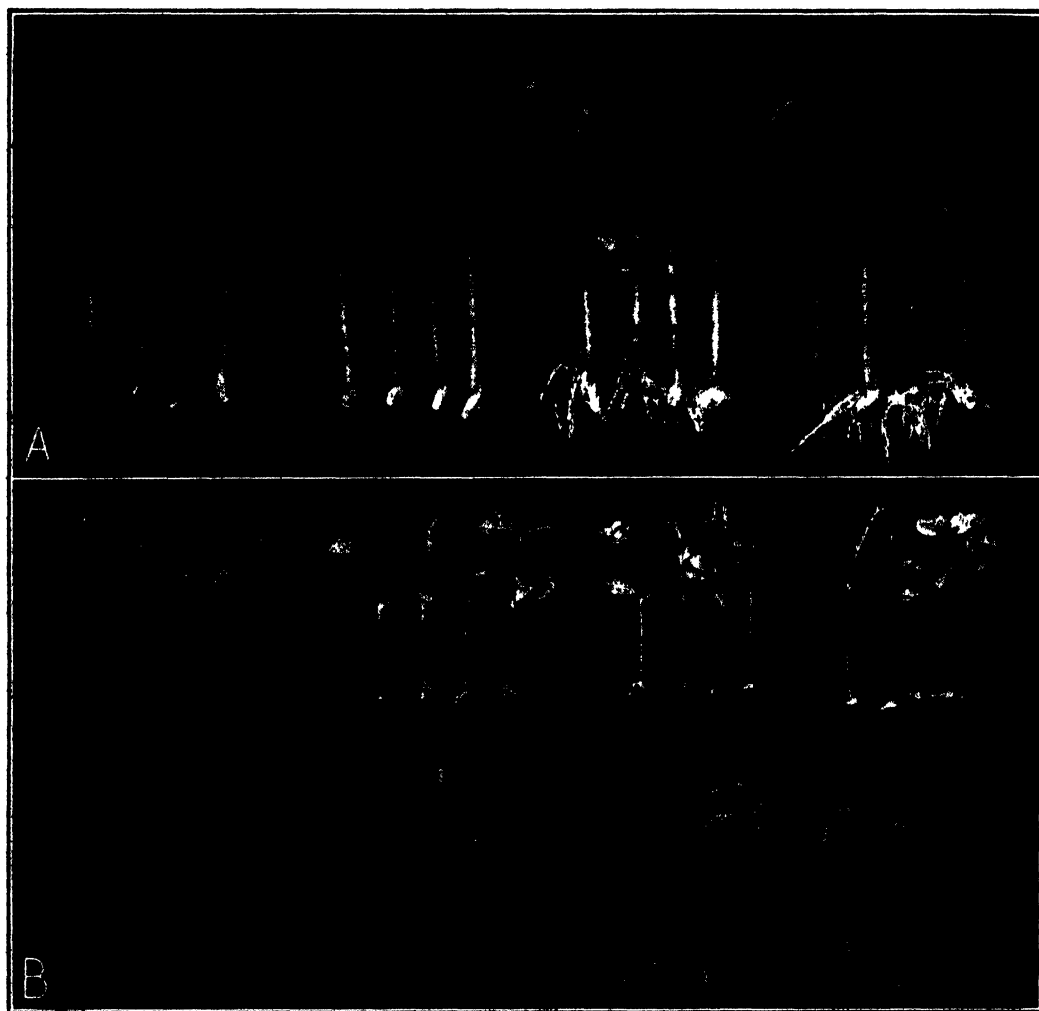


FIGURE 1. A. Hardwood cuttings of *Celastrus scandens* 34 days after a 40 mg./l. treatment with (left to right) control, *o*-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, and indolebutyric acid. B. *Euonymus radicans* cuttings 19 days after a powder treatment with (left to right, upper row) control talc, 1 mg./g. indolebutyric acid, 1 mg./g. naphthaleneacetic acid, 0.25 mg./g. 2,4-dichlorophenoxyacetic acid; (left to right, lower row) control talc, 1 mg./g. mixture of 25 per cent 2,4-dichlorophenoxyacetic acid and 75 per cent indolebutyric acid, same with 25 per cent 2,4-dichlorophenoxyacetic acid, and 37.5 per cent each of indolebutyric and naphthaleneacetic acids.

containing wood two or more years old were more tolerant to all three compounds than cuttings of the current season's growth. Thus the 1 mg./g. powders were best for the cuttings of one-year wood and a 3 mg./g. powder was as good as or better than the 1 mg./g. powder for cuttings of older wood. In contrast to results with the Kurume varieties, 2,4-dichlorophenoxyacetic acid was less effective than indolebutyric acid or naphthaleneacetic acid on the deciduous varieties (*arborescens*, *calendulaceum*, and *canescens*). Powder preparations of 2,4-dichlorophenoxyacetic acid were more effective at a concentration of 3 mg./g. for privet cuttings than either indolebutyric acid or naphthaleneacetic acid at concentrations of 5 to 8 mg./g.

OTHER PHENOXY COMPOUNDS

Results with 2,4-dichlorophenoxypropionic acid and 2,4-dichlorophenoxybutyric acid indicate that these two homologs are much more effective than 2,4-dichlorophenoxyacetic acid and considerably more effective than either indolebutyric acid or naphthaleneacetic acid. In the case of privet 2,4-dichlorophenoxypropionic acid and 2,4-dichlorophenoxybutyric acid were about 30 times more effective than 2,4-dichlorophenoxyacetic acid (Figs. 2 and 3 and Table I), at least 10 times more effective than naphthaleneacetic acid (Fig. 3 and Table I), and greater than 30 times more effective than indolebutyric acid (Table I). 2,4-Dichlorophenoxybutyric acid appeared to be effective over a broader range in concentration than 2,4-dichlorophenoxypropionic acid (Figs. 2 and 3). The propionic and butyric homologs were also more effective than 2,4-dichlorophenoxyacetic acid on *Cotoneaster apiculata*. The corresponding bromine substituted phenoxy compounds were only slightly less effective on privet and *Euonymus*. The bromine substituted lower homolog (2,4-dibromophenoxyacetic acid) was active at 40 mg./l., but not at 10 mg./l., on *Cotoneaster*. This is in contrast to no activity for 2,4-dichlorophenoxyacetic acid in the range 10 to 40 mg./l.

On rose, azalea, ivy, privet, and *Euonymus*, *p*-chlorophenoxyacetic acid was much less effective than the dichloro- and dibromophenoxy compounds. *o*-Chlorophenoxyacetic acid was not only less active than *p*-chlorophenoxyacetic acid but appeared to be practically inactive up to 40 mg./l. for *Celastrus* and *Cotoneaster apiculata*.

Since *Euonymus* responded to treatment with very low concentrations (0.1 mg./l. or less) of the dichloro- and dibromophenoxy acids, it was of interest to determine the relative activities of less effective phenoxy compounds at higher concentrations when applied to the same test object. Seven of the eight phenoxy acids containing a single substituent in the ring were used in concentrations of 10, 32, and 64 mg./l. The other compound (*o*-iodophenoxyacetic acid) was used at a lower range (1, 3.2, and 10 mg./l.)

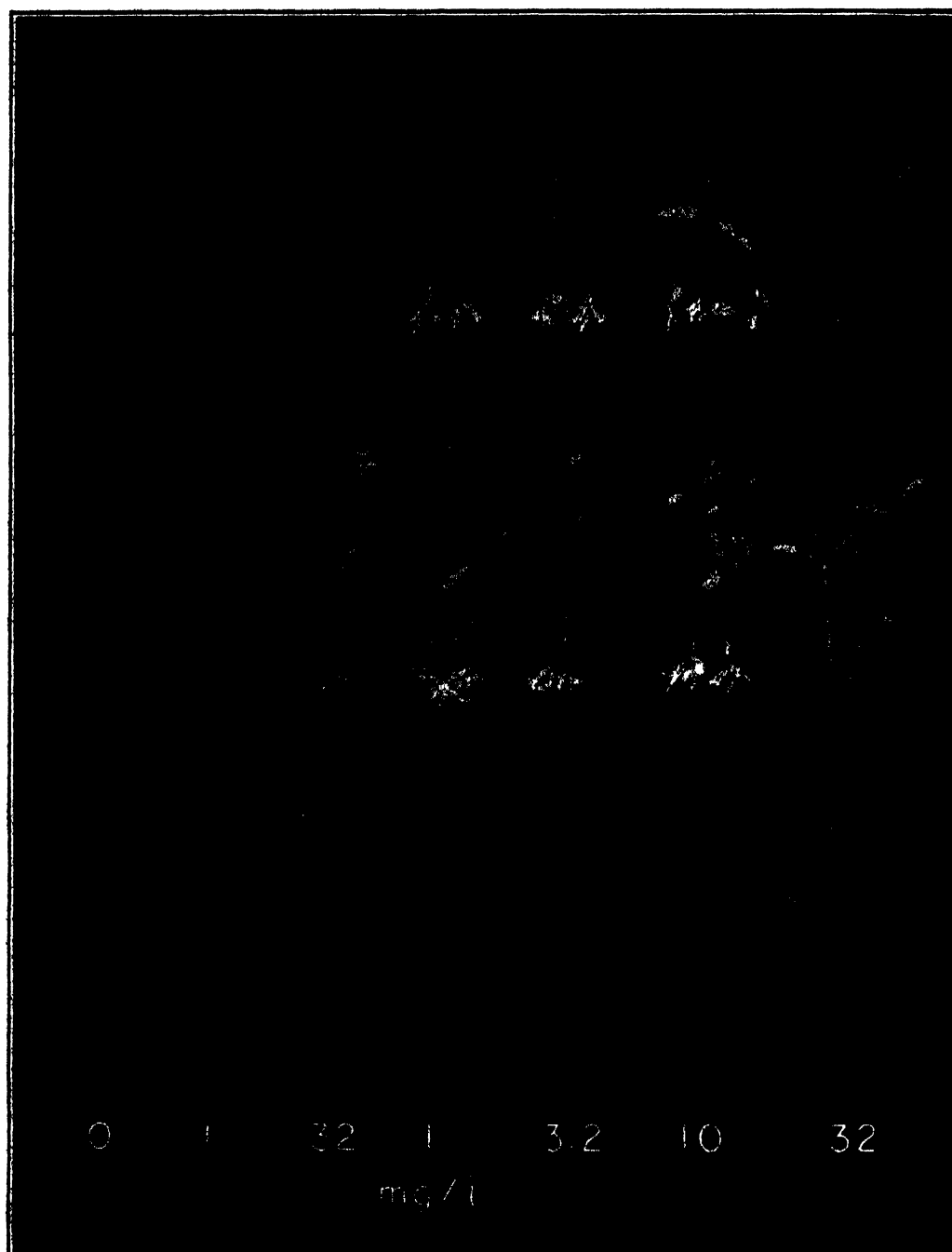


FIGURE 2. Tip cuttings of privet 26 days after a 19-hour treatment with (left to right) control water, 0.1, 0.32, 1, 3.2, 10, and 32 mg./l. of (upper row) 2,4-dichlorophenoxybutyric acid, (middle row) 2,4-dichlorophenoxypropionic acid, and (lower row) 2,4-dichlorophenoxyacetic acid.

because of its relatively low solubility in water. The relative root-inducing activity of these phenoxyacetic acids in descending order was as follows: *p*-chloro-, *p*-bromo-, *o*-chloro-, *p*-amino-, *m*-chloro-, *m*-amino-, *m*-nitro-, and *o*-iodo-. Thus according to the kind and location of the substituent

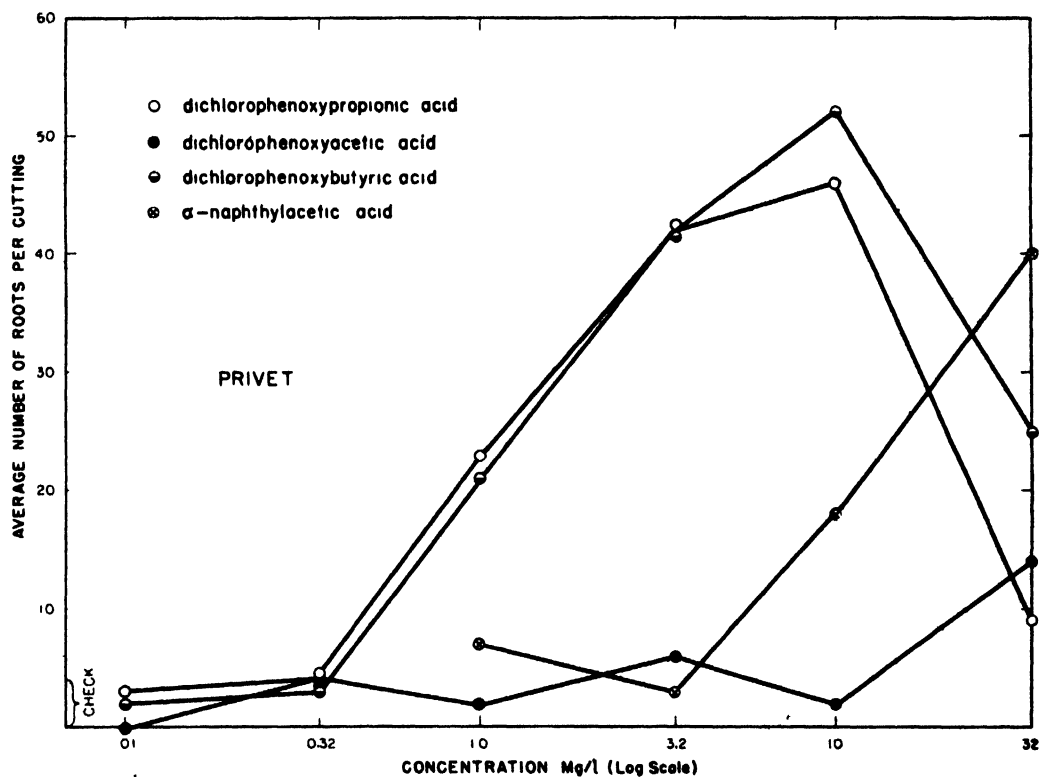


FIGURE 3. Root count curves for privet cuttings showing comparative activity of three dichlorophenoxy acid homologs and naphthaleneacetic (α -naphthylacetic) acid (data from Table I).

in the ring, the root-inducing activities were as follows: para > ortho > meta; Cl > I at the ortho position; Cl > NH₂ > NO₂ at the meta position; and Cl > Br > NH₂ at the para position. These same relationships held also for tomato leaf cuttings when the substances were used in concentrations of 0.1 and 1 mg./l. In this particular tomato leaf test, other phenoxy compounds were used in addition to the eight just mentioned. Phenoxyacetic acid was inactive. Phenoxypropionic and phenoxybutyric acids were slightly active. Xylenoxyacetic and xylenoxypropionic acids were inactive.

Phenoxypropionic acid was more effective than phenoxybutyric acid when used in the range 10 to 40 mg./l. on *Cotoneaster*. Both substances induced fasciated roots, but this response was most pronounced on cuttings treated with phenoxypropionic acid.

MIXTURES

Mixtures containing 2,4-dichlorophenoxyacetic acid and either or both indolebutyric acid and naphthaleneacetic acid were generally more effective than the individual compounds when applied as talc preparations to cuttings of azalea, *Euonymus*, rose, and *Cotoneaster*. Solution mixtures containing three compounds (indolebutyric acid, naphthaleneacetic acid, and either 2,4-dichlorophenoxyacetic acid, 2,4-dichlorophenoxypropionic acid, or 2,4-dichlorophenoxybutyric acid) were more effective than the individual compounds on cuttings of *Euonymus radicans* var. *vegeta*. In addition, solution mixtures containing three dichlorophenoxy acids were more effective than the individual acids for rooting privet cuttings (Table II). Similar synergistic effects were reported previously for other substances (1).

TABLE II

COMPARATIVE ROOT-INDUCING ACTIVITY OF DICHLOROPHENOXY ACIDS APPLIED SEPARATELY AND AS MIXTURES TO CUTTINGS OF PRIVET. AVERAGE NUMBER OF ROOTS PER CUTTING AFTER 22 DAYS

Code letters	Phenoxy acids	Concentration mg./l.					
		0	0.1	0.32	1	3.2	10
A	Dichlorophenoxyacetic	0	0	0	0	0	0
P	Dichlorophenoxypropionic	0	0	0	3	5	15*
B	Dichlorophenoxybutyric	0	0	0	0	7	27**
A+P+B	33.3% each of A, P, B	0	1	2	13**	13**	1
A+P+B	37.5% each of A, P; 25% B	0	0	0	11**	14**	16
A+P+B	45% each of A, P; 10% B	0	0	0	0	12**	12
A+B	75% A and 25% B	0	0	0	0	0	15**
A+B	90% A and 10% B	0	0	0	0	0	0

* Optimum rooting exceeded at a concentration of 10 mg./l. but not reached at 3.2 mg./l.

** Optimum rooting.

That the individual substances in a mixture were exerting separate effects as well as a general activating influence on root initiation was substantiated by certain qualitative responses. In the case of both powder and solution treatments the fasciated root response was induced by mixtures containing 2,4-dichlorophenoxyacetic acid and by 2,4-dichlorophenoxyacetic acid alone. The fasciated root response induced on *Ardisia* cuttings (Fig. 4) was more pronounced on cuttings treated with solution mixtures of 2,4-dichlorophenoxyacetic and indolebutyric acids than with 2,4-dichlorophenoxyacetic acid alone. Indolebutyric acid did not induce this type of fasciated root at any of the concentrations used.

DISCUSSION

The dihalogen propionic and butyric homologs were of nearly equal activity and noticeably effective at 0.01 mg./l. on *Euonymus* and at 0.5

mg./l. on privet. However, the dihalogen propionic homologs were generally more active but were effective over a narrower range of concentration than the corresponding butyric homologs. In general, chlorine was a more active substituent than bromine and the 2,4-dihalogen substituted phenoxy compounds were more active than the monohalogen compounds. Substituents were more active in the para than in the ortho or meta positions.

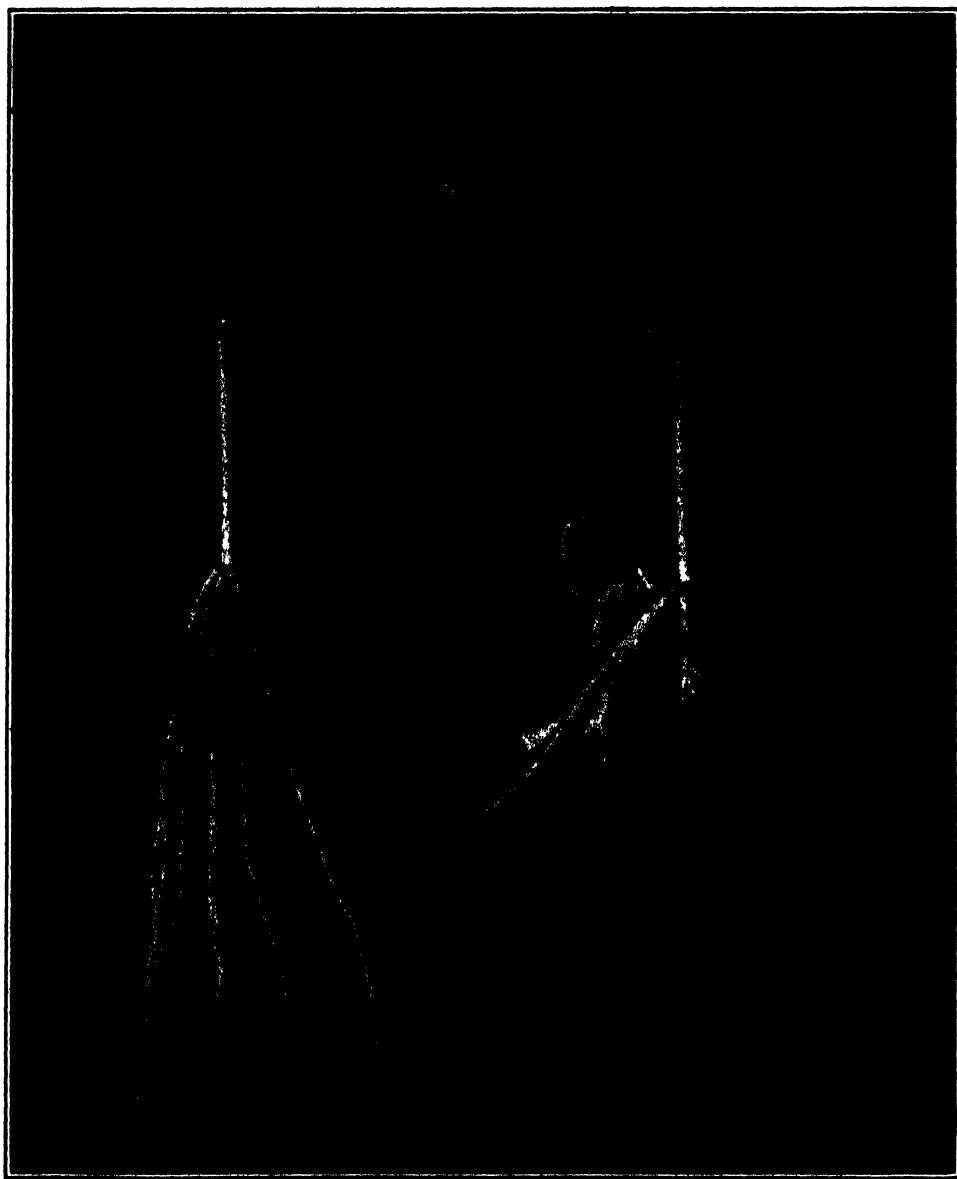


FIGURE 4 *Ardisia* cuttings. Left, control. Right, fasciated roots as usually induced by treatment with 2,4-dichlorophenoxyacetic acid or mixtures of 2,4-dichlorophenoxyacetic acid and indolebutyric acid at concentrations of 2 to 6 mg./l.

From the standpoint of hormone responses in general, the root-inducing response appears to be more closely associated with the cell enlargement response than with the modification of form or "formagenic" response. For example, although phenoxyacetic acid is inactive for modification (2), practically inactive for cell enlargement, and of low activity for root-induction, activation for all three responses may be accomplished by increasing the length of the side chain or by the introduction of certain substituents into the ring. The activity resulting from an increase in length of side chain (phenoxypropionic and phenoxybutyric acids) is not nearly so great as when chlorine or bromine are substituted in the 2,4-positions in the ring. Increasing the length of side chains in the dihalogen phenoxyacetic acids greatly increases the root-inducing activity but causes complete inactivation of the modification response.

Possible practical uses of phenoxy compounds for rooting cuttings of horticultural varieties are at present complicated for a number of reasons. Some of the compounds must be used at much lower concentrations than have been recommended for either indolebutyric acid or naphthaleneacetic acid. The results thus far indicate that the most active phenoxy compounds have a relatively narrow effective range below that which is toxic or which induces undesirable over-treatment effects such as swelling, proliferation, retarded growth of roots, and noticeably fasciated roots. Results with solution and powder mixtures of 2,4-dichlorophenoxyacetic acid (used as 25 per cent of the total concentration) and either or both indolebutyric acid and naphthaleneacetic acid are sufficiently favorable to indicate that these highly active substances may function better in mixtures than when used separately. Besides inducing synergistic effects, the mixtures appeared to induce a type of root system which is qualitatively better than that resulting from the use of the phenoxy compound alone. Results with mixtures of dichlorophenoxyacetic, dichlorophenoxypropionic, and dichlorophenoxybutyric acids indicated that the ratio of the three acids was of considerable importance.

Since the present tests are of a preliminary nature, only approximate differences in relative activities can be given for a limited number of compounds and varieties of cuttings. Additional tests now being carried out are expected to give a more substantial basis for determining threshold values and more comprehensive data relating to structure and the root-inducing response, particularly for substituents other than chlorine and bromine and for substitutions other than at the 2,4-positions.

SUMMARY

1. The root-inducing activity of phenoxy compounds varied from low activity to very high activity depending upon the kind, number, and positions of substituents in the ring and to the relative length of the side chain.

2. Compared with β -indolebutyric and α -naphthaleneacetic acids the root-inducing activity on cuttings of horticultural varieties was as follows for the substituted phenoxy compounds: the monohalogen substituted acids less, (2,4)-dichloro- and dibromophenoxyacetic acids equal or greater, and the corresponding α -propionic and α -butyric homologs 30 to 100 times greater.

3. The descending order of activity for monosubstituted phenoxyacetic acids on *Euonymus* cuttings was: para > ortho > meta; Cl > I at the ortho position; Cl > NH₂ > NO₂ at the meta position; and Cl > Br > NH₂ at the para position.

4. The root-inducing activity of phenoxyacetic acid (practically inactive) was increased by increasing the length of side chain or by substitutions in the ring just as previously reported for the cell enlargement and modification responses. An additional increase in root-inducing activity resulted from increasing the side chain of the dihalogen substituted phenoxyacetic acids although as previously shown these higher propionic and butyric homologs were inactive for modification.

5. Mixtures of three dichlorophenoxy homologs or of dichlorophenoxyacetic acid and either or both β -indolebutyric and α -naphthaleneacetic acids were more effective than the individual compounds. The ratio of the different compounds in the mixture was a limiting factor.

6. All active phenoxy compounds induced marked fasciation of roots at concentrations slightly above optimum.

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