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ERRATA

Page 126, line 12, for 'more toxic that' read 'more toxic than'

Page 129, add footnote 'This work was supported by a fellowship from the Rockefeller Foundation for Medical Research.'

INITIATION AND STIMULATION OF ROOTS FROM EXPOSURE OF PLANTS TO CARBON MONOXIDE GAS[1]

P. W. ZIMMERMAN, WILLIAM CROCKER, AND A. E. HITCHCOCK

Botanists have long been interested in chemicals that stimulate the growth of existing plant organs and have attempted to find something that will initiate roots or buds. Considerable progress has been made in forcing dormant buds and in causing renewed growth of certain tissues of such organs as leaves. Johannsen (7) in 1906 showed that ether and chloroform were effective in breaking dormancy and thereby causing early growth of buds. Stuart (12) showed that ethyl bromide, ethyl iodide, and carbon tetrachloride forced early growth of herbaceous and woody plants. Denny (3, 4) and Denny and Stanton (5) extended these researches to include some new chemicals, among them ethylene chlorhydrin being found especially effective. Another type of research has dealt with stimulation of a particular part of such organs as leaves or growing stems. The researches along this line have recently been reviewed in a paper entitled "Ethylene-induced epinasty of leaves and the relation of gravity to it" by Crocker, Zimmerman, and Hitchcock (1). They report that ethylene is a stimulant for specific leaf tissues while acting as an anaesthetic to other parts of the plant.

The purpose of this paper is to report the results of experiments with carbon monoxide gas which induced root initiation and caused stimulation of preexisting root primordia. It also caused epinasty and abscission of leaves, retarded elongation of stems, and anaesthetized plants as is claimed for ethylene. In addition to these effects carbon monoxide gas induced the formation of *adventitious roots* where they do not normally occur on stems of woody and herbaceous plants. So far as the authors know this is the first report to show that new organs can be induced by the application of a known chemical.

MATERIALS AND METHODS

Carbon monoxide gas used in the experiments was made by heating 100 grams of oxalic acid with 300 cc. concentrated sulphuric acid and collecting the gas over water. The gas comes over in equal parts of carbon dioxide and carbon monoxide. In the early experiments this mixture was used, but in later experiments the gas was scrubbed with soda lime, potassium hydroxide, and barium hydroxide until it was approximately 96 per cent pure carbon monoxide gas. The following is a typical analysis of the gas: carbon monoxide, 95.6 per cent; carbon dioxide, 0.5 per cent; oxygen, 2.0 per cent; nitrogen, 1.9 per cent. The plants were exposed to gas under

[1] Awarded the A. Cressy Morrison Prize in Experimental Biology in 1932, by The New York Academy of Sciences.

bell jars or in Wardian cases. Check plants were kept in comparable conditions without the gas. Concentrations used varied from 0.05 per cent to approximately 50 per cent by volume. When bell jars were used, gas was sometimes left throughout the experiment without being replenished, but where Wardian cases were used they were aired out frequently and a new gas supply introduced. Analyses made of the gas in cases after introducing 1 part of carbon monoxide to 100 parts of air indicated that the concentration dropped rapidly during the daylight period but remained fairly constant at night. Unless replenished frequently, the supply was nearly exhausted after the first daylight period.

RESULTS

INITIATION AND STIMULATION OF ROOT PRIMORDIA ON STEMS

Of the 80 species of plants tested, 27 have shown definite root effects from exposure to carbon monoxide gas. They are as follows: *Amaranthus retroflexus* L. (Green amaranthus), *Brassica oleracea* L. var. *italica* Plenck. (broccoli), *Bryophyllum pinnatum* Kurz., *Celosia argentea* L. var. *plumosa* (cockscomb), *Chrysanthemum coronarium* L., *Coleus blumei* Benth., *Cosmos bipinnatus* Cav., *Cosmos sulphureus* Cav., *Cucurbita maxima* Duchesne (squash), *Fagopyrum esculentum* Moench. (buckwheat), *Forsythia viridissima* Lindl., *Galinsoga parviflora* Cav., *Gossypium hirsutum* L. (cotton), *Helianthus debilis* Nutt. (sunflower), *Hydrangea macrophylla* DC., *Impatiens balsamina* L. (balsam), *Ipomoea batatas* Lam. (sweet potato), *Lycopersicon esculentum* Mill. (tomato), *Myrica carolinensis* Mill. (bayberry), *Nicotiana tabacum* L. var. Burley (tobacco), *Ribes nigrum* L. (black currant), *Salix babylonica* L. (weeping willow), *Solanum pseudocapsicum* L. (Jerusalem cherry), *Solanum tuberosum* L. (potato), *Tagetes erecta* L. (African marigold), *Tagetes patula* L. (French marigold), *Zea mays* L. var. *everta* Bailey (pop corn). The response was manifested in a variety of ways as shown by Table I.

The most evident responses were (a) initiation of roots from young tissues on the stem, (b) stimulation of preexisting root primordia on the stem, (c) secondary soil roots changed in their response to gravity, and (d) various combinations of the three aforementioned types.

Root Initials Induced to Form on Young Portions of Stems

The following 10 species of plants have been induced to grow roots from young stem tissue: (a) tobacco, (b) tomato, (c) two species of marigold, African and French, (d) galinsoga, (e) two species of cosmos, common and sulphur, (f) Green amaranthus, (g) balsam, and (h) hydrangea.

Tobacco. Tobacco plants treated with one per cent carbon monoxide gas for 10 days produced roots on the stem over only a narrow zone a short

TABLE I

INITIATION AND STIMULATION OF ROOTS WITH CARBON MONOXIDE GAS

Species	Initiation of roots*	Stimulation of roots**	Special nodal rooting	Reversal of response to gravity†
Tomato	+	+		
African marigold	+	+		+
French marigold	+	+		+
Turkish tobacco	+			+
Cosmos (common)	+	+	+	+
Cosmos (sulphur)	+	+	+	+
Balsam	+			
Galinsoga	+			
Pop corn			+	+
Buckwheat			+	+
Squash			+	+
Potato		+		
Jerusalem cherry		+		
Chrysanthemum		+		
Broccoli		+	+	+
Sunflower		+	+	
Cotton		+		
Sweet potato		+		
Green amaranthus	+			
Bryophyllum		+		
Hydrangea	+			
Forsythia		+		
Currant		+		
Willow		+		
Bayberry				+
Cockscomb		+		
Coleus	+		+	

* Root initials were induced in young stem tissues of treated plants in contrast to no rooting from comparable regions of controls.

** Forcing of preexisting primorida is called stimulation.

† Roots caused to grow up out of the soil in contrast with control plants.

\+ Positive response.

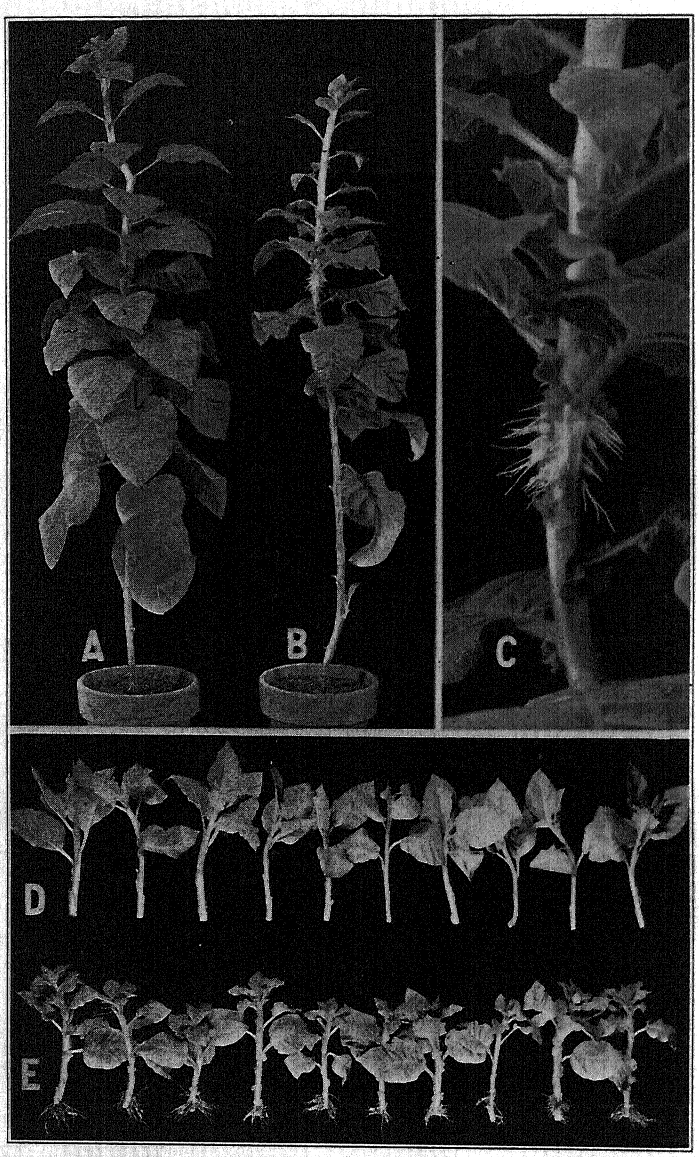


FIGURE 1. *Nicotiana tabacum* (Turkish variety) exposed to one per cent carbon monoxide gas. A. Control plant kept in Wardian case; B. Plant exposed to gas 15 days, then allowed to stand in air two days, after which time it was photographed; C. An enlargement of the rooting region of B; D. Tobacco cuttings from control plants in Wardian case for 10 days. Photographed 5 days after having been placed in rooting medium; E. Cuttings from plants treated with one per cent carbon monoxide for 10 days and then placed in rooting medium for 5 days, after which time they were photographed.

distance below the growing point (Fig. 1, A, B, and C). This important region (region of elongation) was usually approximately one inch below a place on the stem representing the growing point at the time the treatment began. At the time the roots could first be seen they were five or more inches below the new growing point. In no case did roots appear from the young part of the stems of control plants. An occasional root appeared at the base of the stems in both treated and control plants. There was no evidence of root stimulation anywhere except in the young tissue near the growing point. This is in striking contrast to adventitious root growth of tomato and marigold plants exposed to carbon monoxide gas, where the region of response extended over a considerable distance along the stem.

Since no root primordia have been found in microscopic sections of young stems of control plants, this peculiar response of treated plants has been looked upon as a case where the gas has actually induced root initials to form. It is not understood why there is such a limited region that can be forced by the gas. Control tobacco stems cut into five-inch segments and planted in series showed rooting from all regions in 13 days, but the tip cuttings rooted more profusely than others.

Figure 1, D and E, shows two sets of tip cuttings taken from controls and from plants exposed to carbon monoxide gas. Both sets of plants had been in Wardian cases for 10 days before the cuttings were made, the only difference being that one set had been exposed to approximately one per cent carbon monoxide gas. The cuttings from treated plants produced larger root systems in five days than the checks in 15 days. Also, there was a difference in the region from which roots appeared. Those of cuttings from treated plants were scattered for a distance of an inch and a half above the base, whereas cuttings from control plants produced their roots very near the cut surface. These results indicate that root initials had been forced by the gas before the cuttings were made. In cross sections of fresh material they appeared ready to emerge from the bark. In longitudinal sections they were found along the stem for a distance of an inch or more.

A total of 108 tobacco plants of varying sizes were used in 10 different experiments to determine the effect of carbon monoxide on initiation and stimulation of adventitious roots. The response as reported above was nearly constant. In one experiment 27 of the 30 plants exposed to one per cent gas for 10 days produced roots on the stem near the growing point but at no other place. The three remaining plants were in good condition and might have formed roots had the experiment been continued. None of the 28 control plants in this experiment produced roots.

Twenty-five tip cuttings of treated plants produced large root systems in five days (Fig. 1, D and E), whereas control cuttings were just beginning to root in eight days. Fifteen days were required for controls to approach the size of roots produced by the treated cuttings in five days.



FIGURE 2. Tomato plants treated with carbon monoxide gas. A. Control in Wardian case for 11 days; B. Plant exposed to one per cent carbon monoxide gas for 5 days and then held in Wardian case for 6 more days at the end of which time it was photographed. Note the slender root growth made after the plant was removed from gas; C. Control plant; D. Plant with a flask of carbon monoxide sealed over a leaf. Note the epinastic response of the leaves, indicating that the gas is taken in through the leaf and transmitted to all parts of the plant.

Tomato. Figure 2, A and B, shows a characteristic response of a tomato plant exposed to carbon monoxide gas for five days and then kept in a humid atmosphere for six more days. The roots ranged from just visible to one-half inch in length when the plant was removed from the gas but they grew rapidly while in humid atmosphere during the last six days. Plants exposed to gas continuously for 10 days produced many short roots extending up along the stem within two inches of the growing point. Control plants frequently produced roots near the basal part of the stem but in no case did they grow roots from the young tissue as shown by plants treated with gas. Microscopic examinations of young stem tissues revealed many root primordia in treated plants but not in controls. This clearly shows that carbon monoxide gas induced root initials to form in young tomato stems. Once started, the primordia continued to grow, producing *new organs*, adventitious roots, on the stem.

Plants exposed for 24 hours and then removed from the gas produced fewer but much longer roots than plants treated continuously for the same period. The number of root primordia induced to form increased with the time of exposure up to a 15-day period or as long as the plants remained in growing condition. There was some retardation in stem elongation of treated plants but with long exposures roots were produced on stem tissue formed after the experiments began.

Leafless cuttings of young tomato plants produced adventitious roots up along the stems when exposed to carbon monoxide gas about the same as the stems shown in Figure 2 B.

In several cases roots were observed to appear from petiolules of leaves of treated plants. This type of response was not constant under the conditions of our experiments. With proper handling perhaps leaves could be made to produce roots regularly.

The gas was effective over a wide range of concentrations. The lowest used was 0.05 per cent and the highest about 50 per cent. While stimulation was noticeable at 0.05 per cent, the best results were had with a range around one per cent. Fifty per cent concentration stimulated root growth but caused rapid aging of the plants. The effectiveness of the gas did not vary directly with its concentration.

The root responses recorded above occurred while the plants were in high relative humidity. It was not possible to force roots to grow out into dry air. It was possible, however, to force root primordia to form while the plants were standing in dry air. This was accomplished by sealing a flask with carbon monoxide over a leaf of the plant as shown in Figure 2 D. The gas passed into the tissues of the leaf and then diffused throughout the plants as described for ethylene in an earlier paper (14). Since carbon monoxide caused marked epinasty of the leaves, its transmission through the plant was demonstrated (Fig. 2 D). Plants so treated formed many

root primordia along the stem. Fresh material sectioned and examined with the microscope disclosed that roots had broken through the epidermis but they could not grow out into the dry air. A region of the stem covered with moist sphagnum moss produced roots one-fourth inch long in 48 hours. From these results it is seen that the humidity surrounding the stem is not the determining factor for root initiation. It does have, however, a great influence on the amount of root growth outside of the epidermis.

A total of 190 tomato plants were used in 20 different experiments with carbon monoxide gas. In size the plants ranged from six inches to three feet in height. Some had not yet reached the flowering stage while others had half mature fruit. The only difference in root response was that the old plants produced roots more readily than young ones. In all but one experiment 100 per cent of the treated plants responded as described. Even cuttings with all leaves removed showed induced root initiation along the stem. Cuttings with leaves rooted more profusely than leafless cuttings, showing some advantage from the photosynthetic organs.

Marigold. Numerous root primordia were induced to form in young stem tissue of marigold plants treated with one per cent carbon monoxide gas. The control plants frequently grew roots from the basal part of the stems but not from the young portions. Microscopical examinations of stem structures near the apex revealed many primordia in the treated plants but none in controls. The effect of exposure to carbon monoxide for 10 days is well shown in Figure 3. These results show that the gas induced the formation of root primordia to form. The final effect was the production of *organs* (adventitious roots) which were caused by a chemical.

Two species of marigolds, *Tagetes erecta* (African marigold) and *Tagetes patula* (French marigold), have been used in the experiments and found to respond alike. These results are based on 16 different experiments involving 150 plants. The concentrations of gas used and the length of the exposures were the same as described for tomatoes. Aside from the fact that there was some variation in numbers of roots produced by individual plants, the response to gas was nearly constant. A 48-hour exposure to a one per cent concentration was sufficient to initiate roots but with a continuous exposure for 10 days roots were more numerous and came within an inch of the stem apex. The roots could be seen in rows associated somewhat with natural ridges on the stem. When viewed in cross and longitudinal sections with a microscope the primordia appeared one above the other along the bundles and crowding somewhat out into medullary rays. This subject is now being studied with preserved material in the hope of finding more exact information as to the origin of root initials.

Galinsoga. Galinsoga plants form many branches which finally terminate in flower shoots. The last set of branches leading to the flowers were forced to form roots when treated for 10 days with one per cent carbon



FIGURE 3. Initiation and stimulation of adventitious roots of marigold caused by carbon monoxide. Left, control plant. Right, plant exposed to approximately one per cent carbon monoxide gas for 10 days.

monoxide gas. Out of 29 plants so treated, 25 produced numerous roots. The remaining four were in good condition and might have rooted later if the experiment had been continued. None of the control plants formed roots on the stems.

Cosmos. Two species of cosmos, *Cosmos sulphureus* and *Cosmos bipinnatus*, showed a tendency to produce roots from young nodes and internodal regions when exposed to gas. Control plants frequently produced



FIGURE 4. Response of *Hydrangea macrophylla* to carbon monoxide. A. Control plant kept in Wardian case; B. Plant exposed for 25 days to approximately one per cent carbon monoxide. The leaves were removed in order to show the roots to a better advantage. It is evident from the picture that stem elongation was retarded and the leaves which formed while the plant was in gas are abnormally small.

roots from the basal part of the stems and older nodes but the treated plants were forced to initiate roots on much younger tissues. Like tomato, treated plants of *C. bipinnatus* produced roots from some of the leaves though the response was not constant under the conditions of our experiments. *C. sulphureus* plants formed most roots on a region approximately three inches below the growing point. Check plants never formed roots more than two inches above the soil level and they were comparatively few. The most convincing evidence of root initiation came from the fact that

treated plants produced many roots on internodal regions of young stem tissue. This response was in striking contrast to control plants, which did not show this type of response.

Green amaranthus. Scattered roots appeared from internodal regions of plants treated with one per cent carbon monoxide for 15 days.

Balsam. Roots were induced over a narrow zone similar to the response described for tobacco.

Hydrangea. Actively growing shoots of hydrangea produced roots within an inch of the stem apex as shown in Figure 4 B. This active zone was probably the region of elongation at the time the experiment was started. No roots were produced elsewhere on the stem. In this respect the response was like that of Turkish tobacco (Fig. 1 B). Hydrangea stems are distinctly woody and their response to carbon monoxide suggests that other woody stems might be induced to root under proper treatment.

Stimulation of Preexisting Root Primordia

In some cases control plants produced roots from the basal portion of stems or from the older nodes when kept in humid atmosphere. Root primordia were located in microscopic sections of the oldest parts of tomato and marigold stems before they were subjected to gas. These are spoken of as "root germs" or preexisting root primordia. Attention was called by van der Lek (9) to "root germs" in *Salix*, *Ribes*, and *Vitis* stems. Even though such structures existed before the experiments began, the plants subjected to carbon monoxide gas produced visible roots much earlier than controls. This type of response is referred to in this paper as "root stimulation." The phenomenon is illustrated by Figures 2 and 3. Tomato control plants left for long periods of time in humid atmosphere frequently produced roots from the lower half of the stem. Such responses occurred only after 10 or more days in Wardian cases as contrasted with only three days required for treated plants. Eighteen species have been found to exhibit this type of root stimulation (Table I). Seven species showed a tendency to root at the nodes. Corn plants subjected to gas treatment grew roots from four nodes above the ground in 10 days (Fig. 5 B). The check plants showed signs of rooting only from the lowest node during the same period. It was difficult to determine by means of microscopic examination whether root initials were induced in the young nodes or whether primordia existed when the treatment began. It is probable, however, that the gas induced root initials to form on the younger nodes. Six other species (two cosmos, buckwheat, squash, broccoli, and sunflower) also showed evidence of root stimulation at nodes.

Forsythia, *Ribes*, and *Salix* were treated only as cuttings while standing in shallow water. These genera are known to form "root germs" on the stem and they furnished good material to test for root stimulation. All

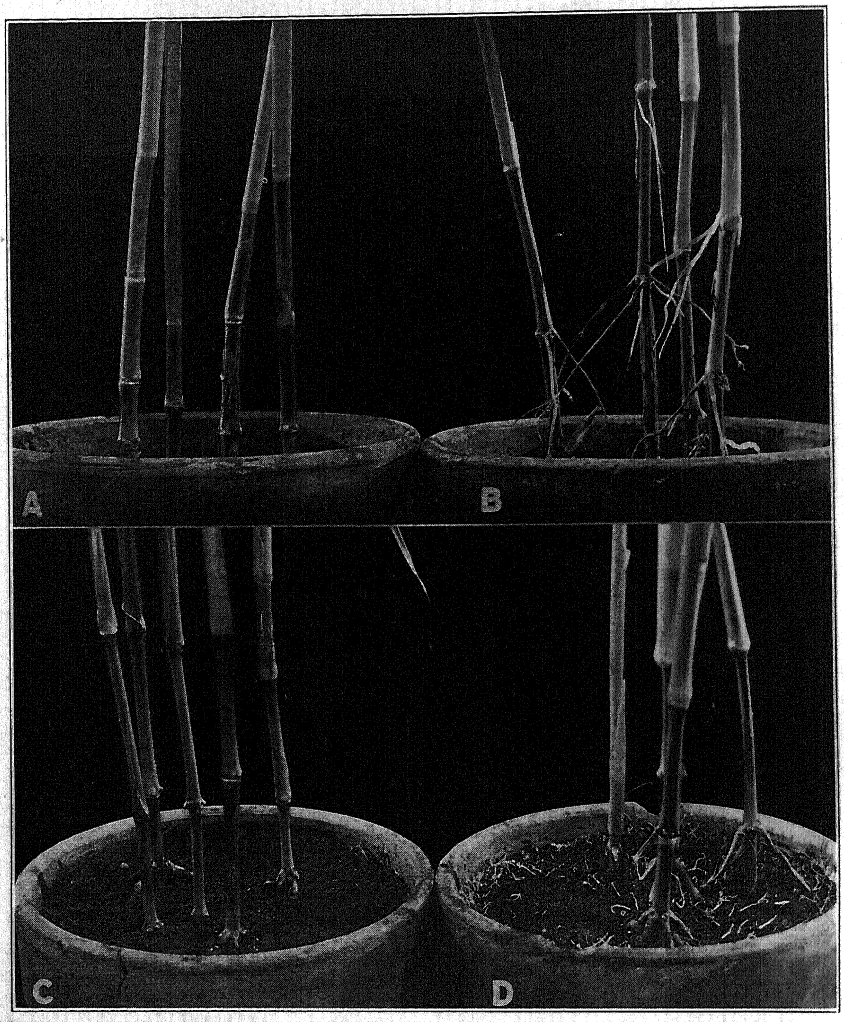


FIGURE 5. The effect of carbon monoxide on root formation of pop corn. A. Control plants in Wardian case for 10 days; B. Plants exposed for 10 days in Wardian case to approximately one per cent carbon monoxide; C. Control plants kept for 6 days in Wardian case; D. Plants exposed for 6 days to carbon monoxide gas. Note the roots growing up out of the soil. This effect was evident 48 hours after the experiment was started.

three types grew visible roots along the stem within five days after being exposed to gas. At this time no roots could be found on control plants. *Ribes* and *Salix* showed a few roots at the end of a 10-day period. *Forsythia* controls grew roots only at the base of stems in water.

MODIFICATION IN ORIENTATION OF LATERAL ROOTS TO GRAVITY

Nine species of plants (Table I) showed a tendency to send roots up out of the soil during treatment with carbon monoxide gas. It appeared



FIGURE 6. Effect of carbon monoxide on root hairs of the tomato. A. Roots that grew for 48 hours in air after having been previously forced with gas; B. The same roots as in A after an 18-hour exposure to one per cent carbon monoxide gas.

to be a kind of modification of normal response to gravity (Fig. 3 and 5 D). Once above the soil these roots produced an abundance of root hairs. Roots of treated corn, cosmos, and broccoli were particularly striking, in places completely hiding the soil from view. This modification in natural orientation of roots to gravity is not unlike the response of stem tips treated with ethylene, especially the epicotyl of pea seedlings (8) which changes from the vertical to nearly a horizontal position when treated with four parts of ethylene to 10,000,000 of air. The extent to which carbon monoxide modifies the direction of growth of roots has not yet been determined. The fact that the gas causes some modification is easily demonstrated.

ROOT HAIRS IN CARBON MONOXIDE GAS

Young roots growing in carbon monoxide gas appeared to have an abnormal supply of root hairs. Plants removed to air showed rapid elongation with few root hairs. Figure 6 A shows the appearance of roots that grew for 48 hours in air after having been forced with gas. Figure 6 B shows the same roots 18 hours after further exposure to gas. The hairs at the region of elongation show first signs of stimulation. Forty-eight hours later the entire root surface was fluffy with root hairs somewhat as shown near the tip in Figure 6 B.

RETARDATION IN ELONGATION OF ROOTS

Tomato plants exposed to gas continuously for 10 days produced many short roots well up along the stems. Those exposed just long enough to force root initials and then removed to humid atmosphere grew fewer but much longer roots than those held constantly in gas. It seems that though the gas induced root initials and stimulated primordia, yet elongation was retarded when the roots were exposed continuously.

CARBON MONOXIDE AS A SPECIFIC FOR ROOTS

The results of experiments to date indicate that adventitious roots are the only organs that can be induced to form by carbon monoxide. In several cases more than the normal number of branches occurred on treated plants. At first it seemed as though adventitious buds had formed, but more careful observations disclosed that the new shoots had arisen from preexisting buds. It appears, therefore, that carbon monoxide is specific for roots.

EFFECTS OF CARBON MONOXIDE ON SHOOTS

During the course of these experiments many results other than effects on roots have been recorded. For example, carbon monoxide causes (a) epinasty of leaves, (b) injury and abscission of leaves and flowers, (c) unusual proliferation of lenticular tissue, (d) retardation in elongation of stems, and (e) modification in the size of leaves developed while the plants are in the gas.

The details for these observations will be included in another paper which will be published soon.

DISCUSSION

Stone (11) reported that roots were stimulated on willow cuttings placed in water that was charged with illuminating gas. Doubt (6) passed illuminating gas through soil in which tomato plants were growing and found that roots had a tendency to grow up out of the soil and that there was an unusual development on the basal part of the stems; roots also appeared from the lower part of tomato stems treated in Wardian cases with

3.5 per cent illuminating gas. We have been unable to verify the results of Stone but those reported by Doubt were easily demonstrated. Since 20 to 40 per cent of illuminating gas consists of carbon monoxide, this constituent probably caused the unusual root effects. Where 3.5 per cent illuminating gas was used in a Wardian case, probably one per cent was carbon monoxide, and that is approximately the concentration we found most effective in causing the response. The same explanation should serve for Stone's results if his method allowed the gas to be held around the cuttings for a long enough period of time. Carbon monoxide gas is so slightly soluble in water that it is difficult to see how an effective concentration could get into the willow cuttings by simply bubbling illuminating gas for a few minutes each day through the liquid. Our results show, however, that carbon monoxide has the capacity to force "root germs" which are preexistent on willow cuttings. Stone's illustrations show that the treated cuttings made considerably more top growth than controls and this alone might account for extra root growth.

Richards and MacDougal (10) treated germinating seedlings and entire plants with high percentages of carbon monoxide and various mixtures of oxygen, nitrogen, carbon dioxide, and carbon monoxide. They did not find the responses reported in our results because the concentration of carbon monoxide used was too high. In general they found that carbon monoxide retarded root growth and caused imperfect development. The newly germinated corn seedlings produced a peculiar thickening at the base of the stem and from this "a considerable number of supernumary roots arose without order and grew out for a millimeter or two" (10, p. 61). These roots showed "a less degree of sensibility to the geotropic stimulus" than normal. This may be similar to the responses shown in Figure 3 and 5 D which are described as a modification in orientation to gravity and to Doubt's (6) results where roots of tomato plants grew up out of the soil. Richards and MacDougal also exposed potted plants to carbon monoxide under bell jars. No mention is made of root growth and it is assumed that no such response was evident. The concentration of the gas used was probably too high to permit of rooting. Went (13, p. 39) claims that in nature there is a "special root producing substance, not specific" manufactured by leaves. He sought to prove the hypothesis by growing cuttings of *Acalypha* with and without grafted leaves. Those with grafted leaves grew roots more readily than leafless cuttings. He also grew cuttings in agar with and without diastase, which was an extract from germinating barley seeds. Some substance associated with the diastase, he claims, stimulated roots on *Acalypha* cuttings. In these cases it is difficult to explain the results on a different basis from those that can be produced by various nutrients.

Curtis (2) increased shoot and root growth of woody cuttings by treat-

ment with potassium permanganate. Potted plants were not subjected to similar chemical treatments. He did not claim that the results constituted induced root initiation. Five possible explanations for the stimulation were offered as follows: (a) that the treatment causes a change in the nature of the food supply of the twig; (b) that it affects the rest period of the cuttings, serving to start growth earlier and thereby causing an apparent stimulation of root growth; (c) that it upsets the balance of food supply between the tops and the roots in favor of the latter; (d) that it retards or inhibits growth of microorganisms; (e) that it increases respiratory activity by catalytically hastening oxidation.

The results published by the foregoing authors show that the growth of roots or shoots was hastened by chemical treatment. In no case, however, was it shown that the chemicals induced roots to form in places where they do not normally occur when given the proper moisture and temperature conditions. The results reported in the present paper show that carbon monoxide actually induced roots to form. Also, since no other organs were induced, it appears that this gas is specific for roots.

SUMMARY

1. Carbon monoxide gas induced definite rooting responses in 27 species of plants.

2. Root initiation was induced by carbon monoxide in young stem tissues of 10 species. Microscopic examination in no case revealed root initials in comparable tissues of control plants.

3. *Hydrangea macrophylla*, *Nicotiana tabacum*, and *Impatiens balsamina* responded to carbon monoxide by producing roots from a short zone on the stem, representing approximately the region of elongation at the time the treatment began. Galinsoga produced roots only from the flower stems.

4. Carbon monoxide stimulated the growth of preexisting root primordia on older parts of stems.

5. Eighteen species were found to show root stimulation from gas treatments. Seven of these species showed a tendency to root especially at nodes.

6. Nine species exhibited some modification in normal orientation of soil roots to gravity during treatment.

7. Root hairs were more abundant on roots in gas than on those in a humid atmosphere without gas.

8. There was some evidence that after roots penetrated the epidermis they were slightly retarded in further elongation by carbon monoxide.

9. Tobacco cuttings taken from tips of plants exposed to gas grew large root systems in five days. It required 15 days to grow comparable roots on control cuttings.

10. Leafless tomato cuttings exposed to gas produced roots similar to those on the stems of treated plants. Leafy cuttings produced more roots than those without leaves.

11. Carbon monoxide is specific for inducing adventitious root formation.

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INSECT TRANSMISSION OF PEACH YELLOWS[1]

L. O. KUNKEL

INTRODUCTION

It has been shown that *Cicadula sexnotata* (Fall.), the gray leafhopper, which is known to transmit aster yellows to a large number of different plants including one species belonging in the Rosaceae, is unable to transmit aster yellows to the peach and is probably not a vector of peach yellows (3). During the past five years a number of other insects were used in attempts to transmit peach yellows. Successful transmissions were obtained with only one of these insects, the leafhopper *Macropsis trimaculata* (Fitch). The object of this paper is to report the results of the tests.

MACROPSIS TRIMACULATA

Macropsis trimaculata was first collected by the writer, who is indebted to Professor Herbert Osborn for its identification, on May 31st, 1931. Nymphs in the second and third instars were found in large numbers on peach trees. The first adult observed appeared in an experimental colony on June 13th. Soon after this date more adults than nymphs were found in peach orchards. By June 27th practically all individuals had become adult. They were numerous in orchards in the vicinity of Yonkers, New York, both in New York and in New Jersey from June 25th to July 10th. During this period from 50 to 100 adults could be caught from orchard trees in about one hour. There was a sharp falling off in numbers in the orchards after July 10th. By July 15th not more than one-half dozen could be taken in an hour. During the last half of July they were still more difficult to find. The last individuals caught during the season of 1931 were taken on August 8th.

This leafhopper has been reported on plums in New York (2) and other states (1). It has been reported on the peach in Virginia (4). During the past two summers it was numerous on both peach and plum trees in the vicinity of New York City. It was more numerous on the peach than on the plum in the early part of the summer. This condition was reversed late in both seasons when it became more numerous on the plum than the peach. It was not collected from other trees.

Macropsis trimaculata is a leafhopper which, so far as the writer has observed, never hops. It can run more rapidly than most other leafhoppers and has unusual ability in hiding. When disturbed it runs away and tries to hide. The adults seldom fly when slightly disturbed. They resort to flight only when closely pressed or when they move for considerable

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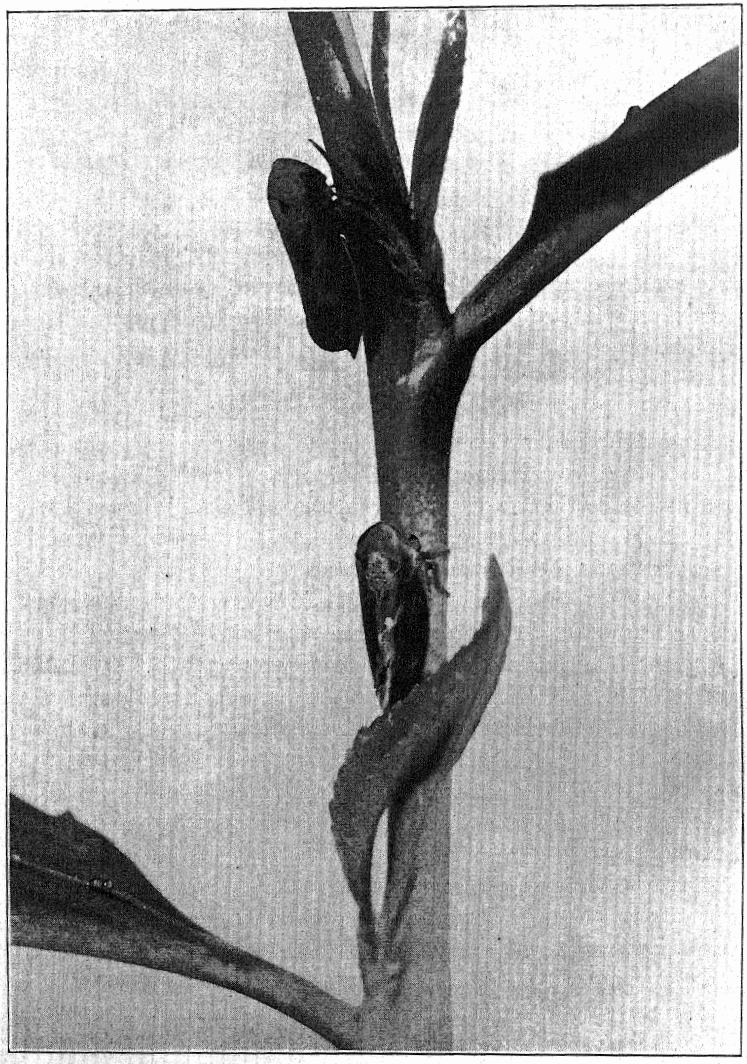


FIGURE 1. Two adults of *Macropsis trimaculata* feeding on a seedling peach tree.

distances in the course of their feeding and breeding activities. When flying they move very rapidly.

Both nymphs and adults feed on twigs and large branches. They are occasionally found feeding on leaves. They prefer to feed on old woody branches rather than on succulent young shoots and on old trees rather than on young ones. Figure 1 shows two adults feeding on a seedling peach tree.

Macropsis trimaculata, like many other leafhoppers which feed on woody plants, produces only one generation a year. The eggs are dormant when first deposited and apparently require the low temperatures of winter to bring them out of the dormant condition. Transmission experiments with such an insect are difficult because they can be made only during the relatively short periods when the yearly broods are feeding.

PLAN OF TRANSMISSION EXPERIMENTS

Nymphs and adults were transferred from both healthy and yellowed orchard trees to insect-proof cages containing potted peach seedlings having the peach yellows disease. After confinement on the yellowed peach seedlings for periods varying from 2 to 21 days, they were transferred to healthy young seedlings growing in other insect-proof cages. Colonies composed of from 2 to 100 individuals were allowed to feed for varying periods of time on healthy seedlings immediately after they had been taken from yellowed trees. After exposure to insect colonies all trees were held in a greenhouse kept free of sucking insects by frequent fumigations. On September 15th they were placed outside of the greenhouse in uncovered cold frames along with check trees and trees exposed to another insect. The trees were placed in the cold frames in order that they might become somewhat hardened before going into the dormant condition. All of the trees were transferred from the cold frames to a greenhouse held at 70° C. about December 1st.

WILTING ASSOCIATED WITH FEEDING OF MACROPSIS TRIMACULATA

If as many as 10 adults are allowed to feed on a young seedling peach tree about one foot high for as long as one week, they cause a sudden wilting of the tree. If the wilting is slight and the insects are removed promptly the tree may recover. If the insects are not removed promptly the tree will die. Wilting begins in the youngest leaves and the trees die from the tips downward. If a colony of as many as 20 insects is allowed to feed for three or four days on a young seedling it will usually show no wilting whatever at the end of this period. The tree may, however, begin to wilt several days after the insects have been removed. The severity of wilting depends on the number of insects used and the length of time they are allowed to feed. The size and age of the tree is also important. The

older the seedling the more insects it will support without wilting. Whether all individuals are equally effective in causing wilting has not been determined. Figure 2 shows five peach seedlings of the same age. Three of the trees show the characteristic wilting caused by *Macropsis trimaculata*. A microscopic examination was made of a number of wilted trees. No bacteria or fungi could be found in the leaf, stem, or root tissues to account for the wilting. It is believed that *Macropsis trimaculata* may inject a deleter-



FIGURE 2. Peach seedlings showing wilt which follows the feeding of *Macropsis trimaculata*. The tree on the left is the check. The tree next to the check has recovered after slight wilting. The three trees on the right are badly wilted.

ious substance into the trees on which it feeds, and that this substance may in some way cause wilting.

TRANSMISSION TESTS

Table I shows the results of transmission tests with *Macropsis trimaculata* made during the summer of 1931. A total of 74 trees which were exposed for varying periods of time from June 3rd to August 7th are listed in the table. A number of trees that wilted and died shortly after exposure are not included. Some of the trees that lived wilted slightly but recovered fully after about two weeks. No record was kept of the exact number of insects composing most of the colonies to which the different trees were

TABLE I

PEACH YELLOWS TRANSMISSION EXPERIMENTS WITH MACROPSIS TRIMACULATA

Trees	Period of exposure	Trees that remained healthy	Trees that became diseased	Date when transmision was recorded
Numbers 1 to 6	June 3 to June 5, 1931	6	0	
Numbers 7 to 12	June 5 to June 9, 1931	6	0	
Number 13	June 5 to July 2, 1931	1	0	
Numbers 14 to 17	June 9 to June 15, 1931	4	0	
Number 18	June 9 to June 13, 1931	1	0	
Numbers 19 to 25	June 10 to June 15, 1931	7	0	
Numbers 26 and 27	June 11 to June 13, 1931	2	0	
Numbers 28 and 29	June 13 to June 15, 1931	2	0	
Number 30	June 13, 1931 until colony died	1	0	
Numbers 31 to 43	June 15 to July 2, 1931	13	0	
Numbers 44 to 48	June 18 to June 20, 1931	5	0	
Number 49	June 18 to July 2, 1931	1	0	
Numbers 50 to 52	June 20 to June 30, 1931	3	0	
Number 53	June 27 to June 29, 1931	1	0	
Number 54	June 29 to July 13, 1931	1	0	
Numbers 55 to 65	July 2, 1931 until colonies died	11	0	
Numbers 66 to 68	July 2, 1931 until colonies died	0	3	January 4th, 1932
Number 69	July 13 to July 15, 1931	0	1	October 15th, 1931
Number 70	July 15 to July 21, 1931	0	1	October 1st, 1931
Number 71	July 15 to July 21, 1931	1	0	
Number 72	July 15 to August 2, 1931	0	1	January 4th, 1932
Number 73	July 21 to August 7, 1931	1	0	
Number 74	July 21 to August 7, 1931	0	1	October 15th, 1931
Numbers 75 to 207	Unexposed checks	133	0	

exposed. Many of the colonies used were as large as the trees would support without severe wilting. A number of trees were exposed until all individuals of the colonies placed on them had died. Since adults usually live for more than a month these exposures were rather long. Yellows was not transmitted to any of the trees exposed during the month of June when most of the tests were made. The seven trees which took the disease were exposed during July. Only 19 trees were exposed during this month. Insects that remained alive after the completion of tests were frequently used in making up colonies for later tests. Some of the colonies that transmitted yellows included individuals that had fed on yellowed peach trees a long time before they were placed on the trees that became diseased. These results suggest that there may be a long incubation period for the virus of peach yellows in this leafhopper.

One of the trees which took the yellows disease was exposed for only two days. Another was exposed for six days. The other five trees were exposed for longer periods of time. Column 5 of the table shows the dates on which yellows was recorded for each of the trees that became diseased. Well marked symptoms were shown by tree number 70 seventy-eight days after it was exposed. This tree showed early symptoms about two weeks before the record shown in the table was made. Tree number 74 showed unmistakable symptoms of yellows eighty-six days after exposure, tree number 69, ninety-four days after exposure, tree number 72, one hundred seventy-three days after exposure, and trees number 66, 67, and 68, one hundred eighty-six days after exposure. The first symptoms of yellows appeared in each case some days before the trees were recorded as diseased.

Symptoms of yellows appeared in the first three trees to show the disease as they began to lose their leaves. Instead of becoming fully dormant as the weather grew cold some of the branches on these trees continued to grow and to produce tender secondary shoots such as are characteristic for yellowed trees. All other trees exposed to *Macropsis trimaculata* and all check trees lost their leaves. As the trees began to grow after being taken from the cold frames to a warm greenhouse, the three that had shown yellows in the cold frames and four others that had been exposed to *Macropsis trimaculata* became badly yellowed. One hundred thirty-three check trees similar to the ones exposed to *Macropsis trimaculata* and 60 trees that had been exposed to another insect were still healthy one year after the tests were made. All trees were held under the same conditions and were treated alike in every way except as regards exposure to insects. Five of the trees that took yellows and one healthy check tree are shown in Figure 3. The results recorded in the table are disappointing in that only 7 of the 74 trees that were exposed took yellows. The reason for this low percentage of transmission is not known. It should be pointed out, however, that young trees such as were used in the tests seldom take yel-



FIGURE 3. Six peach seedlings of the same age. The seedling on the left is one of the check trees. It was not exposed to insects and is healthy. The other five seedlings were exposed to *Macropsis trimaculata*. They have the peach yellows disease.

lows when growing naturally in nurseries. It may be that they are more resistant to infection than the older trees on which *Macropsis trimaculata* prefers to feed. Because of the difficulty of moving and of confining large trees in insect-proof cages or even in greenhouses for periods of time long enough to make transmission tests, small trees only were used.

During the past five years many hundreds of healthy and yellowed seedlings were grown in the same greenhouses. Many healthy and yellowed trees were held side by side on the benches for more than a year. Some were even grown side by side in the same pots. During this time there was not a single case of accidental transmission. The possibility of accidental transmissions in the case of the seven trees that took yellows after exposure to *Macropsis trimaculata* seems very remote.

As was noted above, the trees exposed to *Macropsis trimaculata* as well as the check trees and the trees exposed to another insect were placed in uncovered cold frames on September 15th. Any peach insects prevalent at this season of the year may have fed on the trees although none except the green peach aphid was observed. The trees which had been exposed to *Macropsis trimaculata* were not placed together in the cold frames but were distributed at random among the 133 check trees and 60 trees that had been exposed to another insect. These 193 trees have all remained healthy.

The possibility that some agent capable of transmitting yellows may have been prevalent in the unprotected cold frames after September 15th is recognized. The probability that such an agent would have carried the disease to seven trees exposed to *Macropsis trimaculata* during July but to none of the trees exposed to this insect in the month of June and to none of the check trees or trees exposed to another insect, is small. But even if this had occurred it would not account for the development of yellows in three of the trees in less than one month after they were placed in the cold frames, since the minimum incubation period for peach yellows in seedling trees to which the disease is transmitted by budding is 41 days, under the most favorable conditions for the appearance of symptoms. It is, of course, possible that this period might be less than 41 days when transmission is by means of an insect vector.

Although only seven of the trees exposed to *Macropsis trimaculata* in 1931 became diseased, the conclusion that this insect is a vector of peach yellows seems justified.

INSECTS WHICH HAVE NOT TRANSMITTED YELLOWS

Species of insects used in unsuccessful attempts to transmit peach yellows include the green peach aphid, *Myzus persicae* (Sulzer), black peach aphid, *Aphis persicae-niger* Smith, tarnished plant bug, *Lygus pratensis* (L.), peach borer, *Conapia exitiosa* Say, mealy bugs, *Pseudococcus*

citri Risso and *P. longispinus* (Targioni), froghopper, *Philaenus leucophthalmus* (L.) var. *pallidus* (Zett.), treehoppers, *Thelia bimaculata* (Fabricius) and *Ceresa bubulus* (Fabricius), and leafhoppers, *Graphocephala coccinea* (Forst.), *Empoa rosae* (L.), *Jassus olitorius* Say, *Fieberialla flori* (Stal.), and *Erythroneura obliqua* Say. Several species of aphids and leafhoppers which have not been identified were also tested. Most of the aphids were obtained from rosaceous plants other than the peach. The leafhoppers were taken in small numbers from the peach.

The plan of the experimental tests was the same in all cases. Healthy young seedling peach trees growing in pots in insect-proof cages were exposed to insects that had been confined previously on peach seedlings having yellows or taken from yellowed trees growing in an orchard. Trees exposed to the different insects together with unexposed check trees were kept under observation in greenhouses. The houses were fumigated at frequent intervals to keep them free from insects. All trees were held for at least one year after the exposures were made, and many of them were held for two years.

The green peach aphid and the black peach aphid were used in a large number of tests made during several different years. Both are capable of living and breeding on the peach over long periods of time.

The tarnished plant bug was taken in small numbers from the peach. It was used in a small number of tests.

The mealy bugs were obtained from *Sida rhombifolia* L. They live and multiply on the peach and were used in many attempts to transmit yellows.

The froghopper was obtained from hybrid tea roses. It was never found occurring naturally on the peach. Since, however, it can live indefinitely on this host it was used in a large number of experiments.

The favorite host plant of the treehopper *Thelia bimaculata* is the black locust, *Robinia pseudacacia* L. A few individuals were taken from peach trees. The insect was obtained in large numbers from locust trees. It feeds satisfactorily on the peach but is unable to live on this tree for more than five or six days. It was used in a number of different tests.

The buffalo treehopper occurs naturally in small numbers on the peach. Since it can live for many weeks on this host it was used in a large number of tests.

The peach is a favorite host of the leafhopper *Graphocephala coccinea*, which deposits eggs in the twigs and can live its whole life on young peach seedlings. It was used in a large number of transmission tests.

The rose leafhopper *Empoa rosae* occurs in small numbers on the peach. It was obtained in large numbers from rose bushes. Although it can live a long time on peach leaves, it was used in only a small number of tests.

The leafhopper *Jassus olitorius* occurs naturally on the peach in con-

siderable numbers. The nymphs appear late in July. Adults are to be found from about the 10th of August to the middle of September. A large number of tests were made with this leafhopper during two summers.

Adults of *Fieberialla flori* occur in small numbers on the peach. Nymphs have never been taken from peach trees. Both nymphs and adults occur in considerable numbers on *Spiraea thunbergia* Siebold. They live for several weeks when transferred from *Spiraea* to the peach. Many transmission tests were made with this insect.

The leafhopper *Erythroneura obliqua* occurs in large numbers on the peach late in the summer. It has been used in a considerable number of transmission tests.

Peach yellows was not transmitted by any of these insects under the conditions obtaining in the experiments.

SUMMARY

1. Peach yellows was experimentally transmitted to seven young seedling peach trees by the leafhopper *Macropsis trimaculata*. Only about 10 per cent of the trees exposed to the leafhopper took yellows.

2. The disease was not transmitted by the green peach aphid, black peach aphid, tarnished plant bug, peach borer, mealy bugs, *Pseudococcus citri* and *P. longispinus*, froghopper, *Philaenus leucophthalmus* (L.) var. *pallidus* (Zett.), treehoppers, *Thelia bimaculata* and *Ceresa bubulus*, or leafhoppers, *Graphocephala coccinea*, *Empoa rosae*, *Jassus olitorius*, *Fieberialla flori*, and *Erythroneura obliqua*.

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EFFECT OF SULPHUR COMPOUNDS IN BREAKING THE DORMANCY OF POTATO TUBERS AND IN INDUCING CHANGES IN THE ENZYME ACTIVITIES OF THE TREATED TUBERS[1,2]

LAWRENCE P. MILLER[3]

INTRODUCTION

It has long been known that freshly-harvested tubers of the potato (*Solanum tuberosum* L.) are dormant and require a rest period of from several weeks to three months, depending upon the variety, before uniform sprouting of seed pieces results. Various methods of eliminating this rest period have been suggested and especially favorable results have been obtained by treatments with various chemicals. Recently emphasis has been placed (7, 12) upon three chemicals, ethylene chlorhydrin (CH_2ClCH_2OH), sodium thiocyanate (NaSCN), and thiourea ($NH_2CS NH_2$). Two of these are sulphur-containing compounds and the third, ethylene chlorhydrin, has been shown to have important effects upon the sulphur metabolism, increasing the capacity of the juice of both potatoes and gladiolus to form H_2S from sulphur (15), a reaction characteristic of sulphydryl compounds.

It was accordingly thought desirable to test other organic sulphur compounds with respect to their effect on dormant potatoes. Preliminary results indicating that a large number of such compounds are active in breaking dormancy have been published (21). Further studies have confirmed these results. Compounds found effective are ammonium dithiocarbamate (NH_2CSSNH_4), thiosemicarbazide ($NH_2CSNHNH_2$), ethyl mercaptan (C_2H_5SH), hydrogen sulphide (HSH), thioacetamide ($NH_2 CSCH_3$), thioglycol (CH_2OHCH_2SH), methyl disulphide (CH_3SSCH_3), and various derivatives of dithiocarbamic acid (NH_2CSSH). Thioacetamide differs from all the other chemicals studied in that it retards the germination of non-dormant tubers in addition to breaking the dormancy of dormant tubers. The probable reason for these effects is discussed below.

Juices from potatoes treated with certain of these chemicals were examined as to pH, iodine absorption, catalase and peroxidase activity, and reducing sugar and sucrose content at intervals after treatment, and compared with juices from corresponding check potatoes. In general, the treatments resulted in increases in the activity of the enzymes studied;

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[2] Thesis presented to the Faculty of Purdue University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1933.

[3] The writer is indebted to Prof. H. R. Kraybill of Purdue University and to the members of the staff of the Boyce Thompson Institute for helpful suggestions and criticisms throughout the course of the work.

the extent of the increases was, however, not a measure of the efficacy of the treatments. Large increases in sucrose resulted from the treatments. These increases took place even in completely non-dormant tubers which already had a relatively high sucrose content. The changes in the properties of the juices of treated potatoes in general agree with those previously found for treatments with thiourea and sodium thiocyanate (12).

It was also found that in the case of vapor treatments of whole tubers large increases in the respiratory activity resulted. Preliminary experiments with treatments of cut pieces indicated that these treatments do not bring about such large increases in the respiratory rate.

The effect of adding varying amounts of these chemicals to the juice directly was also studied and it was found that increases in the enzyme activity did not result from such direct additions, indicating that the increases in enzyme activity found in the case of the treatments were due to a tissue response to the treatments and not to any direct effect on the enzymes.

These chemicals when added directly to the juices have a very marked effect in retarding the darkening which normally occurs when potato juice is exposed to the air. This retardation is manifest even in very dilute concentrations of the chemicals; the inhibiting effect is stronger than that of potassium cyanide (KCN) which has long been regarded as a very active inhibitor of oxidase activity. It is not known at the present time whether this is of any particular significance in connection with the breaking of dormancy which results from these treatments.

MATERIALS

Source of the potatoes used. Experiments were conducted in 1930 and 1931. During 1930 potatoes from the following sources were used: early in June, potatoes of the Irish Cobbler variety from South Carolina; in August, Bliss Triumph, Irish Cobbler, and Early Ohio varieties from the Institute gardens; in October, Bliss Triumph from Maine; in November, second crop Irish Cobbler from New Jersey. During 1931 Irish Cobbler potatoes were obtained from South Carolina in early June, potatoes of the Irish Cobbler and Bliss Triumph varieties from the Institute gardens in August, Irish Cobbler potatoes from Long Island in September, and second crop Irish Cobblers from New Jersey in November. In the tables the variety and source of the potatoes used are given throughout. A few experiments were conducted with potatoes purchased in the open market. These are so designated in the tables.

Chemicals Used

The organic sulphur compounds used in this work, wherever possible, were obtained from the Organic Division of the Eastman Kodak Com-

pany. If the compounds could not be procured from this source they were prepared in the laboratory. Usually the procedure employed was one previously published and references to the methods of preparation are given in the section of the paper dealing with the results of the treatments with the compounds in question. In the case of ammonium dithiocarbamate, however, previous methods of preparation were modified to give a purer product and a better yield.

Preparation of ammonium dithiocarbamate. The reaction between ammonia and carbon disulphide in alcoholic solution was first studied by Zeise (31) in 1824. He discovered ammonium dithiocarbamate and identified ammonium thiocyanate (NH_4SCN) and ammonium trithiocarbonate [$(NH_4)_2CS_3$] among the products of the reaction. Debus (5) also prepared ammonium dithiocarbamate and published analyses. Others who have prepared this compound are Mulder (22), Freund and Bachrach (14), and Delépine (6).

Debus (5) reported that an excess of ammonia favors the formation of ammonium thiocyanate and that an excess of carbon disulphide results in the formation of ammonium dithiocarbamate as the main product. The method usually suggested for the preparation of the dithiocarbamate (3, 5, 14, 22) is to add carbon disulphide to alcoholic ammonia and allow the mixture to stand for a number of hours.

After various trials the method of preparation finally adopted was to dissolve the carbon disulphide in a large amount of ether with a little alcohol and to pass gaseous ammonia into this mixture. The flask in which the reaction took place was kept cool in an ice bath. After several hours a large mass of crystals formed and the flask was set aside in a room kept at about $-15°C$. After filtering, more ammonia can be passed into the carbon disulphide-ether mixture and a further yield of crystals obtained. While no attempt was made to determine the maximum yield obtainable by this method, the yields were better than the 55 per cent obtained by Freund and Bachrach (14). Thus in one experiment 200 cc. carbon disulphide were dissolved in 600 cc. ether and 100 cc. alcohol and two crops of crystals separated, weighing 92 and 135 g., and representing a yield of 60 per cent. The crystals were separated in a Büchner funnel and washed first with alcohol and then with ether. The material prepared in this way is entirely free of thiocyanate as shown by the test with ferric chloride and also contains no ammonium trithiocarbonate. In testing for thiocyanate with ferric chloride it is, of course, necessary to add an excess since the ferric chloride reacts with the dithiocarbamate as described below, and the color with thiocyanate does not result until an excess of ferric ion is present. Ammonium trithiocarbonate is very unstable and any crystals of this substance can easily be recognized as they absorb water very rapidly when exposed to the air and turn an orange color.

Ammonium dithiocarbamate reacts with ferric chloride and with iodine in acid solution to form thiuram disulphide ($NH_2CSSSSCNH_2$). Tests showed that both of these reactions may be used as a quantitative method for the volumetric determination of the dithiocarbamate. A solution of ammonium dithiocarbamate acidified with hydrochloric acid can be titrated with ferric chloride using the thiocyanate ion as an indicator. The reaction is a little slow toward the end but the end point is very definite. Similarly, solutions of ammonium dithiocarbamate can be titrated with N/10 iodine in the presence of hydrochloric acid. By these two methods it is possible to determine the dithiocarbamate in the presence of its decomposition product, ammonium thiocyanate. The thiocyanate does not react with iodine in the presence of hydrochloric acid.

The crude crystals obtained by the method described above had a purity above 95 per cent. The impurities probably consisted of water together with a little sulphur. The crystals had a citron-yellow color. In the literature ammonium dithiocarbamate is described as being yellow with the exception that Mulder (22) states that the pure substance is probably white. It seems that none of the workers who have prepared this substance has recrystallized it, having considered it too unstable to withstand recrystallization. It was found that it could readily be recrystallized by dissolving the crude crystals in a minimum of water, filtering, and adding this concentrated aqueous solution to a mixture of alcohol and ether and setting aside at low temperature for crystallization to take place. The product obtained by this method was pure white and analyses indicated a purity of 98.5 to 99.5 per cent.

Ammonium dithiocarbamate is quite unstable and the preparations soon give a positive test for thiocyanate. At this stage the odor of hydrogen sulphide is evident and free sulphur is also present. Ammonium dithiocarbamate thus decomposes into ammonium thiocyanate, hydrogen sulphide, and sulphur. The samples of ammonium dithiocarbamate used in the work were stored in a room kept at a temperature between $-10°$ and $-15°$ C. At this temperature this compound is relatively stable; preparations several months old could still be used and contained relatively little ammonium thiocyanate (about 5 per cent). The preparations recrystallized as described above gave a positive test for thiocyanate after several days but this did not increase in intensity very rapidly. Subsequent tests have shown that the decomposition is much hastened by the presence of moisture; the dithiocarbamate is much more stable if kept dry. Some recrystallized material stored in a desiccator over anhydrous calcium chloride has remained free of thiocyanate and of the odor of hydrogen sulphide for several months.

METHODS

Chemical treatments. In the case of chemicals soluble in water, treatments were made by soaking cut pieces for one hour in a solution of the required concentration. For these treatments the tubers were cut into pieces weighing about 25 g. each and with but one eye to each piece. As checks, corresponding pieces were soaked in distilled water. At the end of the treatment the pieces were planted in soil without rinsing. In the case of chemicals not soluble in water but sufficiently volatile, vapor treatments were made. Whole tubers were placed in glass or earthenware vessels with covers and were exposed to the vapor of the chemical evaporating from cheesecloth placed loosely at the top of the container. Check tubers were placed in similar vessels containing no chemical. After 24 hours the tubers were removed, cut into pieces with one eye each, and planted in soil.

Measuring the effect on dormancy. In testing new chemicals, treatments were made on as many different lots of dormant potatoes as possible. Since potatoes vary considerably in regard to dormancy from lot to lot and since it is relatively easy to break the dormancy of partially non-dormant potatoes, treatments were made on rather small samples from many lots of tubers. Data from such experiments are more valuable than tests on fewer lots of tubers even if very large samples are used. Thus, even if only 12 pieces are used per sample, if the potatoes are very dormant the checks will not begin to grow for from four to ten weeks after treatment while in the case of an effective treatment the treated tubers will grow in from two to three weeks after treatment. If a chemical gives such results with a considerable number of lots of dormant tubers one can safely conclude that it is effective in breaking dormancy.

Early in the investigation data were obtained by counting the number of sprouts above ground several times during the length of the test and thus noting the difference between the treated and check lots. Photographs were also taken at the end of the experiments. Later in the work the number of sprouts above ground were counted at more frequent intervals (every few days) and a figure obtained for the number of days necessary for 50 per cent of the sprouts to come above ground. Such a figure really represents the median of the number of days necessary for all the pieces to show sprouts above ground, and is a good figure to use to compare one treatment with another. Data obtained in this way also bring out the degree of dormancy of the checks. When the experiments were ended the weight of tops produced was also recorded and in many cases photographs were taken. If at this time 50 per cent of the checks had not yet sprouted, as was frequently the case, the checks were not discarded until the required data could be obtained.

Obtaining samples of juice. To obtain samples of juice the seed pieces,

which had been planted in good soil in the interim between treatment and sampling, were washed free of dirt, dried with cheesecloth, and the peel and callus tissue removed. The material was then passed through a food grinder and the juice pressed out through cheesecloth. The determinations were made on the juice thus obtained after the starch had been removed by centrifuging. For each sampling, 10 or 12 pieces were taken from each treatment.

Catalase. For the catalase determinations the apparatus and procedure described by Davis (4) were used except that the Dioxygen was neutralized with calcium carbonate instead of sodium hydroxide. The readings reported in the tables represent the number of cubic centimeters of oxygen liberated by 0.8 cc. of potato juice in one minute.

Peroxidase. For the determination of peroxidase activity previous methods were modified to a considerable degree in order to make the procedure more rapid and permit a number of determinations to be made at the same time. Pyrogallol was used as a substrate (2) but the procedure used by Willstätter (30) was modified to the extent that the reactions were carried out in centrifuge tubes and the precipitated purpurogallin was dissolved in 95 per cent alcohol instead of ether for the colorimetric comparison (12). In the first part of the work relative results only were obtained and peroxidase activities were expressed in terms of the activity of the check juices. Later the procedure was changed so that the peroxidase activity could be expressed as the amount of purpurogallin formed by a definite amount of potato juice under standardized conditions. Determinations made at different times could then be compared with each other.

Centrifuge tubes of about 80 cc. capacity were used. To 20 cc. of 2 per cent pyrogallol were added 20 cc. of a phosphate buffer of pH 6.5 (prepared by mixing 150 parts of M/15 $Na_2HPO_4.12H_2O$ with 350 parts of M/15 KH_2PO_4), and the required amount of potato juice diluted to 20 cc. The amount of potato juice varied from 0.4 cc. to 1.6 cc., depending upon its peroxidase content. The reaction was started by adding 2 cc. of Dioxygen and was allowed to proceed exactly 30 minutes in a water bath at a temperature of 26.7° C. controlled by a Freas thermostat. The reaction was stopped by the addition of 1 cc. of 5 per cent sulphuric acid. After allowing several hours to elapse to permit all of the purpurogallin to separate out, the tubes were centrifuged, the solutions carefully poured off, and the precipitate washed into 50 cc. volumetric flasks with 95 per cent alcohol. After the purpurogallin was dissolved the solution was filtered and compared in the colorimeter with standards containing known amounts of purpurogallin. Purpurogallin dissolves rather slowly in alcohol and if quick solution is desired it is necessary to apply heat. The purpurogallin, however, will dissolve in the cold if allowed to stand for several hours.

The standard was prepared from alcoholic solutions of purpurogallin from previous peroxidase determinations. The alcohol was distilled off, the precipitated purpurogallin filtered, washed with water, and dried at room temperature. The same lot of purpurogallin was used as a standard for all the determinations reported in this paper.

In order to find out within what limits the amounts of purpurogallin thus determined were a measure of the peroxidase present, various determinations were made using varying quantities of potato juice. Such a test is shown in Figure 1, where it is seen that between 10 and 25 mg. the amount of purpurogallin obtained is a linear function of the amount of

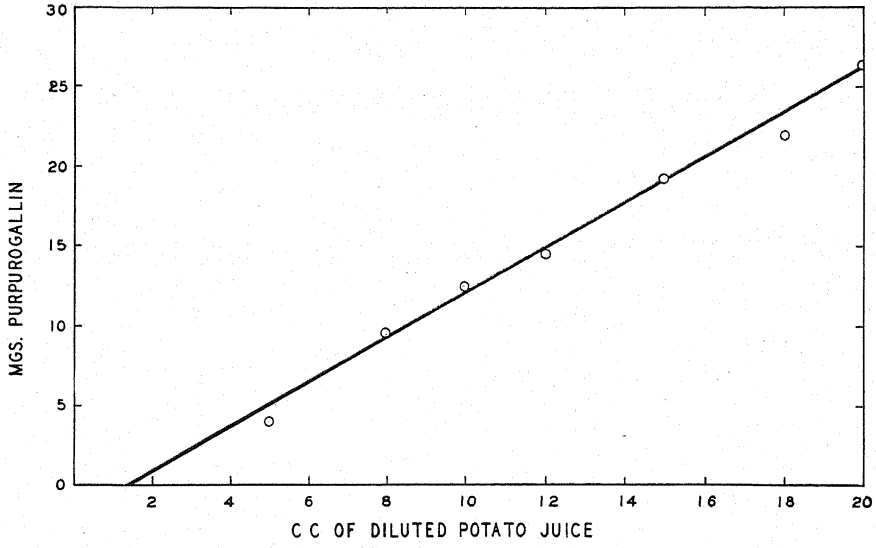


FIGURE 1. Curve showing that the amount of purpurogallin produced is a linear function of the amount of peroxidase present within the limits of 10 to 25 mg.

peroxidase present. Other trials have shown that for larger quantities the curve begins to flatten out and in such cases it is necessary to use less of the sample. It is probable that if a higher concentration of pyrogallol were used the amount of purpurogallin formed would be linear over a wider range. Pyrogallol has an inhibiting effect, however, which becomes quite pronounced if the concentration is increased much over the amount used in this procedure.

The accuracy of the results obtained by this method is indicated by the following test. Eight replicates gave an average of 14.9 mg. of purpurogallin with a minimum of 14.3 and a maximum of 15.7, and a standard deviation of 0.48 mg.

The values for peroxidase activity given in the tables represent the

number of milligrams of purpurogallin formed by 1.6 cc. of potato juice under the conditions described above.

Reduction of iodine. Iodine titration figures given in the tables are the number of cc. of N/100 I absorbed by 5 cc. of potato juice in the presence of 10 cc. of 10 per cent trichloracetic acid, using starch as an indicator.

Hydrogen ion concentration. The quinhydrone electrode was used for the pH determinations.

Methylene blue reduction. Five cc. of potato juice were mixed with 1 cc. of a solution of methylene blue containing 50 mg. per liter and observations made on the rate of decolorization.

Qualitative tyrosinase. Five cc. of a tyrosine solution made by dissolving 0.5 g. tyrosine in 500 cc. each of M/15 KH_2PO_4 and M/15 Na_2HPO_4. $12H_2O$ were mixed with 0.8 cc. of potato juice diluted to 10 cc. Differences in the rate of darkening between treated and check juices were noted.

Reducing sugars and sucrose. Samples of juices for subsequent sugar determinations were added to such a quantity of boiling 95 per cent ethyl alcohol as to make a final concentration of about 70 per cent. When the determinations were to be made the alcohol was evaporated off on the steam bath, the solutions cleared with lead acetate and deleaded with sodium oxalate. Reduction of Fehling's solution was carried out under the conditions described by Quisumbing and Thomas (24) and the cuprous oxide formed was determined by titration with potassium permanganate after solution in sulphuric acid in the presence of ferric ammonium sulphate. Sucrose was hydrolyzed by invertase prepared from yeast after the method of Reynolds (26). The values given in the tables represent milligrams per 5 cc of juice.

Respiration. In certain of the experiments respiration as measured by the carbon dioxide given off was determined. The tubers were put in desiccators placed in a thermostatically-controlled water bath. Carbon dioxide-free air was passed through the desiccators and the carbon dioxide given off was collected in barium hydroxide with the use of Van Slyke-Cullen (29) tubes. These tubes were very satisfactory for this purpose and much more convenient than the Pettenkofer (23) tubes commonly used in respiration studies. The tubes were arranged in series and a sufficient number was used so that the last few tubes in the series showed no precipitate. Each tube contained 50 cc. of saturated barium hydroxide. It was found that if air was passed through at the rate of about 14 liters per hour, two to three tubes absorbed all the carbon dioxide coming off and no appreciable absorption occurred in the subsequent tubes until the barium hydroxide in the first tube was almost exhausted. Because of the formation of the barium carbonate precipitate the progress of the experiment could easily be followed visually and there was no danger of any of the carbon dioxide escaping. At the end of suitable intervals the tubes con-

TABLE I

EFFECT OF AMMONIUM DITHIOCARBAMATE IN BREAKING THE DORMANCY OF POTATO TUBERS (1930)

Concentration, g. per 100 cc.	Number of sprouts above ground at intervals after treatment (12 pieces per treatment)												
	South Carolina Irish Cobblers		Institute Irish Cobblers		New Jersey Irish Cobblers*			Immature Irish Cobblers†		Institute Early Ohio		Maine Bliss Triumph	
	After 25 days	After 41 days	After 28 days	After 45 days	After 35 days	After 35 days	After 37 days	After 31 days	After 106 days	After 28 days	After 45 days	After 25 days	After 42 days
0.75	—	—	8	10	10	12	16**	11	13	9	12	8	12
0.50	11	12	6	8	11	11	14	8	11	7	11	8	11
0.25	4	4	2	6	0	2	—	—	—	5	8	1	6
Check	0	0	0	1	0	0	1	0	1	0	5	0	0

* Three separate treatments of tubers from this lot.
** 16 pieces per treatment.
† 15 pieces per treatment.

taining barium carbonate were disconnected and fresh ones added. Such a change could be made in a few minutes without interrupting the experiment. It was thus possible to determine the total amount of carbon dioxide given off during the course of a week or more. The amount of carbon dioxide was estimated by titrating an aliquot of the barium hydroxide solution after the precipitated barium carbonate had settled and calculating the quantity neutralized by the carbon dioxide, the strength of the barium hydroxide before absorption of carbon dioxide being known.

RESULTS

EFFECT OF AMMONIUM DITHIOCARBAMATE

Effect on Dormancy

Of the sulphur compounds considered in this paper ammonium dithiocarbamate was probably the most efficacious. Tables I, II, and III present

TABLE II

EFFECT OF AMMONIUM DITHIOCARBAMATE IN BREAKING THE DORMANCY OF IRISH COBBLER POTATOES (1931)

Source of tubers	No. of seed pieces in test	Days until 50% were above ground		Growth of tops		
				Days after treatment	Fresh weight, g.	
		Treated*	Check		Treated	Check
Institute	24	19	122	45	367	36
New Jersey						
Exp. 1	12	29	80	72	410	0
Exp. 2	12	<23	71	56	240	0
Exp. 3	12	<25	72	52	203	1

* Treated pieces were soaked in a solution containing 0.75 g. per 100 cc.

TABLE III

EFFECT OF AMMONIUM DITHIOCARBAMATE IN BREAKING THE DORMANCY OF IRISH COBBLER POTATOES (SOUTH CAROLINA 1931)

Conc., g. per 100 cc.	No. of sprouts out of 16 planted				Weight of tops produced, g.			
	Exp. 1*	Exp. 2*	Exp. 3**	Exp. 4**	Exp. 1*	Exp. 2*	Exp. 3**	Exp. 4**
0.75	16	16	—	—	114	117	—	—
0.50	16	16	16	16	121	221	130	185
0.25	14	16	11	7	81	152	110	153
Check	3	2	3	2	27	25	5	25

* 52 days after treatment.
** 56 days after treatment.

data on the results obtained with a total of 16 treatments on nine different lots of dormant potatoes. Included in these tables are all the treatments made on very dormant potatoes, but a few treatments made on potatoes

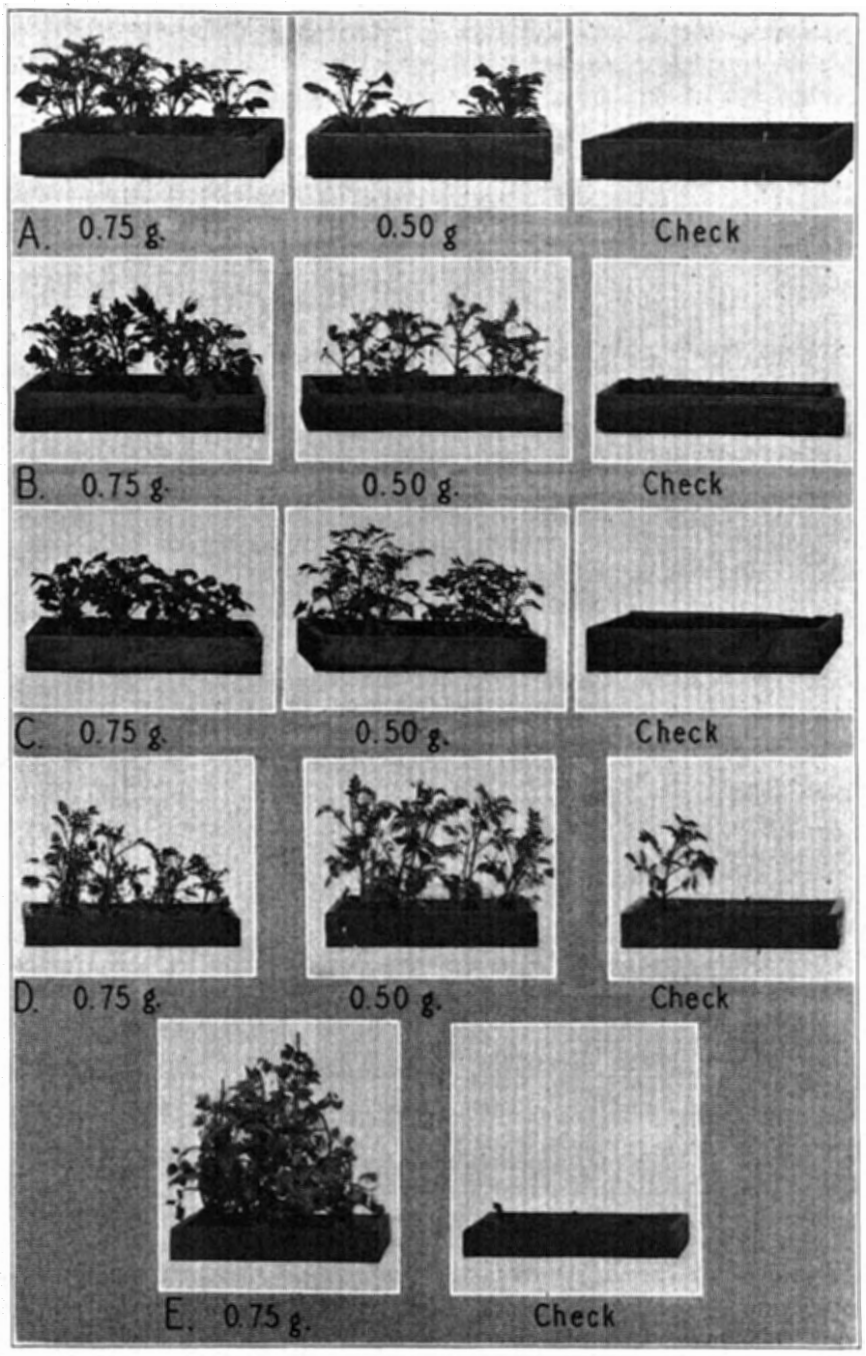


FIGURE 2. The effect of treatments with various amounts (g. per 100 cc.) of ammonium dithiocarbamate in breaking the dormancy of potato tubers. A, Institute Irish Cobbler 1930; B, Institute Early Ohio 1930; C, New Jersey Cobbler 1930; D, Institute Irish Cobbler 1931; E, New Jersey Cobbler 1931.

in which the checks were no longer very dormant and therefore gave considerable growth are not included. It is seen from the data that treatments with this chemical uniformly result in a successful breaking of dormancy. Data are given showing the number of sprouts at various times after treat-

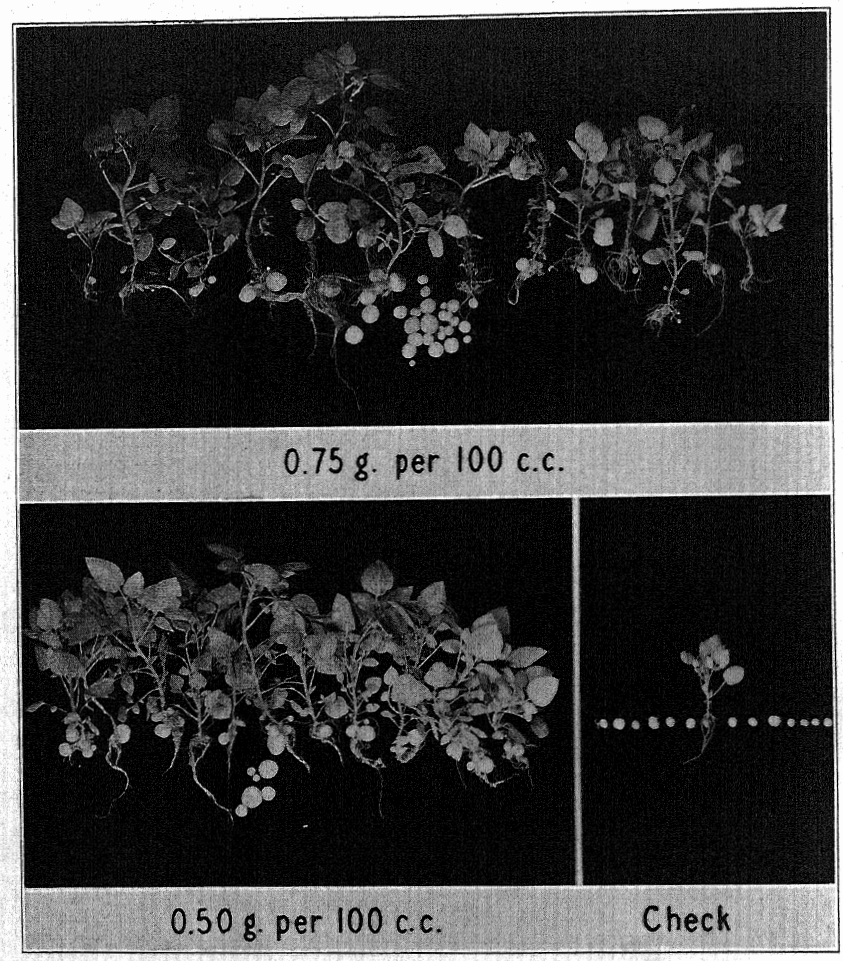


FIGURE 3. The effect of treatments of small immature Irish Cobbler potatoes with ammonium dithiocarbamate. The growth and new crop of tubers produced as a result of the treatments are shown, while in the case of the check only one seed piece has sprouted.

ment and in the case of the treatments of Table II the number of days for 50 per cent to come above ground is given as well as the weight of tops produced. It can be noted from Table II that the very dormant Institute Cobblers and New Jersey Cobblers of the 1931 crops responded well to

these treatments. Although 50 per cent of the checks did not appear above ground until from 71 to 122 days after treatment, the treated samples had a corresponding growth in from 19 to 29 days after treatment. A one-hour soak in a solution containing 0.75 g. per 100 cc. is about the most favorable concentration to use. Treatments with solutions containing 0.50 g. per 100 cc. are also very effective. Concentrations higher than 0.75 g. per 100 cc. often cause injury.

Figure 2 shows photographs of the results of these treatments with five lots of potatoes. The growth produced as a result of these various treatments can be compared with that of the corresponding checks. In Figure 3 are illustrated treatments of very dormant immature Irish Cobbler potatoes harvested from flats in the greenhouse. The top photograph shows the growth of tops and new tubers produced as a result of a treatment with a solution containing one gram per 100 cc. A treatment with a solution containing 0.75 g. per 100 cc. is also shown, as well as the check. It is seen that only one of the checks has produced any growth during this time, while the treated lots have produced new tubers as large as the original seed pieces.

Changes Resulting from the Treatments and Time Relations in the Changes Produced

In Table IV are given the data on changes in pH, iodine titration, and catalase and peroxidase activity resulting from treatments of various lots of dormant and non-dormant tubers with ammonium dithiocarbamate. The number of days for 50 per cent of the pieces to show sprouts above ground and the weight of tops produced are also shown. In Table V are summarized analyses made at various intervals after treatment of dormant Irish Cobbler and Bliss Triumph varieties treated with a series of concentrations of the dithiocarbamate. The upper half of the table shows the results obtained with Bliss Triumph potatoes treated with concentrations of 0.75 g. and 0.37 g. per 100 cc., while the lower half gives the results from Irish Cobbler potatoes treated with 0.75 g. and 0.50 g. per 100 cc. Tables IV and V show that, in general, treatments of dormant tubers result in increases in the catalase and peroxidase activity of the juices. From Table V it follows that these changes do not occur until about 48 hours after treatment. It is also seen that the extent of the changes produced is related to the strength of the treatment, the stronger treatment producing larger increases.

It can be seen from Table IV, where data are given on treatments made on the same lots of tubers at various stages of their dormancy, that when the potatoes are dormant, treatments increase catalase and peroxidase, but when no longer dormant, these increases no longer occur, and the untreated lots then have a higher catalase and peroxidase value than

TABLE IV

EFFECT OF TREATMENTS OF POTATO TUBERS WITH AMMONIUM DITHIOCARBAMATE

Source of potatoes used	Date treated	Sampled days after treatment	Days until 50% were above ground		Tops produced			Expressed juice								Dialyzed juice			
					Days after treatment	Fresh weight, g.		pH		Titration N/100 I		Catalase		Peroxidase		Catalase		Peroxidase	
			Tr.	Ck.		Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.
Institute Cobbler	Sept. 4*	5	24	>55	55	262	77	6.37	6.14	0.8	0.7	20.0	10.3	64.8	24.4	18.0	7.7	51.2	21.2
	Dec. 24	6	16	16	54	377	305	6.37	6.20	0.8	0.4	19.7	17.3	52.1	40.3	3.8	1.7	17.8	<10.0
Long Island Cobbler	Oct. 14	7	<16	22	—	—	—	6.07	5.83	0.6	0.4	12.7	6.2	37.6	22.4	12.2	7.0	43.9	20.4
	Dec. 12	6	14	13	45	350	337	6.00	5.90	0.7	0.4	13.3	17.7	44.4	44.7	10.2	10.2	33.2	24.6
	Jan. 8	3	12	15	56	305	370	5.98	5.83	0.8	0.4	11.8	13.1	49.4	33.8	18.6	9.8	56.2	19.2
New Jersey Cobbler	Nov. 4	7	29	78	72	410	0	6.29	6.20	0.7	0.4	13.8	19.2	44.4	44.4	11.0	11.6	46.4	48.4
	Nov. 16	8	<23	71	56	240	0	6.46	6.51	1.4	0.5	25.1	25.5	69.8	72.0	17.2	15.8	45.7	43.2
	Dec. 5	6	<24	72	52	245	1	6.51	6.42	0.7	0.6	13.6	19.4	62.6	63.6	13.6	14.7	37.0	39.0
	Jan. 9	3	<25	32	55	490	304	6.20	6.14	0.4	0.5	13.8	19.0	66.8	53.2	16.5	12.2	52.4	35.0
Institute Bliss Triumph	Sept. 4*	5	21	27	55	148	78	6.25	6.02	0.7	0.6	17.6	9.0	60.4	18.9	18.0	7.0	51.2	15.9
	Dec. 14	9	18	15	43	124	197	6.14	6.03	1.2	0.5	19.0	27.7	51.1	49.7	21.6	11.4	40.7	25.9
	Dec. 24	7	18	18	43	153	118	6.12	6.03	0.9	0.4	14.5	18.1	44.0	39.6	13.5	17.4	24.9	12.5
Bliss Triumph**	Feb. 17	6	18	36	40	447	120	6.07	5.93	1.1	0.4	19.3	11.2	44.4	19.7	15.7	6.2	34.4	13.0

* All lots were treated with a solution containing 0.75 g. per 100 cc. except the two thus designated which were treated with a solution containing 1.0 g. per 100 cc.

** Bought in the Yonkers market.

TABLE V

TIME RELATION IN EFFECT OF TREATMENTS WITH AMMONIUM DITHIOCARBAMATE. UPPER HALF OF TABLE, POTATOES OF THE BLISS TRIUMPH VARIETY; LOWER HALF, IRISH COBBLER VARIETY

Hours after beginning of treatment	Expressed juice												Dialyzed juice					
	pH			Titration with N/100 I			Catalase			Peroxidase**			Catalase			Peroxidase**		
	0.75*	0.37*	Check	0.75	0.37	Check	0.75	0.37	Check	0.75	0.37	Check	0.75	0.37	Check	0.75	0.37	Check
17	6.12	6.10	5.97	1.7	1.2	0.6	7.3	10.6	10.7	1.25	1.21	1.00	8.6	10.6	7.6	1.25	1.43	1.00
43	6.10	6.00	5.92	1.9	1.4	1.0	28.8	25.6	13.8	1.84	1.53	1.00	30.5	26.1	13.7	2.00	1.59	1.00
67	6.12	6.02	5.97	1.6	1.0	0.7	21.2	18.4	16.3	1.58	1.31	1.00	24.3	21.2	8.6	1.83	1.56	1.00
115	6.12	6.05	5.97	1.1	1.0	0.7	18.1	19.5	19.8	1.21	1.16	1.00	22.1	13.4	7.2	2.65	1.49	1.00
24	6.09	6.00†	5.90	0.9	0.6	0.6	7.5	8.8	7.6	21.7	20.3	15.5	8.3	7.3	6.1	24.0	19.4	17.8
43	6.03	5.98	5.87	0.5	0.5	0.4	14.0	13.2	9.1	28.2	23.6	18.8	14.4	8.9	5.6	30.7	22.8	15.5
139	6.00	5.93	5.80	0.8	0.8	0.4	13.2	11.3	9.0	41.2	35.9	23.9	—	10.8	8.2	—	16.0	18.1
189	6.17	6.07	6.00	0.9	0.8	0.6	20.9	16.3	12.5	51.8	41.5	25.9	23.5	16.1	10.0	52.4	38.8	28.2

* These figures represent the strength of the treatment expressed in grams per 100 cc. solution.

** Peroxidase values in the upper half of the table are relative only, calculated on the basis of the check as 1.00.

† The weaker treatments of the lower part of the table, shown in the middle columns, were made with a concentration of 0.50 g. per 100 cc.

before. This is illustrated, for example, in the case of the first lot of Table IV, Institute Irish Cobblers. In the first treatment made on September 4th when it took 24 days for 50 per cent of the treated lot to appear above ground and more than 55 days for a corresponding growth of the checks, the catalase and peroxidase values of the check were 10.3 and 24.4 respectively, while the treated sample gave values of 20.0 and 64.8 respectively. In the case of the treatment made on December 24th when the treated and check samples grew equally well, the catalase and peroxidase values of the check were then equal to 17.3 and 40.3 respectively, and the values for the treated sample, although about as high as in the case of the earlier treatment, no longer represented such large increases. An increase in peroxidase activity of potatoes as they become non-dormant has previously been reported by Appleman (1).

An examination of Table IV shows that the above holds generally for the various lots of tubers used with the interesting exception of the New Jersey Cobblers. Here the checks were higher in catalase and peroxidase than the checks of any of the other lots of tubers used and the treatments, even when the potatoes were very dormant, did not further increase the activity of the enzymes, although these treatments were successful in breaking the dormancy. These results seem to indicate that catalase and peroxidase activity are not primary factors in dormancy and that dormancy can exist even though these enzymes are relatively highly active. On the other hand, at least with regard to the data of the tubers used in these tests, in most cases a breaking of dormancy does result in increases in the activity of these enzymes.

Tables IV and V show also that the treated lots have a slightly higher pH value than the corresponding checks. Although the differences are small they are so consistent that they undoubtedly are significant. The values for the iodine titrations are also somewhat higher in the treated lots. This is due, to some degree, to the ammonium dithiocarbamate absorbed by the tubers, which reacts with iodine under the conditions under which the titrations were carried out.

The data of Table V show that in the case of the tubers of these tests, which were only partially dormant (50 per cent of the Irish Cobbler checks appeared above ground 31 days after planting), increases in catalase and peroxidase activity from day to day during the first week after treatment occurred to some extent also in the checks, but the increases in the treated lots were more rapid as was also the subsequent germination. For example, it is seen in the lower half of Table V, that during the period from 24 hours after treatment until 189 hours after the treatment the checks increased in catalase from 7.6 to 12.5 and the strongest treatment increased from 7.5 to 20.9. In the case of peroxidase the checks increased from 15.5 to 25.9, while the juice from the strongest treatment increased from 21.7 to

51.8. The changes in the treatment with a solution containing 0.50 g. per 100 cc. were intermediate between the check and the treatment with a solution containing 0.75 g. per 100 cc.

Analyses for reducing sugars and sucrose were made in some cases and these showed consistent increases in the sucrose content. The two treated samples of the lower half of Table V had a sucrose content of 24.1 and 17.9, and 33.0 and 21.4 mg. per 5 cc. juice 139 and 189 hours after treatment respectively, while the sucrose content of the check juice was 8.9 and 12.1 mg. per 5 cc. juice at these periods. No differences between the treated and check samples in reducing sugar content were evident. Analyses of other lots treated with ammonium dithiocarbamate are given in Table VI where

TABLE VI

EFFECT OF TREATMENTS WITH AMMONIUM DITHIOCARBAMATE ON THE REDUCING SUGAR AND SUCROSE CONTENT OF THE EXPRESSED JUICES

Potatoes used	Date treated	Sampled days after treatment	Milligrams in 5 cc. juice			
			Reducing sugars		Sucrose	
			Treated	Check	Treated	Check
Long Island Irish Cobbler	Oct. 14	7	8.3	6.6	31.5	10.6
	Dec. 12	6	11.3	0.2	33.8	14.8
	Jan. 8	3	10.2	0.5	45.8	9.3
New Jersey Irish Cobbler	Dec. 5	6	41.4	26.0	18.0	10.4
	Jan. 9	3	52.2	41.8	19.6	5.9
Bliss Triumph*	Feb. 17	6	12.4	2.3	45.2	21.4

* Purchased in the Yonkers market.

it can be seen that in the case of all of the six treatments shown a significant increase in sucrose occurred, the treated samples having a sucrose content from almost two to almost five times that of the corresponding check. The table shows that treatments of the New Jersey Cobblers which did not give some of the enzyme increases given by the other lots of potatoes used did produce large increases in the sucrose content of the expressed juices.

The Fate of the Ammonium Dithiocarbamate Absorbed by the Treated Tubers

Dormant potatoes treated with freshly recrystallized preparations of the dithiocarbamate or with material that had been kept for several months and had undergone considerable decomposition, or with the freshly prepared unrecrystallized salt, seem to respond equally well. If there is any difference between the efficacy of the pure substance and the partially deteriorated material, it is so small that extensive experiments would be necessary to show such a difference.

Since thiocyanates have been found very effective in breaking dormancy (7) and since thiocyanates are formed when ammonium dithiocarbamate breaks down, the question arises as to what extent the action of the dithiocarbamate may be due to the thiocyanate resulting from its decomposition. In this connection it should be remembered that the effective concentration of ammonium dithiocarbamate (about 0.75 g. per 100 cc.) is below that of the thiocyanates (about 1 g. per 100 cc.). This would indicate that the efficacy of dithiocarbamate in breaking dormancy is not due merely to the thiocyanate.

Freshly prepared solutions of ammonium dithiocarbamate are stable enough to undergo practically no decomposition in the hour during which the pieces are soaked. If such treated pieces are sampled immediately and the expressed juices titrated with iodine it is seen that considerable quantities of ammonium dithiocarbamate are taken up in an unaltered condition. Juice from the outer half of pieces just taken off treatment contains from 30 to 40 mg. per 100 cc. At this stage, however, the juice already contains some thiocyanate. Cut pieces treated with a concentration of 0.75 g. per 100 cc. of the dithiocarbamate were sampled at intervals after treatment and compared with corresponding pieces which had been treated with potassium thiocyanate in concentrations of 0.5 g. and 1.0 g. per 100 cc. The thiocyanate content of the juices was determined with the aid of the red color produced by ferric chloride, the proteins having been previously removed by precipitation with trichloracetic acid. The colors were compared by inspection only, since the potato juices were not clear enough for comparison in the colorimeter. In two such series the highest concentration of thiocyanate reached in the juice from the outer half of the pieces treated with dithiocarbamate was equivalent to 10 mg. of potassium thiocyanate per 100 cc. In the case of the thiocyanate treatments the juice from the outer half of the pieces contained 20 mg. potassium thiocyanate per 100 cc. in the pieces treated with 1.0 g. potassium thiocyanate per 100 cc., and 12 mg. per 100 cc. juice in the pieces treated with 0.5 g. potassium thiocyanate per 100 cc. The concentration of thiocyanate reached in the potatoes treated with 0.75 g. ammonium dithiocarbamate is therefore less than that attained when potatoes are treated with 0.5 g. potassium thiocyanate per 100 cc. This concentration of potassium thiocyanate is below the optimum for breaking dormancy (about 1 g. per 100 cc.).

These results indicate that the efficacy of the dithiocarbamate in breaking dormancy is due to other factors in addition to the formation of thiocyanate in the treated potato.

In connection with these tests it was found that in tubers treated with thiocyanate and dithiocarbamate the positive test for thiocyanate in the expressed juices persists for a long time after treatment and long after the pieces have germinated and show sprouts above ground.

In working with solutions prepared from freshly recrystallized ammonium dithiocarbamate it was found that such solutions remained free of thiocyanate for a considerable time while potato tubers treated with these solutions gave positive tests for thiocyanate in their tissues sooner than the solutions out of contact with the tubers. Further experiments showed that both boiled and filtered, and unboiled potato juice hastened the decomposition of ammonium dithiocarbamate. This property was not further investigated and it is not known what constituent of the juices is responsible for this effect.

Discussion of Results with Ammonium Dithiocarbamate

Experiments with many lots of dormant potato tubers for a period of two years showed that ammonium dithiocarbamate very effectively breaks their dormancy if cut pieces are soaked for one hour in a solution containing 0.75 g. per 100 cc. Treatments of dormant tubers result in increases in the catalase and peroxidase activity of the expressed juices both before and after dialysis through collodion, the catalase and peroxidase values forming a series in line with the series of concentrations used for the treatments, the strongest treatment giving the highest activity. The sucrose content of the expressed juices of the treated samples reaches a value several times that of the check samples. Slight increases in the pH value of the juices also occur. These changes take place in from 24 to 48 hours after the treatment.

Non-dormant tubers treated in the same manner do not show similar increases in catalase and peroxidase activity. The results show, however, that the activity of these enzymes in the check samples is much higher than that of the same lot of tubers when still dormant. On the other hand the treatments increase the sucrose content of the tubers even though they are completely non dormant and have a relatively high sucrose content before the treatment.

It was found that ammonium dithiocarbamate could be readily prepared in a pure state if gaseous ammonia is passed into a solution of carbon disulphide in ether and alcohol at low temperature. Recrystallization results in a pure white product free of ammonium thiocyanate and ammonium trithiocarbonate. This product is stable if kept dry at low temperatures. Ammonium dithiocarbamate decomposes into ammonium thiocyanate, hydrogen sulphide, and free sulphur. The decomposition is hastened by potato juice. The thiocyanate content of potato tubers treated with optimum concentrations of dithiocarbamate is below that of treatments with potassium thiocyanate with concentrations below the optimum. The efficacy of the dithiocarbamate in breaking dormancy is due to other factors in addition to the thiocyanate formed. In potatoes treated with dithiocarbamate and potassium thiocyanate, thiocyanate persists in

the tubers a long time after the treatment, even after the sprouts are above ground.

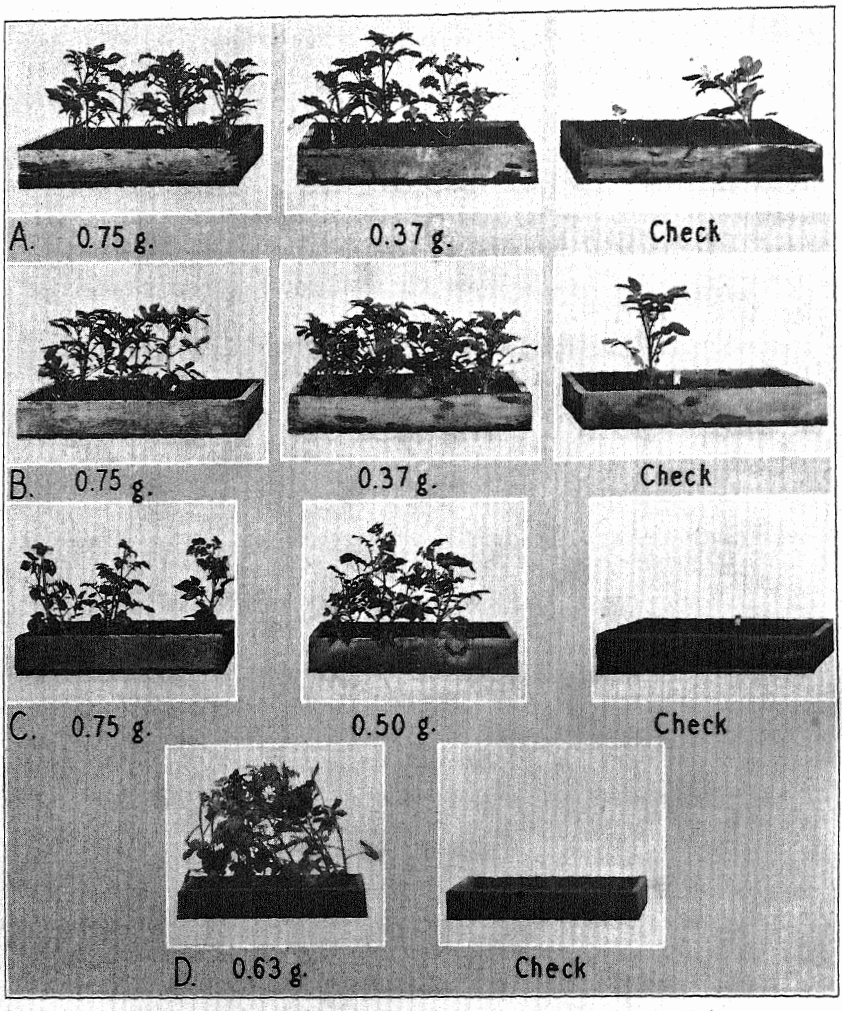


FIGURE 4. The effect of treatments with various concentrations (g. per 100 cc.) of thiosemicarbazide in breaking the dormancy of potato tubers. A, Institute Irish Cobbler 1930; B, Institute Early Ohio 1930; C, New Jersey Cobbler 1930; D, New Jersey Cobbler 1931.

EFFECT OF THIOSEMICARBAZIDE

Effect on Dormancy

Thiosemicarbazide ($NH_2CSNHNH_2$) is related to thiourea in that one of the amino groups of thiourea is replaced by a hydrazine ($-NHNH_2$)

group. The effect of this chemical on dormancy was tested in ten treatments of seven lots of dormant tubers. The growth resulting in four of these experiments is illustrated in Figure 4. In Table VII are summarized the data obtained with treatments of the very dormant lot of New Jersey Irish Cobblers of the 1931 crop. The results show that while this chemical has a considerable action in breaking dormancy it is not as effective as ammonium dithiocarbamate. In many of the treatments not all of the 12

TABLE VII

EFFECT OF THIOSEMICARBAZIDE IN BREAKING THE DORMANCY OF IRISH COBBLER POTATOES (NEW JERSEY SECOND CROP 1931)

Date treated	No. of days until 50% were above ground		Growth record				
			Days after treatment	No. of sprouts		Weight of tops, g.	
	Treated*	Check		Treated	Check	Treated	Check
Nov. 16	23	71	56	11	0	235	0
Dec. 5	25	72	52	11	1	203	1
Jan. 9	25	32	55	12	7	347	304

* Treatments were made with a concentration of 0.63 g. per 100 cc.

pieces responded. This is due in part to the fact that thiosemicarbazide is quite toxic and the concentrations necessary to bring about a breaking of dormancy are close to the concentrations causing injury and subsequent decay of the pieces. Since the concentration producing injury is not exactly the same for all lots of potatoes, it is impossible to choose a concentration which will be effective in all cases and at the same time not produce any injury. If the proper concentration is used, this chemical will break the dormancy of very dormant potatoes. This is shown, for example, in the series at the bottom of Figure 4. The checks did not have 50 per cent sprouts above ground until 71 days after planting while those treated with a solution containing 0.63 g. per 100 cc. showed 50 per cent above ground in 23 days. The photographs were taken 59 days after treatment. In general, the effective concentration is between 0.5 and 0.75 g. per 100 cc.

Changes Resulting from the Treatments

Tables VIII and IX show the changes resulting in the properties of the juices from tubers treated with thiosemicarbazide. A study of Table VIII shows that the treatments result in very large increases in catalase and peroxidase activity, the treated samples in many cases having an activity two, three, or even four times that of the corresponding checks. Many of the treatments of Table VIII belonged to series which also included an ammonium dithiocarbamate treatment so that in many cases it is possible to make a comparison between the treatments of Tables VIII and IV, the

TABLE VIII

EFFECT OF TREATMENTS OF POTATO TUBERS WITH THIOSEMICARBAZIDE

Potatoes used	Date treated	Sampled days after treatment	Days until 50% were above ground		Tops produced			Expressed juice								Dialyzed juice			
					Days after treatment	Fresh weight, g.		pH		Titration N/100 I		Catalase		Peroxidase		Catalase		Peroxidase	
			Tr.	Ck.		Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.
Institute Cobbler	Sept. 4	5	23	>55	55	202	77	6.27	6.14	0.7	0.7	32.3	10.3	106.8	24.4	25.5	7.7	81.2	21.2
	Dec. 24*	6	22	16	54	205	305	6.29	6.20	0.8	0.4	36.6	17.3	104.8	40.3	8.5	1.7	41.7	<10.0
Long Island Cobbler	Oct. 14	7	27	22	—	—	—	6.15	5.83	0.4	0.4	21.4	6.2	74.8	22.4	20.2	7.0	73.0	20.4
	Dec. 12	6	14	13	45	258	337	6.00	5.90	0.7	0.4	33.2	17.7	123.0	44.7	30.7	10.2	98.1	24.6
	Jan. 8*	3	13	15	56	365	370	5.97	5.83	0.8	0.4	15.0	13.1	66.4	33.8	23.1	9.8	68.0	19.9
Institute Bliss Triumph	Sept. 4	5	27	27	55	165	78	6.15	6.02	0.6	0.6	25.8	9.0	80.0	18.9	23.2	7.0	68.0	15.9
	Dec. 24*	7	26	18	43	48	118	6.14	6.03	0.6	0.4	14.7	18.8	45.0	39.6	—	7.4	—	12.5
New Jersey Cobbler	Nov. 16	8	<23	71	56	235	0	6.49	6.51	0.8	0.5	29.2	25.5	99.4	72.0	27.2	15.8	74.2	43.2
	Dec. 5	6	25	72	52	203	1	6.44	6.42	0.5	0.6	25.7	19.4	108.2	63.6	30.1	14.7	82.6	39.0
	Jan. 9*	3	25	32	55	347	304	6.14	6.14	0.5	0.5	16.2	19.0	72.4	53.2	22.0	12.2	63.2	35.0

* All lots were treated with a solution containing 0.63 g. per 100 cc. except those starred which were treated with a solution containing 0.50 g. per 100 cc.

same check appearing in both tables. Such a comparison shows that in general much larger enzyme increases took place in the case of thiosemicarbazide treatments. For example, in the treatment of the Long Island Cobbler potatoes made on October 14, the check had a catalase value of 6.2, while the ammonium dithiocarbamate and thiosemicarbazide treated lots had a catalase activity of 12.7 and 21.4 respectively. Although thiosemicarbazide has a much greater effect on these enzymes than ammonium dithiocarbamate it is not any more effective in breaking dormancy but is in fact somewhat less effective.

It will be noted from Table VIII that with thiosemicarbazide too the New Jersey Cobblers proved somewhat of an exception as to the enzyme changes taking place. Although thiosemicarbazide in the case of the other lots of tubers has been found to be very active in increasing enzyme activity, the treated lots of New Jersey Cobblers did not respond to the same degree. This is especially true of the catalase activity, which was only slightly increased by the treatments. Somewhat larger changes in peroxidase took place. The values for peroxidase and catalase of the treated lots, as pointed out before in the case of ammonium dithiocarbamate, are however quite high; the reason they do not represent larger increases over the corresponding checks is because the untreated checks show high enzyme activities.

TABLE IX

REDUCING SUGAR AND SUCROSE CONTENT OF JUICES FROM IRISH COBBLER POTATOES TREATED WITH THIOSEMICARBAZIDE

Source of tubers	Concn. of chemical, g. per 100 cc.	Milligrams per 5 cc. juice					
		Reducing sugars			Sucrose		
		Days after treatment			Days after treatment		
		3	4	7	3	4	7
Institute 1931	0.65	4.6	9.5	5.7	20.6	41.5	53.7
	0.31	6.0	3.4	11.0	28.8	22.5	50.9
	Check	5.1	1.1	0.5	20.4	21.9	19.0
New Jersey	0.63			28.3			19.1
	Check			26.0			10.4
New Jersey	0.50	60.9			11.0		
	Check	41.8			5.9		

The treated lots also show an increased sucrose content (Table IX), the expressed juice of the treated lots containing from two to three times the amount of sucrose of the corresponding checks. The data from the first part of Table IX indicate that this increase takes place in from three to four days after treatment. Small increases in pH values are also shown by the treated samples (Table VIII).

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Discussion of Results with Thiosemicarbazide

Thiosemicarbazide in concentrations of 0.5 to 0.75 g. per 100 cc. breaks the dormancy of potato tubers. The concentrations breaking dormancy are close to concentrations producing injury. Treatments result in unusually large increases in catalase and peroxidase activity of the expressed juices. Large increases in the sucrose content of the treated juices also result.

EFFECT OF ETHYL MERCAPTAN

Effect on Dormancy

Tests with potatoes. Ethyl mercaptan (C_2H_5SH), the sulphur analog of ethyl alcohol, was found to have considerable effect in breaking the dormancy of potatoes but the results obtained with it were not very uniform, the treatments in some cases being unsuccessful. Treatments were made by subjecting the tubers to the vapor of the chemical for 24 hours and the favorable concentration was found to be about 0.5 cc. per liter. It is probable that one reason why the results were not uniform is that vapor treatments, especially with very low concentrations of chemicals, are more sensitive to other factors, as for example temperature during treatment, than soak treatments. It has been found by Denny (8) that the chlorhydrin dip treatments, which include a 24-hour storage period, are more sensitive to temperature effects than soak treatments with sodium thiocyanate and thiourea. The results obtained thus far, however, permit it to be said that ethyl mercaptan has a considerable action in breaking the dormancy of potatoes. A successful treatment is illustrated in Figure 11 A where it can be seen that concentrations of 0.4 cc. per liter and 0.2 cc. per liter broke dormancy in the case of the Institute Irish Cobblers of the 1931 crop.

When the favorable concentration for the treatments with ethyl mercaptan is compared with that of its oxygen analog, ethyl alcohol, which has been reported by Guthrie (16), it is seen that the concentration of ethyl alcohol necessary is about six times that of the mercaptan.

Tests with lilacs and grapes. To a limited extent the effect of ethyl mercaptan on dormant grape (*Vitis labruscana* Bailey var. Concord) and lilac (*Syringa vulgaris* L. var. Charles X) plants was also studied. Because of the extremely penetrating odor of the mercaptan and the difficulty of confining it in a closed space, no attempt was made to treat whole plants but instead individual twigs and buds of the plants were treated. Separate twigs on a given plant act as units and any one of them can be aroused from its rest period while adjacent twigs remain inactive.

The treatments were made by placing on a piece of cotton within an Erlenmeyer flask (125 cc. and 500 cc. sizes were used) the required amount of the chemical and then inserting the flask over the twig and closing the

opening between the twig base and the mouth of the flask with modelling clay. In Figure 5 A are shown detached lilac twigs which had been treated with concentrations of the mercaptan ranging from 0.1 cc. to 0.7 cc. per liter. From the results in these tests as well as with others not here shown, it was found that the most favorable concentration is around 0.5 cc. per liter. Dormant grapes also responded to treatments with ethyl mercaptan. Favorable results were obtained with concentrations varying from 0.2 cc. to 1.0 cc. per liter.

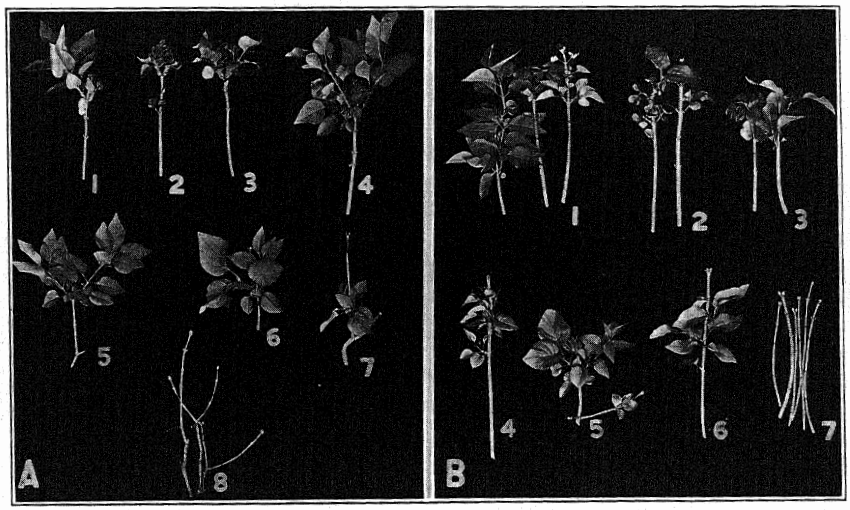


FIGURE 5. Lilac twigs which had been treated with chemicals. A, 1, 2, 3, 4, 5, 6, 7, and 8 were treated with ethyl mercaptan in the following concentrations expressed as cc. per liter: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and check respectively; B, 1, 2, 3, 4, 5, 6, and 7 treated with methyl disulphide: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 cc. per liter, and check respectively.

Tests with gladiolus. In several experiments with gladiolus (*Gladiolus* sp. var. Alice Tiplady), treatments of 0.4 cc. and 0.2 cc. per liter for 24 hours produced favorable responses. For example in one test in which 50 per cent of the checks did not grow until 80 days after planting, lots treated with 0.4 cc. and 0.2 cc. showed 50 per cent sprouts above ground 43 and 40 days after treatment respectively. The treatments, however, produced a certain amount of injury and some of the tops produced were not very vigorous. In experiments in which treatments with ethylene chlorhydrin (9) were compared directly with ethyl mercaptan treatments, the chlorhydrin treatments gave the better results. The experiments show, however, that ethyl mercaptan treatments have a considerable action in breaking the dormancy of gladiolus.

Changes Resulting from the Treatments and Time Relations in the Changes Produced

Treatments of potato tubers with ethyl mercaptan resulted in large increases in catalase and peroxidase activity of the expressed juices. In two different series of treatments the activity of these enzymes was determined at daily intervals after treatment up to seven days. The data are given in Table X. The upper half of the table shows analyses of treatments of Bliss Triumph potatoes belonging to the same series as is illustrated in Figure 11 A. The lower half of the table shows the data obtained in treatments of Irish Cobbler potatoes. These were not as dormant as the Bliss Triumph potatoes of Figure 11 A, but they were dormant enough to show a considerable difference in growth between treated and check samples. Treatments at two concentrations are shown for each variety, 0.4 cc. and 0.2 cc. per liter in the case of the Bliss Triumph variety and 0.6 cc. and 0.3 cc. in the case of the Irish Cobbler variety.

It is seen from the upper half of Table X that catalase values are depressed immediately after treatment, the check lot showing a value of 12.5 cc. oxygen while the lots treated with 0.4 cc. and 0.2 cc. gave values of 5.2 and 6.7 respectively. The tubers at this stage contained ethyl mercaptan throughout the tissue as shown by the nitroprusside test. However, the depressing effect was not removed by dialysis of the juice in collodion bags for four hours since in the dialyzed samples the treated lots are still considerably lower than the check. The table shows that by the fifth day after treatment the treated samples were higher than the check, the stronger treatment giving a juice having a value a little over twice that of the check. Similar differences persisted in the dialyzed samples. Relative values only were obtained for the peroxidase activities but peroxidase also was lower in the treated samples immediately after treatment, but increased to a value higher than the check five days after treatment.

The results obtained in the treatments of the Irish Cobbler potatoes shown in the lower half of Table X are similar. The checks of this series were not very dormant and showed some increase in catalase and peroxidase activity from day to day during the first week after planting, as was also the case with the partially dormant checks in an ammonium dithiocarbamate treatment as shown in the lower half of Table V. The increases in the treated samples from day to day were, however, considerably greater than those of the check. Thus the catalase activity of the check increased from 4.5 to 16.5 from the time immediately after treatment until seven days after treatment, while the sample treated with 0.6 cc. per liter increased from 0.9 to 25.8 cc. The peroxidase values show an increase in the case of the check from 22.5 mg. immediately after treatment to 47.7 mg. seven days later, while the stronger treatment increased from 16.2 to 89.3. The changes taking place in the case of the weaker treatment

TABLE X

TIME RELATIONS IN EFFECT OF ETHYL MERCAPTAN TREATMENTS OF POTATO TUBERS. UPPER HALF OF THE TABLE, BLISS TRIUMPH VARIETY; LOWER HALF, IRISH COBBLER

Hours after beginning of treatment	Expressed juice												Dialyzed juice					
	pH			Titration with N/100 I			Catalase			Peroxidase**			Catalase			Peroxidase**		
	Tr.*	Tr.*	Ck.	Tr.	Tr.	Ck.	Tr.	Tr.	Ck.	Tr.	Tr.	Ck.	Tr.	Tr.	Ck.	Tr.	Tr.	Ck.
23	6.25	6.20	6.20	2.8	2.4	1.5	5.2	6.7	12.5	0.57	0.60	1.00	9.4	9.4	16.2	0.67	0.67	1.00
44	6.34	6.37	6.15	1.7	1.4	1.5	14.2	16.6	11.5	0.86	1.04	1.00	12.5	16.9	11.6	1.20	1.59	1.00
63	6.34	6.29	6.09	1.7	1.2	1.2	17.6	17.5	12.4	1.20	1.07	1.00	17.2	14.8	11.7	1.63	1.40	1.00
111	6.34	6.27	6.10	1.5	1.3	0.8	28.3	18.6	12.2	1.32	1.06	1.00	25.7	19.0	13.8	1.57	1.32	1.00
135	6.27	6.24	6.02	1.6	1.6	0.7	22.4	23.0	11.8	1.27	1.25	1.00	18.1	17.6	13.2	1.27	1.22	1.00
159	6.44	6.31	6.10	2.0	2.0	1.0	27.6	21.9	11.6	2.00	1.55	1.00	23.2	13.4	10.3	1.85	1.00	1.00
24	6.27	6.17	6.24	2.4	1.7	1.3	0.9	1.7	4.5	16.2	17.1	22.5	1.6	2.5	4.2	13.7	14.4	19.4
46	6.25	6.17	6.00	1.1	1.1	0.7	1.5	2.6	3.5	20.3	21.8	23.2	0.7	1.9	2.5	13.8	16.3	18.1
94	6.29	6.10	6.03	1.2	0.7	0.7	12.2	9.2	8.0	48.4	41.4	33.4	10.7	8.6	5.7	43.8	33.7	27.4
120	6.34	6.22	6.02	1.3	0.8	0.5	29.7	22.6	15.6	64.1	57.0	35.3	26.4	19.8	12.6	62.5	51.5	32.6
144	6.32	6.14	6.05	1.3	1.0	0.7	29.0	24.4	19.3	75.6	64.1	46.0	24.5	17.3	13.1	69.5	57.5	36.2
168	6.34	6.44	6.10	1.0	0.8	0.6	25.8	19.0	16.5	89.3	66.3	47.7	22.8	13.6	10.3	73.4	49.9	37.0

* The treated samples in the upper half of the table were treated with concentrations of 0.4 cc. and 0.2 cc. per liter respectively; in the lower half with 0.6 cc. and 0.3 cc. respectively.

** Peroxidase values in the upper portion of the table are relative only, calculated on the basis of the check as 1.00.

both as to catalase and peroxidase activity were intermediate between those of the check and the stronger treatment.

Changes in pH values and iodine-reducing power also occurred. The pH change amounted to about 0.3. The increased iodine titration values, given by the treated samples immediately after treatment, are no doubt due in part to the absorbed ethyl mercaptan which reacts with iodine. However, after the second day the tubers no longer contained any mercaptan as shown by the nitroprusside test and the increased iodine value must be due to other substances, perhaps similar to the reducing substances formed as a result of chlorhydrin treatments (12).

Enzyme activities were also determined in still another series of treatments, the potatoes being completely non-dormant in this case. In this series as in those of Table X the catalase and peroxidase values of the treated samples were depressed as compared with the check, immediately after treatment, but four days after treatment were much higher than those of the check. The effect of ethyl mercaptan treatments on the reducing sugar and sucrose content was determined only in one case in which analyses were made five days after treatment. Five cc. of the juices from the tubers treated with 0.6 cc. per liter and 0.3 cc. per liter had a sucrose content of 23.2 and 20.5 mg. respectively as compared with 17.0 mg. present in the check.

The respiratory rate of tubers treated with various concentrations of ethyl mercaptan was determined by the method previously described. The potatoes were treated in the desiccators used for the respiration determinations and when the 24-hour treatment period was over a stream of carbon dioxide-free air was passed through the desiccators and the carbon dioxide in the air leaving the containers determined. In order to avoid the error resulting from a reaction between the mercaptan and the barium hydroxide used to absorb the carbon dioxide, the stream of air coming out of the desiccators containing the treated samples was first passed through an iodine solution which oxidized the mercaptan to the disulphide. Any of the vapor of the disulphide passing through the barium hydroxide tubes would not affect the determinations. The barium hydroxide tubes were changed about every 24 hours. The carbon dioxide absorbed during this time was then determined and plotted, the milligrams of carbon dioxide per 100 g. of tissue per hour being plotted against the number of hours since the beginning of the treatment, the hour plotted being the time halfway between the beginning and the end of the period during which the carbon dioxide was being absorbed. When the tubes were taken off they were immediately replaced by fresh ones and thus a continuous record of the carbon dioxide output was obtained. Curves plotted in this manner obtained from treatments with 0.35 cc. per liter and 0.15 cc. per liter together with an untreated sample are shown in Figure 6.

A prompt and pronounced rise in the respiratory rate takes place when tubers are treated with this chemical. The first determination recorded in Figure 6 is one covering the carbon dioxide given off during the 24 hours of the treatment and 16 hours thereafter and plotted at 20 hours. During this time the treated samples gave off three times the carbon dioxide given off by the check. The respiratory rate subsequently increased until about 53 hours after the beginning of the treatment a value four to five times that of the check was reached. Subsequently the respiratory rate gradually

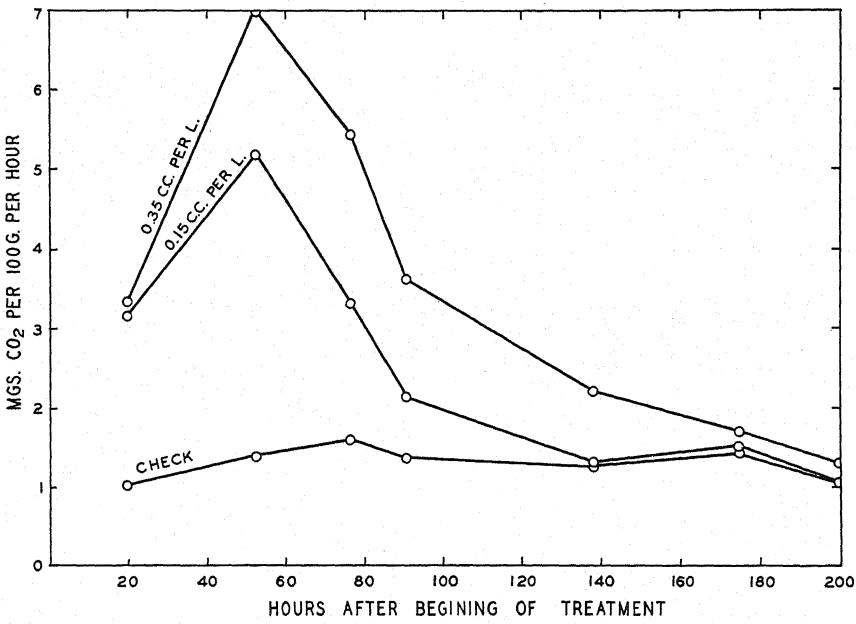


FIGURE 6. The effect of ethyl mercaptan treatments on the respiratory rate of potato tubers.

fell until 175 hours after treatment when the treated samples were respiring at only a very slightly higher rate than the checks.

The data represented by the curves in Figure 6 are also given in Table XI together with those of another test, the curves of which are not reproduced. The results of the additional test confirm those of Figure 6. It can be seen from the table that the curve representing the respiratory rate of the treatment with 0.25 cc. per liter has the same shape as the curves from the treated lots of the test of Figure 6. The absolute amounts of carbon dioxide given off per 100 grams tissue in seven days are also given in the table. It is seen that for the seven-day period, a treatment with 0.35 cc. per liter has resulted in a production of carbon dioxide three times that of

the check, a treatment with 0.25 cc. per liter about two and one-half times that of the corresponding check and a treatment with 0.15 cc. per liter about twice that of the check.

TABLE XI

EFFECT OF TREATMENTS WITH VARIOUS CONCENTRATIONS OF ETHYL MERCAPTAN ON THE RESPIRATORY RATE OF POTATO TUBERS

Exp. 1				Exp. 2		
Hours after beginning of treatment	Mg. CO_2 per 100 g. per hour			Hours after beginning of treatment	Mg. CO_2 per 100 g. per hour	
	0.35 cc. per l.	0.15 cc. per l.	Check		0.25 cc. per l.	Check
0 – 40.5	3.31	3.18	1.00	0– 42	5.28	1.64
40.5– 65.5	6.94	5.16	1.34	42– 67	6.95	1.89
65.5– 89.5	5.41	3.29	1.54	67– 91	5.66	2.11
89.5–113.5	3.61	2.07	1.28	91–115	3.51	1.48
113.5–161.0	2.20	1.28	1.23	115–141	2.54	1.46
161.0–189.0	1.68	1.51	1.43	141–165	2.18	1.40
189.0–213.0	1.30	1.04	1.01	165–189	1.77	1.34
Grams CO_2 per 100 g. in 7 days	0.64	0.46	0.21		0.70	0.29

Discussion of Results with Ethyl Mercaptan

Ethyl mercaptan breaks dormancy in potato tubers but the results obtained are not as uniform as those resulting from treatments with ammonium dithiocarbamate and thiosemicarbazide. Ethyl mercaptan also breaks dormancy in grapes and lilacs and has some effect on dormant gladiolus.

Treatments result first in a depression of the catalase and peroxidase activity of the juices from treated tubers. This effect persists after dialysis through a collodion membrane. After about four to five days the catalase and peroxidase activity of the treated samples reaches a value two to three times that of the corresponding checks. The respiratory rate is increased to from three to four times that of the check by treatments with concentrations which break dormancy. The respiratory rate of the treated tubers then gradually drops until it reaches that of the check about a week or ten days after the treatment.

EFFECT OF HYDROGEN SULPHIDE

Effect on Dormancy

Another sulphur compound tested for its effect on dormant potatoes was hydrogen sulphide. Treatments were made by weighing out definite quantities of sodium sulphide ($Na_2S.9H_2O$) and liberating the hydrogen

sulphide with an excess of sulphuric acid. Since sodium sulphide is very hygroscopic it is important that the salt weighed out has been kept in a sealed bottle; otherwise the concentration of hydrogen sulphide produced can not be controlled with sufficient accuracy. The effective range for these treatments was found to be narrow. Tubers in the higher concentrations were blackened throughout their whole volume at end of the 24-hour treatment period. It was found that concentrations below which this blackening occurred were not effective in breaking dormancy.

In Figure 7 is illustrated the resultant growth from four lots of dormant potatoes treated with various concentrations of hydrogen sulphide. The concentrations are expressed in terms of the amount of sodium sulphide weighed out per liter of air space in the container used for the treatments. An examination of the photographs shows that while hydrogen sulphide does have an effect in breaking dormancy its efficacy is not as great as that of some of the other sulphur compounds previously discussed. The effective concentration is in the neighborhood of 0.4 g. sodium sulphide per liter.

Changes Produced by the Treatments

In Table XII are given values obtained for catalase and peroxidase activity, pH, iodine titration, reducing sugar and sucrose content of tubers treated with concentrations ranging from 0.5 g. per liter to 0.3 g.

TABLE XII

EFFECT OF TREATMENTS OF WHOLE TUBERS WITH HYDROGEN SULPHIDE GAS

Grams of $Na_2S.9H_2O$ per liter of air space	Expressed juice						Dialyzed juice	
	Reducing sugars	Sucrose	pH	Titration N/100 I	Catalase	Peroxidase	Catalase	Peroxidase
0.5	62.8	73.5	6.36	1.7	28.6	46.0	20.6	36.2
0.4	53.5	59.4	6.24	1.7	20.7	36.8	14.2	25.6
0.3	57.7	53.5	6.12	1.4	15.2	29.8	7.8	15.8
Check	53.6	29.3	5.85	0.4	12.4	24.2	4.6	12.4
0.5*	67.5	52.1	6.24	1.0	22.2	60.0	12.6	25.6
0.4	70.4	41.6	6.14	0.7	19.7	39.8	9.0	22.2
0.3	70.0	44.1	6.10	0.5	17.8	39.8	8.0	18.9
Check	62.9	37.4	6.03	0.4	18.5	40.6	7.8	16.6

* Treatments shown in lower half of table were made on same lot of tubers shown in the upper half but 2½ months later.

per liter. The potatoes used were from a lot of the Bliss Triumph variety purchased in the open market in February 1932. The enzyme and other changes produced by the treatments were very large, the catalase and peroxidase activity of the highest treatment being about double that of the check. There occurred also a pH change of about 0.5 and a greatly

increased iodine titration. The sucrose values of the treated samples are from two to almost two and one-half times those of the check.

In the lower part of Table XII are given results obtained from this

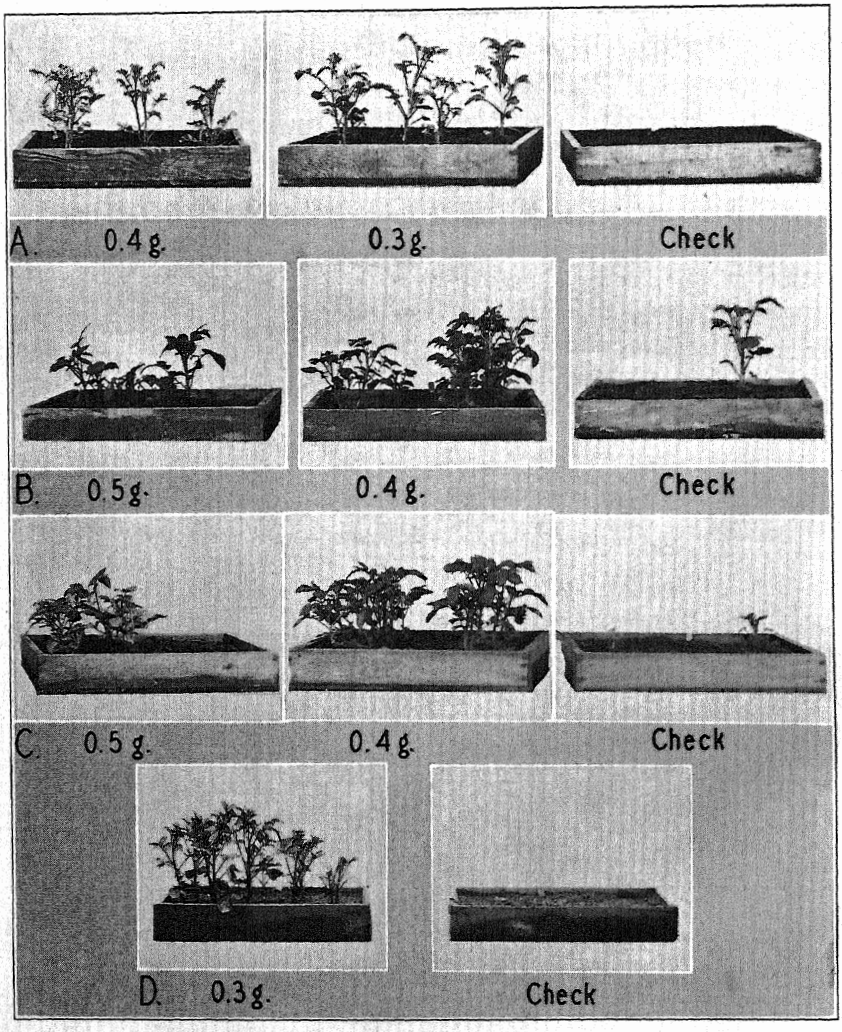


FIGURE 7. The effect of hydrogen sulphide in breaking the dormancy of potato tubers. Concentrations are expressed as grams of $Na_2S.9H_2O$ per liter of air space. A, South Carolina Cobbler 1930; B, Institute Cobbler 1930; C, Institute Early Ohio 1930; D, South Carolina Cobbler 1931.

same lot of tubers treated with the same concentrations two and one-half months later. It is seen that similar changes occurred except that the

changes were not as large. An examination of the tables shows, however, that the catalase and peroxidase values of the checks have increased in the interval between the two treatments. Such an increase in these enzymes as the potatoes lose their dormancy was also pointed out in connection with the data of Tables IV and X. Although the peroxidase value of the non-dormant check (40.6) is almost as high as the value resulting from the strongest treatment of the dormant tubers (46.0), treatment with the hydrogen sulphide liberated from 0.5 g. sodium sulphide per liter induced a further increase of about 50 per cent. The tubers also increased in pH value and sugar content in the interval between the two treatments.

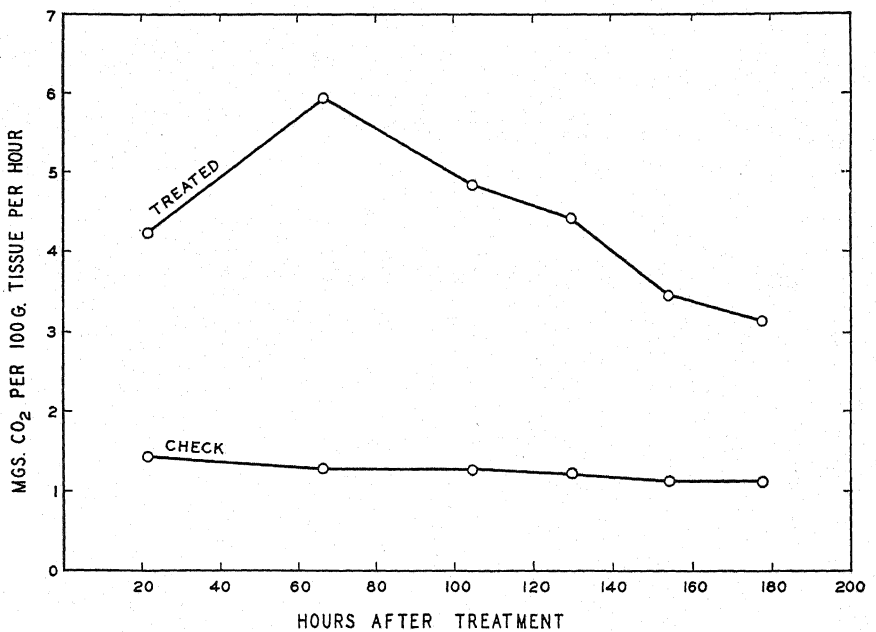


FIGURE 8. The effect of hydrogen sulphide on the respiratory rate of potato tubers.

Table XII shows that although this lot of tubers was relatively high in sucrose content, treatments with hydrogen sulphide produced increases. Thus in the treatment shown in the lower half of the table, although the check juice contained 37 mg. of sucrose in 5 cc., the strongest treatment resulted in an increase to 52 mg. in 5 cc.

Effect on Respiration

The respiratory rate of tubers treated with hydrogen sulphide was determined by the method previously described, the carbon dioxide given off during the time of treatment also being included. The stream of air coming

from the treated container was first passed through cadmium sulphate in order to absorb the hydrogen sulphide. By the use of this reagent it is possible to absorb hydrogen sulphide in acid solution and thus prevent any carbon dioxide from being lost. In Figure 8 are shown the curves representing the respiratory rate of a lot of tubers treated with a concentration of hydrogen sulphide corresponding to 0.3 g. sodium sulphide, and that of a check lot. This treatment resulted in a marked rise in the respiratory rate which was evident even when the first reading was made. The first values plotted at 22 hours and calculated from the amount of carbon dioxide given off during the 24 hours of the treatment and for 10 hours subsequently, show the treated lot to be respiring about three times as rapidly as the check. The respiratory rate of the treated lot increased further until it reached a value a little over four times that of the check about 65 hours after the beginning of the treatment. The rate gradually fell from that time on, but 178 hours after the beginning of the treatment it was still almost three times that of the check.

The total amount of carbon dioxide given off by the treated sample per 100 g. of tissue in seven days amounted to 0.79 g. while the check gave off 0.21 g. during this time. The average increase of the treated sample for the seven-day period thus amounts to almost 400 per cent.

Discussion of Results with Hydrogen Sulphide

Treatments with hydrogen sulphide are somewhat effective in breaking the dormancy of potato tubers. Concentrations corresponding to amounts of $Na_2S.9H_2O$ ranging from 0.3 to 0.5 g. per liter break dormancy successfully. Treatments of both dormant and non-dormant tubers result in increases in pH, iodine titration, catalase and peroxidase activity, and sucrose content of the expressed juices. Hydrogen sulphide also has a marked action in increasing the respiration of potato tubers.

EFFECT OF THIOACETAMIDE

The case of thioacetamide (NH_2CSCH_3) is very interesting in that this substance was found to break the dormancy of very dormant potatoes and to retard the sprouting of non-dormant tubers. A study of the data on sprout emergence after treatment showed that growth did not result until a considerable time after treatment both in dormant and non-dormant potatoes; the result being that when the tubers are not dormant the checks grow considerably sooner than the treated lots. An examination of the juices of treated tubers showed that a considerable quantity of thioacetamide was absorbed by the seed pieces during treatment, and analyses of the tubers at intervals after treatment showed that the concentration of thioacetamide decreased very slowly. Sprout emergence did not occur until the thioacetamide content of the pieces was practically nil.

Effect on Dormancy

The experiments show that in the case of very dormant potatoes thioacetamide has a considerable effect in breaking dormancy. The tests were not as uniformly successful as with ammonium dithiocarbamate and thiosemicarbazide, but good results were obtained in many cases. In Figure 9

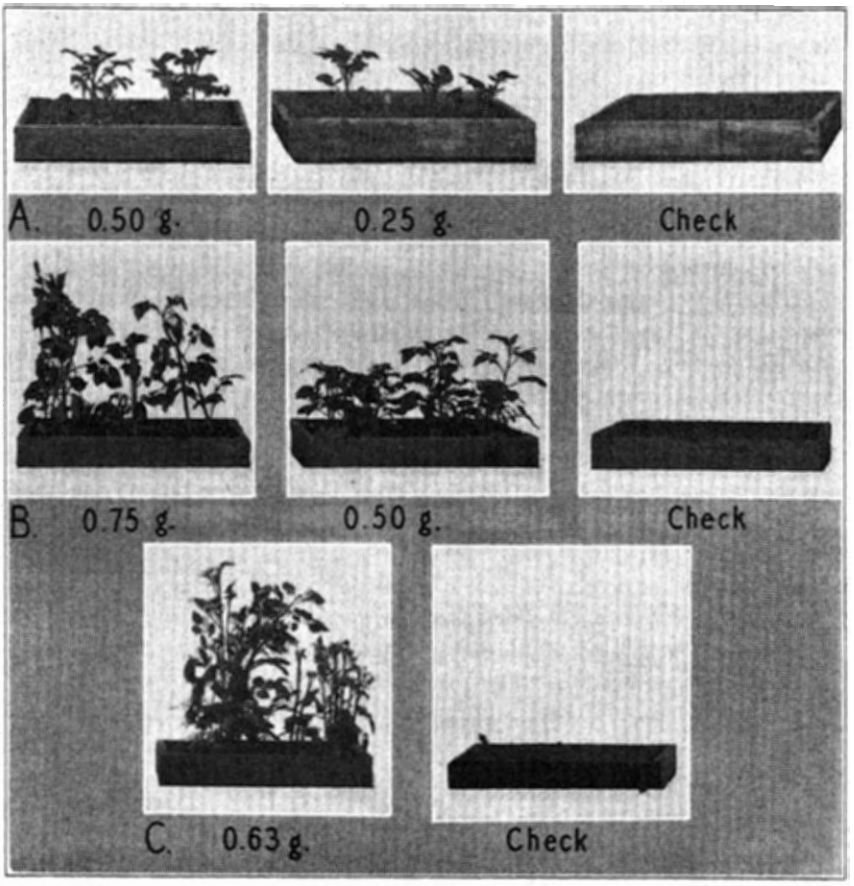


FIGURE 9. The effect of treatments with various concentrations (g. per 100 cc.) of thioacetamide in breaking dormancy. A, Institute Cobbler 1930; B, New Jersey Cobbler 1930; C, New Jersey Cobbler 1931.

are shown the results of treatments of three lots of potatoes: A, Institute Cobblers 1930; B, New Jersey Cobblers 1930; and C, New Jersey Cobblers 1931. The effective concentrations were found to be in the neighborhood of 0.5 g. per 100 cc. Concentrations as high as 1.0 g. per 100 cc. usually caused rot, although some successful treatments were made with a concentration

of 0.9 g. per 100 cc. It is seen in Figure 9 that thioacetamide had a considerable effect in breaking the dormancy of the very dormant lot of New Jersey Cobblers of the 1931 crop. Although the treated lots did not show 50 per cent above ground until 51 days after treatment, the checks did not come up until 78 days after treatment, and the treated lot produced 274 g. of tops before the checks began to grow. Immature Cobblers have also shown a response to treatments with thioacetamide and produced a new crop of tubers before the checks sprouted, such as is illustrated in Figure 3 for an ammonium dithiocarbamate treatment.

Effect in Retarding Sprouting of Non-Dormant Tubers

Some tests in which thioacetamide retarded the sprouting of non-dormant tubers are illustrated in Figure 10. Figure 10 A shows a treatment with 0.63 g. per 100 cc. compared with a check and Figure 10 C shows a series of decreasing concentrations beginning with 0.9 g. per 100 cc. It is seen that the resultant growth forms a series corresponding with the concentrations of the chemical used but that the minimum growth is obtained with the highest concentration and the maximum in the check. Further data on the retarding action of thioacetamide treatments are given in column 4 of Table XIII in which the number of days until 50 per cent of the sprouts appeared above ground is given. Included also are a few cases of very dormant potatoes where the treated lots grew before the checks.

This retarding effect of thioacetamide is due to a delay in the sprouting of the seed pieces. Once growth is started it proceeds quite normally. This is illustrated in Figure 10 D and E where a treated flat is shown when the sprouts were just emerging (D) and again 22 days later (E). It is seen that the treated tubers have made good healthy growth in the meantime.

Various trials with acetamide (CH_3CONH_2), the oxygen analog of thioacetamide, showed that it does not produce similar effects. Figure 10 B shows a photograph of one such test and it is seen that the growth in the treated and check lots is about equal.

Changes in the Properties of the Juices Produced by Thioacetamide Treatments

Since thioacetamide differs from the other substances discussed in this paper in that sprouting is considerably retarded as a result of the treatments, it is interesting to study the changes produced and compare these with the studies made in the case of the chemicals which induce a prompt growth of the buds. In Table XIII are summarized analyses made on both dormant and non-dormant tubers treated with thioacetamide. Even though growth does not take place until from five to seven weeks after treatment, the changes produced are very similar to those occurring in treatments with ammonium dithiocarbamate and thiosemicarbazide when

50 per cent of the sprouts appear above ground in from two to three weeks. The thioacetamide treatments produce similar slight increases in pH and similar increases in the peroxidase activity. In many cases the catalase values are about equal or even lower in the treated lots than in the checks but these results do not differ markedly from those obtained with am-

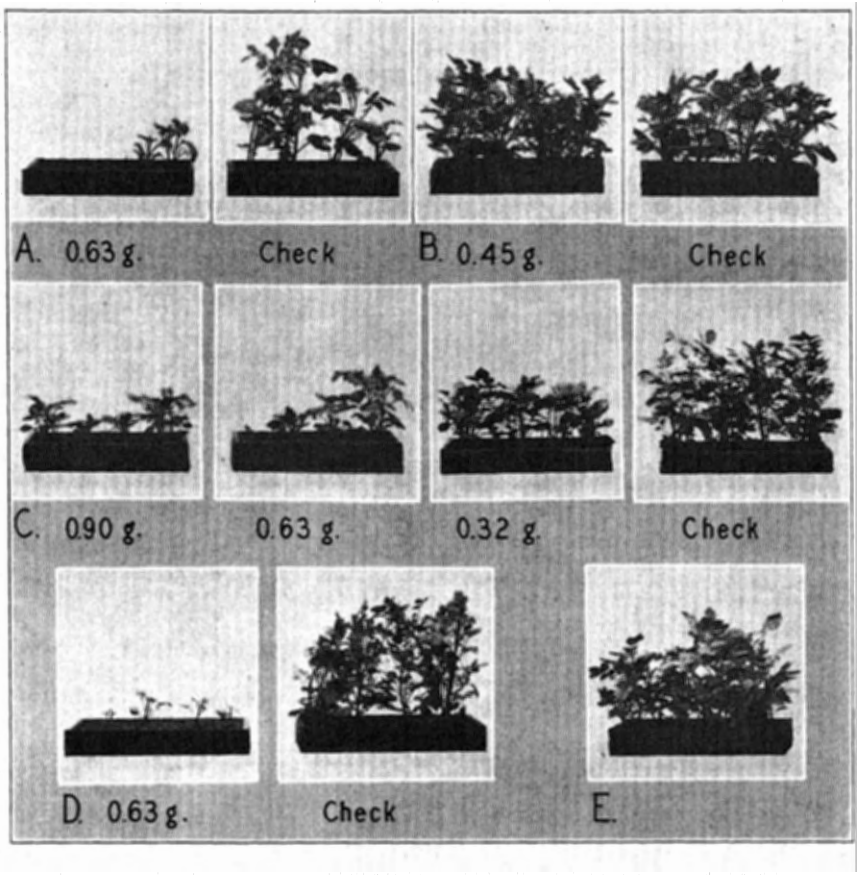


FIGURE 10. The effect of various concentrations (g. per 100 cc.) of thioacetamide and acetamide on the sprouting of non-dormant potato tubers. A, C, and D, Thioacetamide treatments of non-dormant tubers; B, An acetamide treatment. The treated flat in D is shown a second time in E 22 days after the first photograph was taken.

monium dithiocarbamate which does not retard the sprouting. The values obtained for the iodine titrations are quite high and indicate the absorption of much of the chemical. This will be discussed more fully below.

Especially interesting are the data on the sugar content given in Table XIV. The treatments induce quite a remarkable increase in the sucrose

TABLE XIII

EFFECT OF TREATMENTS OF POTATO TUBERS WITH THIOACETAMIDE

Potatoes used	Date treated	Sampled days after treatment	Days until 50% were above ground		Tops produced			Expressed juice								Dialyzed juice			
					Days after treatment	Fresh weight, g.		pH		Titration N/100 I		Catalase		Peroxidase		Catalase		Peroxidase	
			Tr.	Ck.		Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.
Long Island Cobbler	Oct. 14	7	44	22	—	—	—	5.95	5.83	1.6	0.4	11.6	6.2	37.6	22.4	8.7	7.0	31.0	20.4
	Dec. 12	6	32	13	45	65	337	6.02	5.90	2.8	0.4	22.8	17.7	64.5	44.7	17.3	10.2	56.4	24.6
	Jan. 8	3	46	15	56	79	370	6.02	5.83	4.5	0.4	15.2	13.1	49.4	33.8	14.7	9.8	33.1	19.9
New Jersey Cobbler	Nov. 4	7	51	78	72	274	0	6.29	6.20	1.5	0.5	13.5	19.2	45.2	44.4	9.9	11.6	40.0	48.4
	Nov. 16	8	>95*	71	56	80	0	6.47	6.51	0.6	0.5	20.0	25.5	72.7	72.0	17.9	15.8	44.6	43.2
	Dec. 5	6	>76**	72	52	16	1	6.44	6.42	2.5	0.6	19.0	19.4	67.9	63.6	15.4	14.7	37.0	39.0
	Jan. 9	3	>55***	32	55	75	304	6.15	6.14	2.8	0.5	11.8	19.0	52.4	53.2	11.4	12.2	37.2	35.0
Bliss Triumph	Dec. 14	9	37	15	43	9	197	6.17	6.03	2.9	0.5	21.2	27.7	51.4	49.7	15.2	11.4	32.9	25.9
	Dec. 24	7	39	18	43	9	118	6.19	6.03	3.0	0.4	15.2	18.8	44.7	39.6	8.8	7.4	21.7	12.5
Institute Cobbler	Dec. 24	6	58	16	54	—	305	6.22	6.20	4.0	0.4	22.9	17.3	56.1	40.3	4.3	1.7	15.8	<10
Bliss Triumph†	Feb. 17	6	28	36	40	122	120	6.07	5.93	1.6	0.4	13.5	11.2	33.8	19.7	7.2	6.2	22.8	13.0

* Three came up after 55 days and none grew thereafter up to 95 days; ** Three after 49 days, none grew thereafter; ***Three 41 days after treatment.

† Purchased in the Yonkers market.

content of the juices, even in the cases where the tubers are completely non-dormant and already contain much sucrose. It will be noted that when treatments were made with a series of concentrations, the sucrose content of the juices also formed a series corresponding with the concentrations of the treatments.

TABLE XIV

REDUCING SUGAR AND SUCROSE CONTENT OF JUICES FROM POTATOES TREATED WITH THIOACETAMIDE

Potatoes used	Grams of thio-acetamide per 100 cc.	Days until 50% were above ground	Mg. in 5 cc. juice	
			Reducing sugars	Sucrose
Long Island Cobbler treated Jan. 16	0.90	32	16.6	52.7
	0.63	37	17.6	44.0
	0.32	32	16.7	37.1
	Check	16	0.0	8.8
Long Island Cobbler treated Mar. 12	0.90	*	17.9	90.7
	0.45	25	29.1	74.0
	0.225	16	23.1	53.0
	0.112	14	14.5	35.2
	Check	12	3.5	21.9
New Jersey Cobbler	0.63	*	56.6	12.4
	Check	32	41.8	5.9
Bliss Triumph**	0.50	28	16.0	46.2
	Check	36	2.3	21.4
Bliss Triumph**	0.50	29	62.5	61.6
	Check	40	48.6	41.5
Green Mountain	0.50	29	67.9	13.5
	Check	13	49.3	4.7

* More than 50 per cent of these pieces rotted.
** Two different lots purchased in the Yonkers market.

The Thioacetamide Absorbed by the Tubers

It has been found by Ray and Dey (25) that in acid solution one molecule of thioacetamide reacts with two molecules of iodine. The iodine titrations reported then can be taken as a measure of the thioacetamide content of the tubers. An iodine titration of 5 cc. would indicate a concentration of thioacetamide about 1/200 molar. Treated and check pieces were divided into outer and inner halves and iodine titration figures determined at intervals after treatment. It was found that large amounts are absorbed and that it takes 20 days or more until the titration in the treated lot is as low as that of the check. For example, in one test 5 cc. of the juice from the outer half of the pieces gave an iodine titration value of 14.0 cc. immediately after treatment. This value fell to 4.2 cc. in 72 hours, was still 1.0 cc. after 283 hours, and 0.6 cc. after 523 hours. Juice from corresponding check pieces

gave a value of 0.4 cc. Considerable thioacetamide also penetrated into the inner half of the treated pieces. Sprout emergence never occurs before the titration values of the treated juices are about the same as those of the checks. These results indicate that the presence of thioacetamide prevents the growth of the sprouts.

It was found that in the case of thiourea, too, large amounts of the chemical are absorbed and remain in the tubers for a long time, but growth takes place even though the tubers still show a high thiourea content.

Recently Elmer (13) has reported that if apples are placed in the same room with potatoes or in closed containers containing potted seed pieces, the development of sprouts is greatly retarded. It is not known to what substance this retardation is due. This phenomenon is exceedingly interesting; however, it does not seem probable that there is much of a similarity between the retarding effect of apples and that of thioacetamide.

Discussion of Effects of Thioacetamide

Potato tubers treated with the proper concentration of thioacetamide sprout within about five to eight weeks after treatment. If the potatoes are very dormant, sprouting of the treated tubers takes place before growth of the checks and if non-dormant the treated lots do not sprout until a considerable time after the emergence of the checks. Although the sprouting is so long delayed after treatment the changes resulting from the treatments do not seem to be markedly different from those obtained with chemicals which cause prompt growth. The evidence indicates that treatments with thioacetamide bring about the changes associated with the breaking of dormancy but that growth does not begin until the thioacetamide absorbed by the tubers is no longer present.

EFFECT OF OTHER SULPHUR COMPOUNDS ON DORMANCY

A large number of other chemicals were tested as to their effect in breaking the rest period of potatoes. These chemicals were not tried on as many lots of dormant tubers as the ones discussed previously in this paper and in some cases the preparations used were of undetermined purity. Some of the results with these chemicals will be discussed briefly in this section, however, since the results collectively add considerably to the evidence already presented showing that many sulphur-containing compounds are active in breaking dormancy.

Thioglycol

Monothio-ethylene glycol ($HSCH_2CH_2OH$) was prepared from sodium sulphide and ethylene chlorhydrin according to the method described by Rosen and Reid (28). The fraction coming over between 50° and 60° under

12 mm. pressure was used for the treatments. When used as a one-hour soak treatment, concentrations of 1.0 to 3.0 g. per 100 cc. were effective.

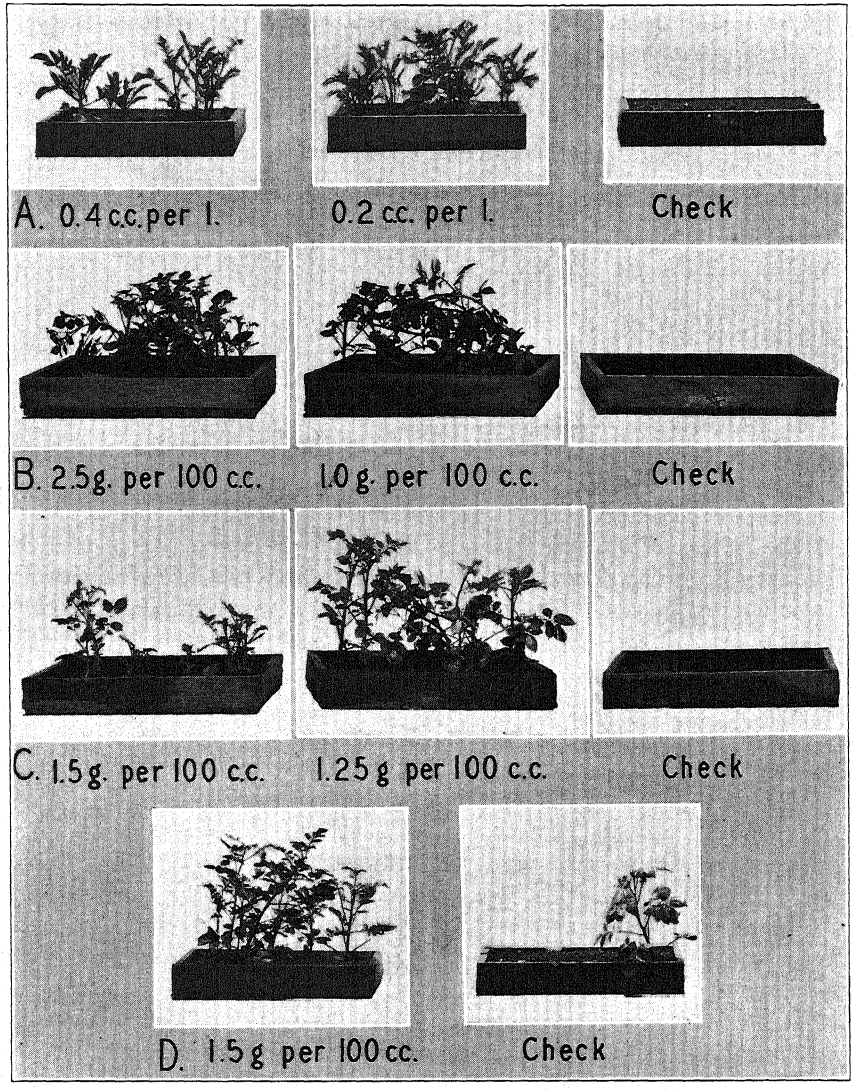


FIGURE 11. Effect of various sulphur compounds in breaking dormancy. A, Ethyl mercaptan; B, Thioglycol; C, Sodium azido-dithiocarbonate; D, Potassium sulphocarbonate.

A treatment of the New Jersey second crop Irish Cobblers of the 1930 season is illustrated in Figure 11 B. Treatments with 2.5 g. and 1.0 g. per

100 cc. are shown. Treatments with 2.5 g., 2.0 g., 1.0 g., and 0 g. per 100 cc. produced tops weighing 132, 174, 137, and 0 grams respectively, 59 days after treatment. This chemical was also effective in breaking dormancy in a lot of immature Irish Cobblers treated in March 1931. Concentrations of 3.0, 1.5, 0.75, and 0 g. per 100 cc. produced 10, 9, 5, and 1 sprouts above ground out of 15 planted, 46 days after treatment.

Sodium Azido-Dithiocarbonate

Treatments with sodium azido-dithiocarbonate (N_3CSSNa) were also effective in breaking dormancy. This compound was prepared from sodium trinitride (NaN_3) and carbon disulphide following the procedure described by Browne and Hoel (3) for the preparation of the corresponding potassium salt. Three lots of dormant tubers were treated with this chemical and breaking of dormancy resulted in each case. Effective concentrations were found to be between 0.75 and 1.25 g. per 100 cc. In Figure 11 C are shown photographs of treatments of New Jersey second crop Irish Cobblers of the 1930 crop.

From the data previously given on the effect of sulphur compounds one would expect a compound so closely related to ammonium dithiocarbamate and the other sulphur compounds discussed, as is sodium azido-dithiocarbonate, to break dormancy. Tests with sodium trinitride indicate that this substance has no effect on dormancy. This would indicate, perhaps, that the trinitride ($-N_3$) group is not particularly active in the azido-dithiocarbonate in regard to the effect on dormancy. It should be noted also that sodium azido-dithiocarbonate is quite unstable and one of its decomposition products is sodium thiocyanate. Certainly some of its efficacy is due to the formation of the thiocyanate in the treated tubers.

Thioacetic and Thioglycollic Acid

A number of tests were made with thioacetic (CH_3COSH) and thioglycollic ($CH_2SHCOOH$) acids. Treatments of potatoes of the 1930 crop were quite successful, especially with thioglycollic acid. Effective concentrations were in the neighborhood of 0.5 cc. per liter. Subsequent trials with other lots of potatoes, however, did not give very good results. It is quite probable that the acidity of the chemicals produces an injurious effect at concentrations lower than those necessary for breaking dormancy in very dormant tubers.

Derivatives of Dithiocarbamic Acid

In a manner analogous to the reaction between ammonia and carbon disulphide to form ammonium dithiocarbamate, primary and secondary amines generally react with carbon disulphide to form compounds of the type $NHRCSSNH_3R$ and $NR_2CSSNH_2R_2$ (18); and if one molecule each

of two different amines is used, mixed derivatives are formed, the less basic of the two always uniting with the carbon of the dithiocarbamic acid (20). Extensive experiments were not conducted with these compounds but results obtained in tests made up to this time indicate that many of these derivatives break dormancy. Thus in an experiment with methyl ammonium methyl dithiocarbamate ($CH_3NHCSSNH_3CH_3$) with Bliss Triumph potatoes of the 1930 crop, concentrations of 0.75 g., 0.37 g., 0.18 g., and 0 g. per 100 cc. resulted in 8, 9, 2, and 3 sprouts above ground respectively, 66 days after treatment.

A number of tests conducted with diethylammonium diethyl dithiocarbamate [$(C_2H_5)_2NCSSNH_2(C_2H_5)_2$], recrystallized from toluene and petroleum ether, indicated that this substance has little or no effect on dormancy, concentrations as high as 5 g. per 100 cc. being tried. On the other hand, the product obtained when one molecule of diethylamine and one molecule of ammonia reacted with carbon disulphide broke dormancy quite readily. In the case of Irish Cobbler potatoes of the 1931 crop a concentration of 1.5 g. per 100 cc. resulted in 228 g. of tops after 48 days while the corresponding check produced 50 g. of tops during this period. It is possible, of course, that this product contained some ammonium dithiocarbamate as an impurity.

Potassium Sulphocarbonate

Treatments with solutions of potassium sulphocarbonate ($KSCO_2$) containing 1.5 g. per 100 cc. were somewhat effective in breaking dormancy. Such a treatment made on Irish Cobbler potatoes of the 1931 crop is illustrated in Figure 11 D. The photograph was taken 55 days after treatment. The treated sample had produced a weight of tops equal to 165 g. during this time while the tops from the check weighed 35 g.

Methyl Disulphide

Results of treatments with methyl disulphide (CH_3SSCH_3) were not very uniform but breaking of dormancy resulted in some cases, the effective concentration being about 0.25 cc. per liter used as a 24-hour vapor treatment. No effect was obtained from treatments with methyl sulphide [$(CH_3)_2S$].

Preliminary experiments conducted with lilacs indicate that methyl disulphide also breaks their dormancy. A treatment of lilac twigs is shown in Figure 5 B. A response to the treatment resulted from concentrations ranging from 0.1 to 0.6 cc. per liter, although the treatments gave some evidence of injury.

Potatoes treated with methyl disulphide gave a positive nitroprusside test indicating that some mercaptan had been formed. Further studies showed that potato tissue readily reduces both methyl and ethyl disul-

phide to the corresponding mercaptans. The juice does not seem to bring about the reduction as readily as the tissue. Boiled tissue is not as active as unboiled tissue. It is possible that the mechanism which reduces these disulphides is similar to the one reducing the oxidized form of glutathione (19).

DIRECT EFFECT OF THE CHEMICALS ON ENZYME ACTIVITY

Catalase and Peroxidase

In connection with the increases in catalase and peroxidase activity which take place when potato tubers are treated with these sulphur compounds, the question naturally arises as to whether any of these increases result as a direct effect of the chemicals; that is, will the chemicals when added directly to the expressed juice increase the catalase and peroxidase activities?

Various concentrations of the chemicals were added to potato juices and catalase and peroxidase activity determined after the chemical had been in contact with the juice for one hour and again after a longer period of contact. Although the various chemicals differed considerably with respect to the concentration necessary to produce any effect, all the chemicals tested were alike in exhibiting no stimulating effect. The first measurable effect of the contact between the chemicals and the enzymes in each case was a decrease in the catalase or peroxidase activity as compared with the juice to which no chemical was added.

In Table XV are shown the approximate concentrations of ammonium dithiocarbamate, thiosemicarbazide, ethyl mercaptan, hydrogen sulphide, and thioacetamide necessary to reduce the catalase and peroxidase activity

TABLE XV

DIRECT EFFECT OF SULPHUR COMPOUNDS UPON ENZYMES OF POTATO JUICE

Chemical	Mg. per 100 cc. juice required to reduce activities one-half	
	Catalase	Peroxidase
Ammonium dithiocarbamate	50	450
Thiosemicarbazide	80	300
Ethyl mercaptan	90	90
Hydrogen sulphide	10	20
Thioacetamide	800	400

of potato juice by one-half. It should be borne in mind that these values are approximate, since the concentrations of the chemicals necessary to produce this effect vary with the time of contact and also to some extent with different lots of juice. This is especially true of those chemicals which are quite unstable and whose decomposition products also affect the enzymes.

The table shows that the chemicals differ greatly in regard to their action on catalase and peroxidase, a concentration of hydrogen sulphide of 10 mg. per 100 cc. juice producing the same effect on the catalase activity as 800 mg. of thioacetamide per 100 cc. juice. There is no correlation between the effect of these chemicals measured in this way and their efficacy in breaking dormancy.

It is, of course, quite impossible to duplicate conditions present when the tubers are treated with the chemicals, by adding the chemicals to the expressed juice, even in studying direct effects only. In the case of the treated tubers the chemicals are in contact with the tubers for a very long period as compared with the tests made on the expessed juices. If the enzyme changes noted in the treated tubers were due to a direct effect, however, one would expect the change to be the greatest immediately after treatment when the concentration of the chemical in the tuber is the greatest. As a matter of fact the time relation tables previously given show that in most cases the treated and check tubers are about equal in catalase and peroxidase activity for from 24 to 48 hours after treatment and it is not until later than the treated lots show greater activity than the corresponding checks. The only chemical in which a depressing effect was noted immediately after treatment was ethyl mercaptan. This effect may have been due to a direct action since the tubers at this stage gave a strong nitroprusside test. However, dialysis of the juices did not increase the activity of the juices from the treated potatoes sufficiently to equal that of the check juice.

None of the chemicals tested increased the catalase and peroxidase activity of the juices when added directly to the juices. This fact, together with the fact that the increases in catalase and peroxidase do not occur until a considerable time after treatment, shows that the chemicals bring about the increases which result from the treatments in an indirect way; that is, through some response given by the potato tissue to the treatments.

These results agree with those previously reported for treatments with ethylene chlorhydrin, sodium thiocyanate, and thiourea (12). Enzyme changes resulting from the treatments were not caused by any direct action of these chemicals on the juices. Denny, working with amylase, found that changes resulting from treatments with ethylene chlorhydrin, sodium thiocyanate, potassium cyanide, potassium chloride, and potassium nitrate were not correlated with any direct effect on the juices (11).

Tyrosinase

In a previous paper (12) it has been reported that a characteristic of juices from potatoes treated with ethylene chlorhydrin, sodium thiocyanate, and thiourea was that such juices were lighter in color than the

corresponding check juices. Thiourea was especially active in preventing the darkening of potato juice exposed to the air.

In the case of thiourea this is a direct effect in that when the chemical is added directly to the juice the darkening is retarded. This is also a property of the sulphur compounds reported on in the present paper. This retardation takes place in very dilute concentrations of the chemical in question.

The darkening of potato juices is stated to be due to the action of tyrosinase which acts on tyrosine to form colored products, the first products being reddish in color and these later becoming black. Tests were made to determine the lowest concentrations of these chemicals which would retard the action of tyrosinase. Potato juice was dialyzed in collodion bags for several hours, after which it no longer darkened when exposed to the air unless a suitable substrate was added. For the tests here reported 5 cc. of such dialyzed potato juice representing 0.5 cc. of original juice were mixed with 5 cc. of a tyrosine solution containing 0.5 g. of tyrosine dissolved in one liter of a M/15 phosphate buffer of pH 6.8. To this were added 5 cc. of a solution of the chemical to be tested. A series of concentrations was set up, beginning with a relatively strong solution with the concentration decreasing by one-half in each subsequent container. The 15 cc. mixtures were placed in 50 cc. beakers and arranged in series so that one could observe at what concentrations the darkening was taking place at a slower rate than that of the checks containing no chemical.

It was found that under the conditions described above, the lowest concentration of ammonium dithiocarbamate, thiosemicarbazide, thioacetamide, and thiourea showing any retarding effect was about the same for all these chemicals and was equal to 1/62,000 molar. In the case of ammonium dithiocarbamate which has a molecular weight of 110 this represents about 0.03 mg. present in the 15 cc. of reaction mixture. The retarding effect is presumably not due to any direct chemical action between the tyrosine or any of the products of tyrosinase action since the amount of tyrosine present equals 2.5 mg., or about 80 times that of the ammonium dithiocarbamate causing the retardation. The lowest concentration of hydrogen sulphide retarding the coloration was 1/27,000 molar. It is interesting to compare these concentrations with the lowest concentration of potassium cyanide (KCN) resulting in retardation since potassium cyanide has long been regarded as very active in retarding oxidase activity. Tests made at the same time and under the same conditions as those on the sulphur compounds showed that the lowest concentration of potassium cyanide retarding the blackening was 1/19,000 molar, or about three times that of thiourea and related compounds.

This property of these compounds to retard tyrosinase activity might

be used under certain conditions as a qualitative test as extremely small amounts could be detected in this way.

Just what significance should be attached to this property of these compounds with respect to their effect on dormancy is not known at this time. A sufficiently rapid method for the quantitative determination of tyrosinase activity has not been available to permit of tyrosinase determinations on a large number of samples. Qualitative tests have been made on much of the treated material, but the results have not been very uniform. It can be stated, however, that in many cases where the treated juices contain amounts of the chemical used for the treatment sufficient to show much retardation of the blackening, such juices after dialysis show a greater tyrosinase activity than the corresponding checks. Treatments with chlorhydrin, which does not itself appreciably retard tyrosinase activity, result in the formation of substances which retard tyrosinase. On the other hand, observations made in the course of handling potato juices indicate that dormant potatoes have less tyrosinase activity than non-dormant potatoes.

DISCUSSION

From the evidence at hand as to the changes taking place after dormant potato tubers are treated with these various chemicals it is impossible to single out any particular change or set of changes as directly connected with the breaking of dormancy. It can only be stated that the changes measured result from the initiation of activity induced by the treatments. If any one of these changes is *the* important one with respect to the dormancy mechanism, further work is necessary to prove this.

The results obtained with catalase and peroxidase changes agree with those previously found (12, 16) in that there is no correlation between the extent of the changes produced and the efficacy in breaking dormancy. On the other hand, it should be remembered that the breaking of dormancy by the treatments practically always brings about some increase in catalase and peroxidase activity, and that as dormancy is naturally broken the tubers increase in catalase and peroxidase activity. Treatments of thoroughly non-dormant tubers do not bring about such a large increase in catalase and peroxidase activity.

Since the chemicals used in these experiments react with iodine in acid solution it is impossible to use this test as a measure of reducing substances formed in connection with the treatments, such as result from treatments with ethylene chlorhydrin. However, the reducing capacities of the juices could be measured by their action on methylene blue. Tests showed that the differences in reducing capacity were correlated with the pH changes resulting. Where the pH change was only slight, 0.2 or less, the differences in reducing capacity between treated and check juices were very small.

Only treatments with hydrogen sulphide and ethyl mercaptan, which brought about larger pH changes, resulted in considerable differences in the reducing action of the juices with regard to methylene blue, the treated juices having the greatest reducing power.

Increases in respiration have been considered by Rosa (27) and Appleman (1) as important factors in breaking dormancy. Results obtained with hydrogen sulphide and ethyl mercaptan treatments have shown that such treatments bring about large increases in respiration. In these cases increases were brought about by substances reducing in nature while Rosa (27) emphasizes the action of oxidizing agents (sodium nitrate and ferric chloride) in increasing respiration. Studies being made on the effect on respiration of the treatments of cut pieces have not been completed, but the results obtained thus far indicate that these treatments do not produce increases as large as those produced by ethyl mercaptan and hydrogen sulphide. Of course, these cut pieces have a very high initial respiratory rate as a result of the cutting of the pieces; the checks respire at a higher rate for a considerable period after the cutting than whole tubers treated with ethyl mercaptan and hydrogen sulphide. The cutting of the tubers in this manner does not break dormancy, of course, although it increases the respiration enormously.

It has been pointed out that although treatments with ethylene chlorhydrin, sodium thiocyanate, and thiourea are about equally effective in breaking dormancy, a study of the enzyme activities and other changes resulting from treatments has shown that these chemicals differ markedly in the extent to which they bring about these changes (12). It has been found by Denny (10), however, that all three of these chemicals produce marked increases in the sucrose content of the treated tubers, the sucrose content of such tubers being from two to three times that of the corresponding checks. The results with respect to the sucrose changes were found to be much more uniform with regard to the three chemicals under consideration than the other changes measured.

The sulphur compounds considered in the present paper also produced large increases in sucrose and the chemicals as a group were more uniform in regard to their power to cause this change in the treated tubers than with respect to other changes determined. Contrary to the results with catalase and peroxidase, treatments of non-dormant tubers resulted in about as large increases as treatments of dormant tubers, even though such tubers already contained considerable sucrose. Thioacetamide treatments which actually delay the sprouting of non-dormant tubers brought about equally large increases.

Although in most cases no direct comparisons have been made between the sulphur compounds here studied and their oxygen analogs, evidence so far at hand shows that these sulphur compounds are much more active in

breaking dormancy than related oxygen compounds. It was found that acetamide does not produce the results given by thioacetamide. Urea and potassium cyanate are much less effective than thiourea and potassium thiocyanate (16). A much higher concentration of ethyl alcohol than of ethyl mercaptan is necessary to produce an effect. And, hydrogen sulphide is much more active than the corresponding oxygen compound.

It is not known at this time what property common to these sulphur compounds is largely responsible for their dormancy-breaking activity. It may be noted, however, that all these compounds contain sulphur in such a form as to be readily oxidized by iodine. In this connection it should be noted too that non-sulphur compounds which break dormancy have been found to induce the formation of reducing substances, as measured by reaction with iodine in acid solution, in the juice of treated potatoes (16). In the case of treatments with chlorhydrin, glutathione has been isolated from treated potatoes (17).

SUMMARY

1. Experiments conducted over a period of two years have shown that many organic sulphur compounds break the dormancy of freshly-harvested potato tubers. Extensive tests have shown that ammonium dithiocarbamate, thiosemicarbazide, hydrogen sulphide, and ethyl mercaptan are effective. Other compounds not so extensively tested but found effective are thioglycol, sodium azido-dithiocarbonate, methyl disulphide, potassium sulphocarbonate, and various derivatives of dithiocarbamic acid. Preliminary tests have shown that methyl disulphide and ethyl mercaptan also break dormancy in lilacs and grapes.

2. The effect of treatments with thioacetamide was found to be different from any of the other chemicals studied in that this chemical breaks the dormancy of very dormant tubers and retards the sprouting of non-dormant tubers. Treated tubers, whether dormant or non-dormant, show 50 per cent sprouts above ground in from five to eight weeks after treatment. If the tubers are very dormant, the treated tubers grow before the checks; if non-dormant, the check lots grow from three to six weeks before the treated lots. It was found that considerable quantities of thioacetamide were absorbed by the tubers during treatment and that sprouts did not appear until the thioacetamide was no longer present. The chemical changes resulting from the treatments were similar to those resulting from treatments with chemicals which produce a prompt growth of buds. It appears, therefore, that the presence of the thioacetamide retarded growth. Acetamide did not give similar results. Tubers treated with potassium thiocyanate and thiourea also retain considerable quantities of these chemicals long after treatment but growth is not retarded by the presence of these substances.

3. The juices expressed from dormant and non-dormant potatoes treated with ammonium dithiocarbamate, thiosemicarbazide, thioacetamide, hydrogen sulphide, and ethyl mercaptan were examined as to their catalase and peroxidase activity, pH, iodine-reducing capacity, and reducing sugar and sucrose content at intervals after treatment but before visible sprouts appeared. The changes produced by all these chemicals were similar qualitatively but showed quantitative differences.

4. Treatments of dormant tubers resulted in increases in catalase and peroxidase activity amounting to from a few per cent to several hundred per cent. The largest increases in these enzymes are given by the thiosemicarbazide treatments. Large increases resulted also from the treatments with hydrogen sulphide and ethyl mercaptan, and lesser increases with the ammonium dithiocarbamate and thioacetamide treatments.

5. As dormancy was broken naturally during storage the tubers increased in catalase and peroxidase activity and treatments of such non-dormant tubers with these chemicals were not as effective in increasing the activities of these enzymes as when the tubers were dormant. The chemicals that were most active in increasing the enzyme activities of the dormant tubers also were most active in affecting the non-dormant tubers.

6. The increases in enzyme activity took place in from 24 to 48 hours after treatment. When potato tubers which were only partially dormant were planted the catalase and peroxidase activity of juices from such tubers also increased from day to day during the first week after planting but treated samples increased at a faster rate than the corresponding checks. When tubers were treated with various concentrations of the chemicals the enzyme activities of the juices from such treatments differed in the same order as the concentrations used for the treatments, the highest concentration resulting in the largest increases.

7. It is not possible, however, to judge the efficacy of a chemical in breaking dormancy by the extent in which the catalase and peroxidase activity of juices from tubers treated with the chemical are increased. Of the chemicals studied thiosemicarbazide produced increases two and three times that of the other chemicals used but it was not more effective in breaking dormancy.

8. Experiments showed that the catalase and peroxidase activity of potato juice could not be increased by the direct addition of various amounts of ammonium dithiocarbamate, thiosemicarbazide, thioacetamide, ethyl mercaptan, or hydrogen sulphide to the juice. The increases resulting from the treatments must, therefore, be due to some response of the intact and living potato tissue to the treatments.

9. Juices from potatoes treated with ammonium dithiocarbamate, thiosemicarbazide, and thioacetamide gave slightly higher pH values than the corresponding check juices. This increase amounted to from 0.1 to 0.2

pH. Somewhat larger increases, 0.2 to 0.4 pH, were given by the hydrogen sulphide and ethyl mercaptan treatments.

10. The sucrose content of potatoes treated with these chemicals was from two to four times that of the corresponding checks. These increases took place in both dormant and completely non-dormant tubers, even though such tubers were already relatively high in sucrose. This response as a result of the treatments was more consistent than the enzyme changes.

11. Treatments of whole tubers with ethyl mercaptan and hydrogen sulphide brought about large increases in respiration, the treated samples having a respiratory rate as much as four and five times that of the check samples, about 48 hours after treatment. Subsequently the rate fell until it reached a value about the same as the check a week or ten days after treatment. The increase in the respiratory rate took place very quickly. It was found that Van Slyke-Cullen tubes can be used for the absorption of carbon dioxide by barium hydroxide. These are much more convenient to use than the Pettenkofer tubes commonly employed in respiration studies.

12. Tubers treated with ammonium dithiocarbamate contain considerable thiocyanate resulting from the decomposition of the ammonium dithiocarbamate. The concentration of thiocyanate reached in connection with treatments with optimum concentrations of ammonium dithiocarbamate is less, however, than that resulting from treatments with potassium thiocyanate below the optimum for the breaking of dormancy. It is, therefore, concluded that the effect of ammonium dithiocarbamate in breaking dormancy is due to other factors in addition to the thiocyanate resulting from its decomposition.

13. A method for the preparation and recrystallization of ammonium dithiocarbamate is described. It has been found that ammonium dithiocarbamate, previously described as very unstable, can be kept for months without decomposition if stored *dry* at low temperature.

14. The sulphur compounds used in this work are very active in retarding the tyrosinase action of potato juice. In tests conducted with dialyzed juice at pH 6.8 concentrations of ammonium dithiocarbamate, thiosemicarbazide, thioacetamide, and thiourea as low as 1/62,000 molar retarded the darkening resulting from adding tyrosine to the dialyzed juice. The lowest concentration of hydrogen sulphide retarding the darkening was 1/27,000 molar. These compounds are more active in retarding tyrosinase than potassium cyanide, the lowest concentration which retarded under comparable conditions being 1/19,000 molar. It is not known whether this property of these compounds is of any special significance in connection with their effect in breaking dormancy.

15. Methyl disulphide and ethyl disulphide are readily reduced to the corresponding mercaptans by potato tissue.

16. Willstätter's method for determining peroxidase activity, using pyrogallol as a substrate, was modified so that it was possible to run a considerable number of determinations at the same time. The purpurogallin formed was separated by centrifuging and subsequently determined colorimetrically in alcoholic solution. The limits within which the amount of purpurogallin formed, under the conditions of the determinations, is a linear function of the amount of peroxidase present, were determined.

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EFFECT OF CHEMICAL TREATMENTS OF DORMANT POTATO TUBERS ON THE CONDUCTIVITY OF THE TISSUE AND ON THE LEACHING OF ELECTROLYTES FROM THE TISSUE[1]

JOHN D. GUTHRIE

It has been shown by Denny (1) that ethylene chlorhydrin, potassium thiocyanate, and thiourea are very effective in breaking the dormancy of potato tubers. For this reason it was thought desirable to study the effect of these chemical treatments on the electrical conductivity of the tissue and on the leaching of electrolytes from the tissue when placed in water.

METHODS

In all cases, freshly-harvested potato tubers (*Solanum tuberosum* L.) were used. The ethylene chlorhydrin treatments were made by the vapor method, which consists in placing whole tubers in a closed container with a definite amount of ethylene chlorhydrin for 24 hours, and by the dip method, which consists in dipping cut pieces of the tubers in a solution of ethylene chlorhydrin, draining off the excess solution and placing the pieces in a closed container for 24 hours. All concentrations are expressed in terms of the 40 per cent stock solution of ethylene chlorhydrin which was used throughout the experiments. The potassium thiocyanate and thiourea treatments were made by soaking cut pieces of the tubers in solutions of these chemicals for one hour. Twelve pieces from each treatment were planted and the weight of tops determined after a definite interval. This was done in order to be sure that the potatoes were dormant and that the treatments were effective.

The wiring diagram of the apparatus used in determining the conductivity of the tissue is shown in Figure 1. It is the usual arrangement for conductivity measurements except for the rheostat V, which was of the type commonly used in radio sets and had a resistance of about 300 ohms. The introduction of this variable resistance in the lead from the source to the resistance box was very important, since null points could not be obtained without it. The source used was a high frequency buzzer. For the estimation of the conductivity of the tissue, cylinders 18 mm. in diameter were cut from the tubers and pieces 14 mm. long were cut from these with a razor, using two pieces of hard rubber 14 mm. wide fastened together at right angles as a guide. Three such pieces were put together for each determination and placed between KCl electrodes in the electrode holder shown in Figure 2. The procedure was first to fill the lower electrode vessel with N/10 KCl and place the first cylinder of tissue on it, avoiding air bub-

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 55.

bles. The other two cylinders of tissue were then piled upon the first and the upper electrode vessel placed on these. After the upper hard rubber plate and the weight were put in place the upper vessel was filled with N/10 KCl from a pipette. The tissue was now washed down with a stream of water from a wash bottle, the known resistance brought to approximately that of the tissue, and the bridge adjusted to as near minimum sound as

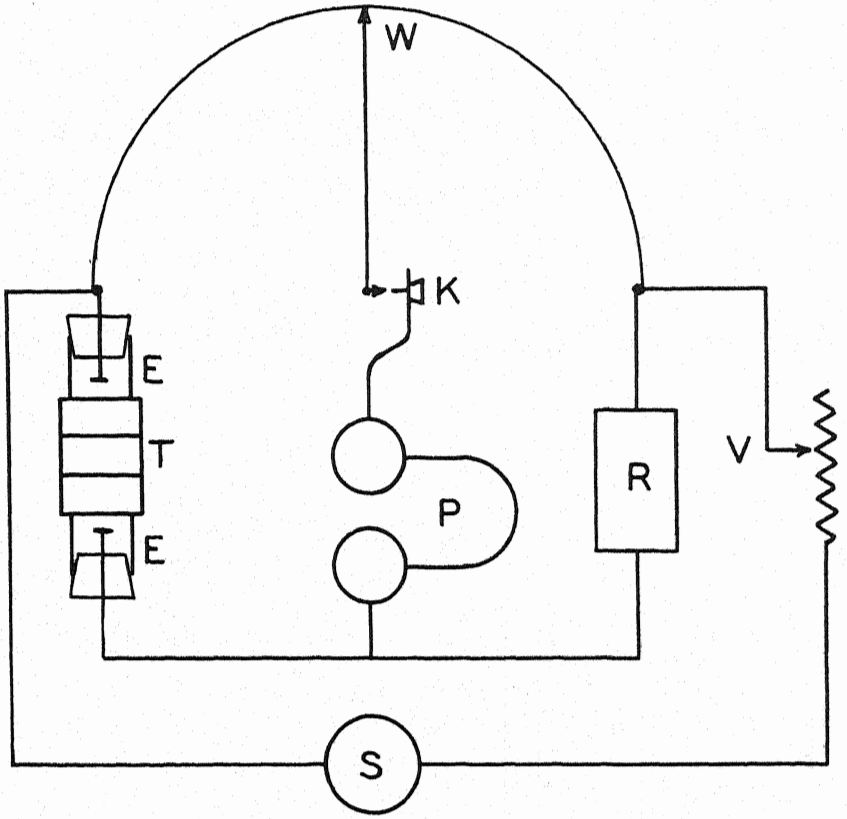


FIGURE 1. Diagram of apparatus for measuring the conductivity of potato tissue. T, tissue; E, KCl electrodes; W, slide wire bridge; K, key; P, phones; R, known resistance; V, variable resistance; S, source.

possible. The rheostat V was now adjusted to minimum sound and the bridge adjusted to the null point. The tissue was repeatedly washed with a stream of water from a wash bottle and readings taken until constant values were obtained.

In the procedure used for studying the leaching of electrolytes from the tissue, cylinders were cut from the tubers with a 14 mm. cork borer and then cut crosswise with the same cork borer. Ten pieces obtained in this

manner were washed three times with water, allowed to stand in water for five minutes, and washed again. They were then dried with cheesecloth and

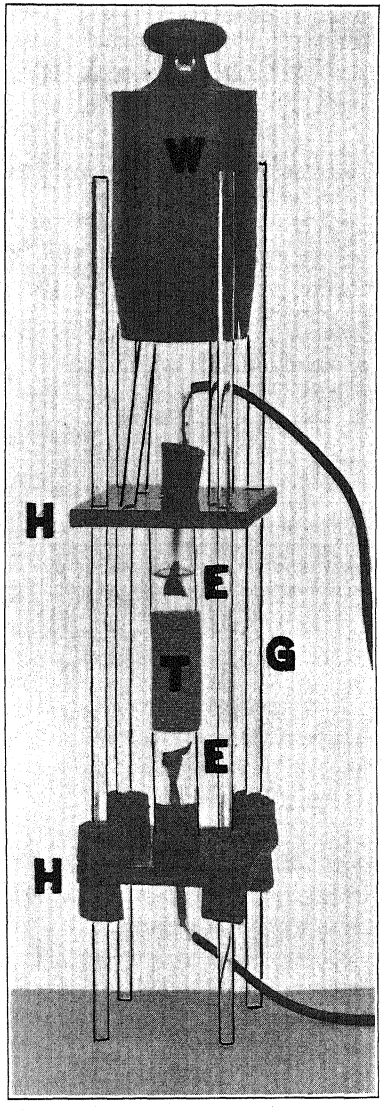


FIGURE 2. Apparatus for holding electrodes and tissue for measurement of conductivity. T, tissue; E, KCl electrodes; W, kilogram weight; G, glass rods; H, hard rubber plates.

placed in test tubes with 25 cc. of water, stoppered with corks, and rotated for 4 hours. The solution was then poured off and its conductivity determined in a Freas cell.

RESULTS

Conductivity of the Tissue

In order to estimate the individual variability of the tubers and to find the magnitude of the differences to be expected, a series of ethylene chlorhydrin vapor treatments were made and conductivity determined on the tissue of individual tubers. The results are shown in Table I. The difference

TABLE I

PRELIMINARY EXPERIMENT WITH ETHYLENE CHLORHYDRIN, SHOWING THE VARIATION OF INDIVIDUAL TUBERS

	Amount of ethylene chlorhydrin per liter*			
	1 cc.	½ cc.	¼ cc.	Check
Conductivity of tissue in reciprocal ohms X 10,000, determined 3 days after treatment on individual tubers	2.11 2.08 1.54 1.86 1.70 1.96	2.04 1.78 2.28 1.63 1.63 1.78	1.56 1.70 1.80 1.61 1.72 2.03	1.77 1.64 1.82 1.67 1.14 1.45
Average	1.88	1.86	1.75	1.58
Standard deviation of single determination	±0.22	±0.26	±0.17	±0.26
Standard deviation of the mean	±0.09	±0.11	±0.07	±0.11
Weight of tops after 9 weeks, g.	69	43	9	7

* 24 hour vapor treatments of variety Irish Cobbler.

between the 1 cc. per liter treatment and the check is 0.30. Calculated according to Fisher's adaptation of "Students" method (2, p. 107) for the comparison of the means of small samples, the odds that this difference is significant are about 20 to 1. The odds, that the differences between the check and the 1/2 cc. and 1/4 cc. per liter treatments are significant, are 10 to 1 and 5 to 1 respectively. In subsequent experiments it was decided to use three pieces from individual tubers in each determination. Usually three such determinations were made on each lot, thus obtaining the average of nine tubers. In the experiments reported in Table I, the potatoes were stored in paper sacks until the determinations were made. In subsequent experiments with ethylene chlorhydrin vapor treatments, the whole tubers were planted in moist soil until the determinations were made. This evidently reduced the variability, since the standard deviations calculated from the replicate determinations were smaller in the following experiments than those reported in Table I.

The results of additional whole tuber vapor treatments are shown in Table II. In most of these experiments three determinations were made on each lot, each determination being made on three pieces taken from different tubers. The values in the table therefore represent the average of

TABLE II

EFFECT OF ETHYLENE CHLORHYDRIN TREATMENTS OF DORMANT POTATO TUBERS ON THE CONDUCTIVITY OF THE TISSUE

24 hour vapor treatments	Conductivity of tissue in reciprocal ohms × 10,000										Weight of tops, g.†			
	Irish Cobbler						Bliss Triumph							
	Series 2			Series 3			Series 4		Series 5					
	Days after treatment*			Days after treatment*			Days after treatment*		Days after treatment*		Series			
	0	1	6	1	3	6	0	5**	0	2	2	3	4	5
1 cc. per liter	1.72	2.19	1.67	1.57	1.87	1.52	2.10	1.88	1.92	1.93	188	117	45	124
1/3 cc. per liter	1.71	1.79	1.57	1.61	1.67	1.49	2.04	1.96	1.95	1.93	157	89	0	112
1/9 cc. per liter	1.66	1.85	1.69	1.51	1.48	1.50	2.02	2.06	2.04	1.77	61	22	0	29
Check	1.68	1.67	1.53	1.52	1.50	1.47	2.12	1.81	1.85	1.70	25	2	0	0

* Time measured from end of 24 hr. treatment.

** Conductivity values in these columns represent averages of 2 determinations. All other values represent averages of 3 determinations.

† Series 2, 3, 4, and 5 were weighed after 6, 7, 7, and 4 weeks respectively.

nine tubers, with the exception of two series of determinations in which only six tubers were used. From these duplicate and triplicate determinations it was possible to calculate the standard deviation of a single determination by the formula $\sigma = \sqrt{\frac{\sum d^2}{(N-1)(M-1)}}$, where d is the deviation of each determination from the mean, M is the number of determinations in each replicate, and N is the number of replicates. It was found that σ for a single determination representing three tubers was 0.091 for the Irish Cobbler potatoes and 0.076 for the Bliss Triumph potatoes. The differences necessary to give odds of 100 to 1 were therefore found to be 0.19 for the Irish Cobbler potatoes and 0.16 for the Bliss Triumph potatoes for the values representing the average of three determinations. Using these considerations to interpret the data in Table II, it will be noted that the 1 cc. per liter treatment in the case of the Irish Cobbler tubers produced significant increases in conductivity which appeared one to three days after treatment and tended to disappear about the sixth day after treatment. The 1/3 cc. per liter treatment also showed increases in conductivity which were smaller than obtained with the 1 cc. per liter treatment, but considered collectively can also be regarded as significant. In the case of the Bliss Triumph treatments the increases observed were much smaller than with the Irish Cobbler and can only be considered significant when considered collectively, all the treated samples being higher than the check when sampled two or five days after treatment.

As additional evidence of the effect of ethylene chlorhydrin treatments, dip treatments were made on Irish Cobbler potatoes. The results are shown in Table III. The standard deviation of a single determination for this

TABLE III

EFFECT OF ETHYLENE CHLORHYDRIN TREATMENTS OF DORMANT POTATO TUBERS ON THE CONDUCTIVITY OF THE TISSUE

24 hour dip treatments	Conductivity of tissue in reciprocal ohms × 10,000				Weight of tops after 8 weeks, g.	
	Irish Cobbler					
	Series 6		Series 7			
	Days after treatment		Days after treatment		Series	
	5	8*	1*	6	6	7
40 cc. per liter	1.64	1.78	1.84	1.75	382	238
20 cc. per liter	1.66	1.66	1.94	1.80	139	168
10 cc. per liter	1.54	1.73	1.68	1.73	93	94
Check—H_2O	1.52	1.63	1.64	1.65	15	20

* Conductivity of values in these columns are averages of 2 determinations. All other values are averages of 3 determinations.

series was found to be 0.076. In the sample taken only one day after treatment differences of 0.20 and 0.30 were observed for the 40 cc. per liter and 20 cc. per liter treatments respectively. Since two determinations were made the standard deviation of the difference is 0.076 and therefore the odds are over 100 to 1 in both cases that the differences are significant. It will be noted that the difference tends to become less at a later date.

The effect of potassium thiocyanate treatments are shown in Table IV. In this experiment the standard deviation of each determination representing three cut pieces was calculated to be 0.085 for the Irish Cobbler potatoes and 0.057 for the Bliss Triumph potatoes. For the Irish Cobblers the difference necessary to give odds of 100 to 1 is 0.22 where two determinations were made, 0.18 where three determinations were made, and 0.16 where four determinations were made. It will be noted that significant increases in conductivity were observed in all the 20 g. per liter treatments with the exception of the 7 day sample of Series 8. The increases observed for the 10 g. per liter treatments may also be regarded as significant when considered collectively. Although the 5 g. per liter treatment was effective in breaking dormancy, the conductivty change produced was very small. The increases obtained with the Bliss Triumph tubers were much smaller and only the 20 g. per liter treatment produced a significant increase in conductivity.

The effect of thiourea treatments is shown in Table V. The standard deviation of a single determination calculated from replicate determinations was found to be 0.082 for the Irish Cobbler tubers and 0.059 for the Bliss Triumph tubers. For the Irish Cobbler potatoes the difference necessary to give odds of 100 to 1 is 0.21 where two determinations were made, 0.17 where three determinations were made, and 0.15 where four determinations were made. Contrary to the results obtained with ethylene chlorhydrin and potassium thiocyanate treatments of Irish Cobbler tubers, where an increase in conductivity resulted from the treatments, there is little evidence of such a change with thiourea. In fact there is some evidence that a decrease in conductivity occurs six days after treatment, since if the 6 day samples of Series 12 and 13 are considered together, the decrease observed is statistically significant. Samples taken at other times show slight increases in conductivity that are of doubtful significance as are also the differences observed in the Bliss Triumph Series. In any case the conductivity changes produced by thiourea are very small and cannot be correlated in any way with the subsequent growth of the tubers.

Leaching of Electrolytes from the Tissue

The effect of ethylene chlorhydrin treatments on the leaching of electrolytes from tissues is shown in Table VI. From the duplicate determinations made in these experiments with ethylene chlorhydrin and the follow-

TABLE IV

EFFECT OF POTASSIUM THIOCYANATE TREATMENTS OF DORMANT POTATO TUBERS ON THE CONDUCTIVITY OF THE TISSUE

1 hour soak treatments	Conductivity of tissue in reciprocal ohms × 10,000										Weight of tops, g.†			
	Irish Cobbler							Bliss Triumph						
	Series 8		Series 9			Series 10		Series 11			Series			
	Days after treatment		Days after treatment			Days after treatment		Days after treatment						
	4	7	3*	6**	9	3	5	2**	4**	7**	8	9	10	11
20 g. per liter	1.73	1.56	2.05	1.89	1.84	1.66	1.77	1.90	1.81	1.72	112	275	193	185
10 g. per liter	1.57	1.56	1.97	1.63	1.63	1.62	1.68	1.71	1.72	1.74	79	413	179	270
5 g. per liter	1.58	1.59	1.88	1.56	1.56	1.63	1.60	1.76	1.83	1.53	66	187	130	199
Check—H_2O	1.48	1.52	1.78	1.59	1.56	1.49	1.57	1.78	1.73	1.61	8	13	10	80

* Conductivity values in these columns are averages of 4 determinations.
** Conductivity values in these columns are averages of 2 determinations.
† Series 8, 9, 10, and 11 were weighed after 9, 7, 6, and 7 weeks respectively.

TABLE V

EFFECT OF THIOUREA TREATMENTS OF DORMANT POTATO TUBERS ON THE CONDUCTIVITY OF THE TISSUE

1 hour soak treatments	Conductivity of tissue in reciprocal ohms × 10,000									Weight of tops, g.†			
	Irish Cobbler						BlissTriumph						
	Series 12		Series 13			Series 14	Series 15						
	Days after treatment		Days after treatment			Days after treatment	Days after treatment			Series			
	6	8	3*	6*	9*	7	1	3	6**	12	13	14	15
20 g. per liter	1.40	1.54	1.93	1.63	1.62	1.55	1.85	1.84	1.68	213	234	197	121
10 g. per liter	1.43	1.54	1.77	1.71	1.58	1.63	1.83	1.76	1.70	106	160	283	92
5 g. per liter	1.52	1.47	1.85	1.55	1.55	1.62	1.88	1.71	1.68	68	89	156	55
Check—H_2O	1.55	1.46	1.80	1.76	1.51	1.60	1.91	1.74	1.65	3	45	77	33

* Conductivity values in these columns are averages of 4 determinations.
** Conductivity values in these columns are averages of 2 determinations.
† Series 12, 13, 14, and 15 were weighed after 9, 7, 6, and 5 weeks respectively.

TABLE VI

EFFECT OF ETHYLENE CHLORHYDRIN TREATMENTS OF DORMANT POTATO TUBERS ON THE LEACHING OF ELECTROLYTES FROM THE TISSUE

24 hour vapor treatments	Specific conductivity of leachings × 10,000													
	Irish Cobbler									Bliss Triumph				
	Series 2*			Series 3*			Series 16*			Series 4*			Series 5*	
	Days after treatment**			Days after treatment**			Days after treatment **			Days after treatment**			Days after treatment**	
	0	1	6	0	1	3	0	2	7	0†	1†	5	0	2
1 cc. per liter	2.34	3.00	2.48	2.02	1.98	2.14	2.34	2.64	1.65	4.23	4.68	2.54	3.36	3.56
1/3 cc. per liter	2.08	2.21	2.21	1.91	2.02	1.85	2.02	2.31	2.02	2.68	3.66	2.77	2.97	3.52
1/9 cc. per liter	1.98	2.14	2.44	1.65	1.88	1.85	2.18	1.91	1.58	3.82	3.40	2.61	3.00	3.28
Check	2.02	1.81	1.88	1.68	1.91	1.75	2.08	1.82	1.58	2.81	2.71	2.58	2.48	2.87

* Growth data for Series 2, 3, 4, and 5 are given in Table II. Series 16 weighed 291, 165, 67, and 47 g., respectively, after 8 weeks.

** Time measured from end of 24 hour treatment.

† Values in these columns represent 1 determination. All other values are the average of 2 determinations.

TABLE VII

EFFECT OF POTASSIUM THIOCYANATE AND THIOUREA TREATMENTS OF DORMANT POTATO TUBERS ON THE LEACHING OF ELECTROLYTES FROM THE TISSUE

1 hour soak treatments	Specific conductivity of leachings × 10,000											
	KSCN treatments					Thiourea treatments						
	Irish Cobbler			Bliss Triumph		Irish Cobbler				Bliss Triumph		
	Series 10*			Series 11*		Series 14*		Series 17*		Series 15*		
	Days after treatment			Days after treatment		Days after treatment		Days after treatment		Days after treatment		
	1	3	5	0	2	4	7	2	4	1**	3**	6
20 g. per liter	1.68	1.75	1.65	2.50	2.64	1.95	1.48	1.85	1.72	2.34	3.06	2.84
10 g. per liter	1.58	1.75	1.65	2.31	2.44	1.85	1.32	1.88	1.61	3.30	2.28	2.71
5 g. per liter	1.49	1.65	1.65	2.51	2.54	1.75	1.32	1.68	1.61	3.00	2.38	2.84
Check—H_2O	1.65	1.42	1.32	2.58	2.38	1.68	1.32	1.72	1.49	2.48	2.74	2.58

* Growth data for Series 10 and 11 are given in Table IV, for Series 14 and 15 in Table V. Series 17 weighed 211, 245, 258, and 160 g., respectively, after 8 weeks.

** Values in these columns represent 1 determination. All other values are the average of 2 determinations.

ing ones with potassium thiocyanate and thiourea, the standard deviation for a single determination was calculated to be 0.15 for the Irish Cobbler tubers and 0.31 for the Bliss Triumph tubers. Therefore the difference necessary to give odds of 100 to 1 where two determinations were made is 0.39 for the Irish Cobbler potatoes and 0.80 for the Bliss Triumph potatoes. Where only one determination was made the difference necessary for odds of 100 to 1 is 1.10 for the Bliss Triumph potatoes. Using these values to interpret the data in Table VI, it will be noted that ethylene chlorhydrin treatments produced significant changes in the leaching of electrolytes from the tissues. Even the 1/9 cc. per liter treatment in Series 2 produced a significant increase. An increase may be noted in the 1 cc. per liter treatments immediately after treatment. This increase becomes larger on the first or second day after treatment and then diminishes, being less in samples taken after five days or longer.

The effect of potassium thiocyanate and thiourea on the leaching of electrolytes from the tissue is shown in Table VII. The differences produced by these chemicals is smaller than that produced by ethylene chlorhydrin treatments. In the case of potassium thiocyanate treatments the data must be considered as a whole in order to show a significant difference. This small difference does not appear until after the first day from the time of treatment. The slight increases observed with thiourea are of doubtful significance, although the 20 g. per liter treatment probably produced an increase. However, the changes in the leaching of electrolytes from the tissue produced by potassium thiocyanate and thiourea are so small that it is unlikely that they are connected in any way with the breaking of dormancy.

SUMMARY

1. Ethylene chlorhydrin treatments of dormant potato tubers produced small, but significant increases in the conductivity of the tissue and increased the leaching of electrolytes from the tissue when placed in water.

2. Potassium thiocyanate treatments produced changes somewhat smaller, but similar to those produced by ethylene chlorhydrin.

3. The changes observed with thiourea treatments were very small and in most cases could not be considered significant.

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OXYGEN REQUIREMENTS OF NEUROSPORA SITOPHILA FOR FORMATION OF PERITHECIA AND GROWTH OF MYCELIUM

F. E. DENNY

Perithecia in *Neurospora sitophila* (Mont.) Shear & Dodge are not formed in single spore cultures but only when mycelia showing opposite sex-reaction are brought together under suitable conditions. A point at issue leading up to the present experiments was whether the mycelia of the two opposite strains must come into contact in order that perithecia may be produced. Moreau and Moruzi (2) inoculated one strain into one arm of a U-tube containing the nutrient substratum, and the complementary strain into the other arm. Perithecia were formed in one of the arms, but the authors were certain that the mycelium of the opposite strain had not grown around through the U-tube from the other arm, and, therefore, that perithecia had been formed without actual contact of the mycelia. According to their view, chemical substances of the nature of hormones, diffused from one strain through the agar, and, upon reaching the opposite strain, induced the production of perithecia.

In repeating these experiments Dodge (1) found that, in autoclaved corn meal agar in which the air content was low, the mycelium grew very slowly downward from the surface in each arm of the U-tube, that this downward advance of growth could continue only when the agar cracked away from the side of the tube; and that perithecia were formed in the U-tube at the place of meeting of the mycelia advancing from opposite sides. He concluded that the cracking away of the agar from the sides of the tube admitted air, and that this air was necessary both for the growth of the mycelium and for the union of the two strains to form perithecia; in other words, that perithecia are formed only when the mycelia of two strains of opposite sex-reaction meet in the presence of air.

The present study was undertaken at the suggestion of Dr. B. O. Dodge who furnished the cultures. The experiments had for their object the measurement of the oxygen concentrations needed for perithecial formation and for growth of mycelium of *Neurospora sitophila.*

METHODS

The strains of *Neurospora sitophila* used were, for the most part, Nos. 56–2 and 56–6, but in some experiments cultures of No. 56–3 were also included. Bits of the mycelium were inoculated into tubes of corn meal agar which were then stored in a cold room at 3° C. from about 30 minutes after inoculation until they were placed in the containers for adjusting the concentration of oxygen. In the later experiments for determining the oxygen requirements for the growth of mycelium the inoculated tubes were put

at once in water at 0° C. until they were placed in the experimental flasks.

In the preliminary experiments desiccators with volumes varying from 5000 to 7000 cc. were used, and different concentrations of oxygen within the containers were obtained by repeatedly evacuating them and filling them with nitrogen from a tank of the compressed gas, admitting sufficient air to the vessels after the final evacuation to produce the desired concentration. When this adjustment had been made the vessels were at approximately atmospheric pressure. In order to insure against leakage of gas the desiccators were immersed in water. Every three to four days the desiccators were evacuated and refilled with the appropriate amounts of nitrogen and air, this being done in order that the respiration of the cultures

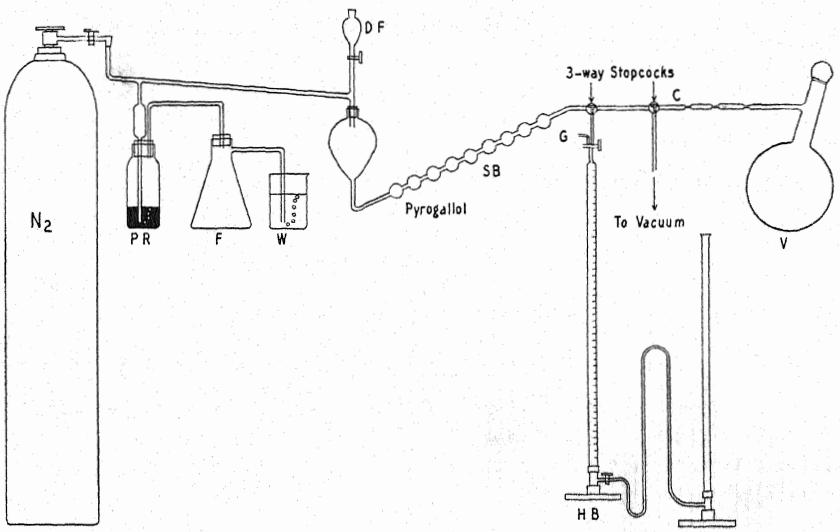


FIGURE 1. Apparatus for removing air from the flask, V, (which contained the cultures of the fungus), and replacing it with a measured amount of air, oxygen, or other gas from the burette, HB, and with nitrogen from the cylinder, N_2. Thus, the cultures were maintained at approximately atmospheric pressure with different proportions of gases.

would not reduce the oxygen concentration to values much below that at which it was desired to carry out the experiment.

The nitrogen gas in the commercial cylinder contained small amounts of oxygen and in order to experiment with low concentrations of oxygen the procedure was modified to permit scrubbing of the nitrogen gas and thus to remove this small amount of oxygen from it. Desiccators were discarded in favor of distilling flasks in order to take further precautions against leakage of air into the containers.

For the experiments at low oxygen concentrations the apparatus shown in Figure 1 was used. The nitrogen from the tank was passed first through pyrogallol before being admitted into the flask, V, containing the tubes of

fungi; a measured amount of air or oxygen was passed from the Hempel burette, HB, into the flask, V; by means of the three-way stopcocks the flask, V, was connected with the vacuum, nitrogen tank, or gas-measuring burette, and in this way the gas in the vessel, V, was removed and replaced with the desired proportion of nitrogen and oxygen; for very small amounts of oxygen, air was diluted with nitrogen in a separate round-bottom distilling flask, usually of three-liter capacity, and after connection was made with the Hempel burette at G, the desired amount of the diluted air was drawn into the burette, HB; a mercury pressure-regulator, PR, prevented excess pressure of gas upon the system, and the rate of bubbling in the water at W indicated when the vessel, V, had been filled with gas; DF is a dropping-funnel permitting pyrogallol to be dropped into the solution of KOH previously placed in the scrubbing-bulbs, SB, the alkaline pyrogallol thus being formed only in the absence of oxygen, or at least in the presence of only the small amounts that existed in the nitrogen gas.

Small test tubes 75 × 12 mm. were used for the culture of the fungi; corn meal agar slants were prepared in these tubes which were inoculated with bits of mycelium, placing side by side the implants of two strains of different sex-reaction if the experiment involved the formation of perithecia, and using inoculum from only one strain if merely the growth of mycelium was being tested. Figure 2 A shows one of these tubes at the end of an experiment with perithecia showing at the surface of the agar. In starting an experiment several of these tubes were dropped into a Pyrex distilling flask, as shown in Figure 2 B, the piece of rubber tubing on the end of the tube preventing breakage; the flask was closed by a rubber stopper which was sealed with Dekhotinsky cement; a piece of glass tubing, previously drawn out to a capillary at three places, was sealed into the side-arm of the distilling flask, using a small quantity of Dekhotinsky cement. The flask containing the tubes of fungi was connected with the apparatus in Figure 1 at C, either by means of heavy rubber tubing if the amount of oxygen used in the test was relatively large, or by sealing with Dekhotinsky cement if the experiment involved very low concentrations of oxygen.

An example of the general procedure is as follows: From the volume of the distilling flask containing the cultures of fungi the required amount of oxygen is computed, and this amount of oxygen or the amount of air needed to furnish this amount of oxygen, is measured into the burette, HB; the flask, V, is connected to the vacuum and evacuated for the length of time shown by previous tests to be required to produce approximately the lowest pressure of which the vacuum system is capable; the vessel, V, is then connected with the nitrogen tank and filled with nitrogen gas, proper adjustment of the rate of flow being obtained by the valve on the nitrogen tank and by one of the three-way stopcocks leading to the flask; failure of the bubbles of gas to rise in the scrubbing-bulbs, SB,

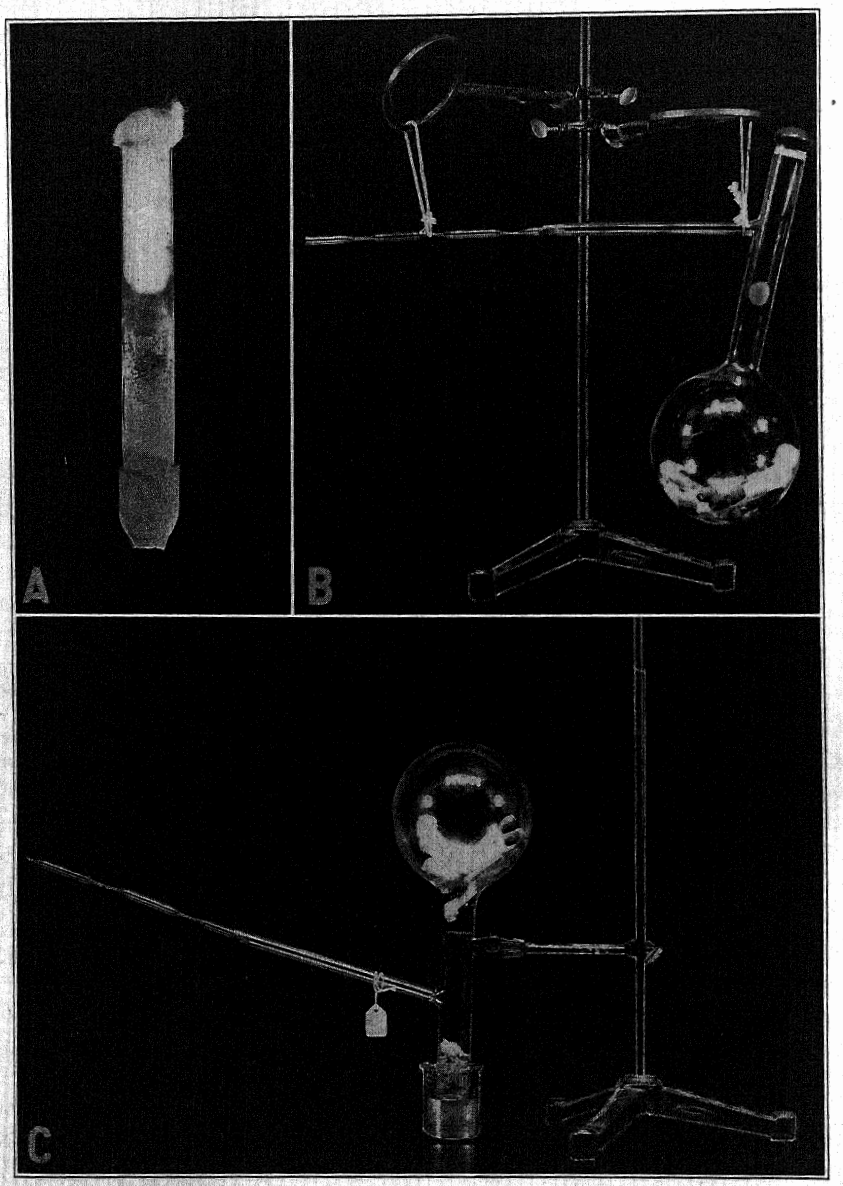


FIGURE 2. Fungi were inoculated into small test tubes, A, which were placed in the distilling flask as shown at B; after the flask was connected to the apparatus of Figure 1 and was filled with the desired amounts of oxygen and nitrogen, the capillary tube was sealed, and the flask was inverted over mercury as shown at C.

and rapid increase in the rate of bubbling at W shows when the flask, V, is filled with gas; the flask, V, is emptied and filled in this way several times, the number of times depending upon the amount of oxygen that is being experimented with, and requiring a larger number the lower the amount of oxygen; after the final evacuation the flask, V, is connected with the burette, HB, and the measured amount of oxygen, or air, is admitted; the connection is made with the nitrogen tank and nitrogen is admitted until the flask is full; the capillary tube nearest C is sealed with a flame; the flask is inverted over mercury as shown in Figure 2 C. The other capillary places in the glass tube in the arm of the distilling flask are used in subsequent renewals of the gas within the flask, these renewals being necessary to prevent depletion of the oxygen within the flask as a result of respiration of the fungi. In these experiments renewals were made two times each week.

RESULTS

OXYGEN REQUIREMENT FOR FORMATION OF PERITHECIA

In the preliminary experiment using large desiccators, and with the fungus cultures inoculated into 30 cc. test tubes with corn meal agar, perithecia were formed within one week at 20.8, 9, 6, and 2 per cent oxygen by volume, but none was formed in a desiccator which was filled with nitrogen and which contained a layer of alkaline pyrogallol. This experiment was repeated using oxygen concentrations of 1.8, 0.6, and 0.2 per cent by volume. At the end of ten days perithecia had formed at 1.8 per cent, but no perithecia were produced at 0.6 and 0.2 per cent O_2 even at the end of 30 days. The tubes not showing perithecia were then placed in air at room temperature and after five days perithecia were found in some but not all of the tubes. To those still not showing perithecia, fresh corn meal decoction was added, and five days later perithecia were found in nearly all of the tubes.

The experiments were then continued with the distilling flask containers such as shown in Figure 2. This facilitated the examination of the cultures from time to time in order to note the time of appearance of perithecia, as it had been noted in the preliminary experiments that at lower oxygen concentrations the perithecia required a longer time for formation. A test was carried out with oxygen concentrations of 2.4, 1.2, 0.6, 0.3, and 0.1 per cent using two-liter Pyrex flasks similar to that in Figure 2 B except that the corn meal agar was placed not in the small test tubes but directly in the bottom of the flask, the inoculations of the two strains of fungi being made upon the surface of the agar. The cotton plugs used in the sterilization process were pushed downward into the neck and side-arm of the distilling flasks after inoculation of the agar in order to permit the stopper and glass side-tube to be inserted; connection with the apparatus of Figure 1 was then made. At 2.4 per cent O_2 perithecia were visible in 7 days, at 1.2 per

cent O_2 in 12 days, and at 0.6 per cent O_2 in 16 days. After 11 days only slow growth of mycelium and no perithecia were obtained in 0.1 per cent O_2, and this flask was given an O_2 concentration of 2.4 per cent; perithecia were formed 7 days later. After 30 days since no perithecia were formed at 0.3 per cent O_2, air was admitted to this flask; perithecia were found in it four days later.

Using the procedure as described for Figures 1 and 2, oxygen concentrations in the series 20.8, 9.4, 3.75, 1.5, 0.6, and 0.24 per cent were tested. Perithecia were formed in the first four in this series after 4, 7, 9, and 12 days, respectively. At 0.6 per cent O_2, out of 18 small tubes in two different flasks 8 showed perithecia. No perithecia were formed at 0.24 per cent O_2 even after 30 days.

Effect of temperature. The above experiments were carried out at room temperature which varied from about 20° C. to about 25° C. Tests were made also at 10° C. and 15° C. using oxygen concentrations varying from 0.3 per cent to 20.8 per cent, and at 31° C. with the oxygen being varied from 10 to 80 per cent. No perithecia were formed within 30 days at 10° C. at any oxygen concentration. At 15° C. only 2 out of 16 tests resulted in perithecia, and these were at 1.2 per cent and 20.8 per cent, showing no definite relation between perithecial formation and oxygen concentration. At 31° C. perithecia were formed in nearly all the tests and in all the oxygen concentrations used; however, the perithecia formed less readily at 31° C. than at room temperature.

OXYGEN REQUIREMENT FOR GROWTH OF MYCELIUM

It was found that at 0.3 per cent O_2 (by volume) good growth of the mycelium was observable after 16 hours. When the oxygen concentration was reduced to 0.05 per cent or lower the rate of growth was much retarded; in such cases it was found advisable to use a hand lens to observe the growth in the tubes inside the flasks. At oxygen concentrations of the order of 0.01 per cent it was necessary to continue the test several days after which time it could be seen that a small amount of growth had taken place. In fact, a slow growth was usually observable after several days in the tubes in the flasks which received only nitrogen that had passed through the pyrogallol scrubber in Figure 1, the hand lens revealing the extension of branches of mycelium from the original spot of implantation a short distance into the agar. In order to prevent completely the spreading of the mycelium it was necessary to have a layer of alkaline pyrogallol continuously inside the container, and even in such cases growth was observable if the tubes were large test tubes (30 cc.) containing much agar, or if the vessel was a desiccator of large size (7000 cc.). Probably the oxygen absorbed in the agar and not removed by the vacuum pump is a factor; or perhaps sufficient oxygen passed through the pyrogallol scrubber to sup-

port a small amount of growth. At any rate the procedure used was not capable of making precise measurements at very low oxygen concentrations. More complete removal of oxygen from the nitrogen gas, and a more favorable medium than agar for releasing absorbed oxygen would be necessary to justify a continuance of the experiments on this phase of the subject.

Effect of carbon dioxide. Since there may be an accumulation of carbon dioxide in the agar tubes as a result of the respiration of the fungus, some tests were made on the effect of increasing amounts of CO_2 upon the growth of the fungus. The experiments were carried out with cultures at 0.3 per cent oxygen (which is approximately the lowest value at which good growth occurs) to which varying amounts of CO_2 from a cylinder of the compressed gas were added. A concentration of 10 per cent CO_2 by volume did not depress the growth of the mycelium to an observable amount, and at 50 per cent CO_2 the growth was only slightly retarded. It was considered possible that in the CO_2 from the tank a small amount of O_2 might have been present, and that the favorable effect of this small amount of O_2 may have overcome any retarding action that the CO_2 could exert. Consequently, in the next experiment the CO_2 gas was brought first into contact with "Oxsorbent," a commercial product that absorbs O_2 without absorbing CO_2. This oxygen-free CO_2 was then added to the flasks already containing 0.3 per cent O_2; the CO_2 concentrations tested were 2, 8, and 32 per cent by volume; no differences were observed in the rate of growth except that possibly there was a slight retardation at 32 per cent.

DISCUSSION

These experiments corroborate the view of Dodge (1) that the presence of oxygen is an important factor in the ability of the strains of *Neurospora sitophila* of opposite sex-reaction to unite to form perithecia. Failure to obtain perithecia in the U-tubes until the agar cracked away from the sides of the tube admitting air to mycelia of the two strains would be consistent with the results of the present experiments on the oxygen requirements for formation of perithecia.

Dodge (1) also found that the downward growth of the mycelium in the arm of the U-tube was very slow, and finally practically ceased until the agar cracked away from the side of the tube and admitted air. The present experiments help to explain the slow growth downward since it was found that as the oxygen concentration was reduced the rate of growth was much retarded. In the present experiments growth of mycelium could be stopped only by special precautions to exclude oxygen. Possibly in these experiments the conditions were more favorable for the absorption of larger amounts of oxygen in the agar, and this residual gas, which could not be removed by evacuation, was too high for complete stoppage of growth.

SUMMARY

1. The oxygen requirement of *Neurospora sitophila* for the formation of perithecia was measured. At room temperature the lowest oxygen concentration at which perithecia formed readily was about 1 to 2 per cent by volume. Reducing the oxygen below 0.5 per cent inhibited the formation of perithecia, at least during a period of 30 days.

2. At a temperature of 10° C., regardless of the amount of oxygen, no perithecia were formed within 30 days, and at 15° C. they were found only in an occasional culture. At 31° C. perithecia were formed somewhat less readily than at room temperature.

3. The mycelium grew rapidly at oxygen concentrations of 0.3 per cent or higher. As the oxygen content was lowered the rate of growth was retarded until in atmospheres very low in oxygen, 0.01 per cent or lower, spreading of mycelium from the place of inoculation could be observed only after several days. However, inhibition of growth was obtained only when the cultures were held in an atmosphere in which a layer of alkaline pyrogallol was maintained continuously.

4. Carbon dioxide was not effective in preventing growth of mycelium; at 0.3 per cent oxygen, even 32 per cent carbon dioxide retarded growth only slightly.

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ELIMINATING THE USE OF CALCIUM CARBONATE IN PREPARING PLANT TISSUE FOR ANALYSIS

F. E. DENNY

Since many plant tissues which contain sucrose have an acid reaction, and since sucrose is hydrolyzed by acids, the practice of adding calcium carbonate to the samples of tissue in order to neutralize the acids and prevent the inversion of sucrose has become almost universal.

It is customary, also, to drop the tissue for analysis into boiling alcohol in order to inactivate the enzymes, and to separate the soluble portion from the insoluble by the use of alcohol at about 70 to 80 per cent.

It would be desirable, if possible, to avoid the use of calcium carbonate, since its presence in the sample interferes with the analysis of the insoluble residue, requiring that an allowance, possibly inexact, be made in weighing out samples of the powdered tissue, and making very difficult or impossible the examination of this residue for ash constituents.

Experiments carried out between November 1931 and June 1932 using 13 different species of plants, including such types of tissue as leaf, tuber, root, and fruit, showed that for these tissues of these species the addition of calcium carbonate was not necessary, and that the sucrose values were the same when the analyses were carried out in the presence and in the absence of it.

Tests were made of the capacity of the organic acids, citric, malic, oxalic, and tartaric, at different pH values, to invert sucrose in alcoholic solution. From these results, the conditions necessary to avoid hydrolysis of sucrose in the extraction of tissues with alcohol were found. Experiments with inulin indicated that its behavior toward organic acids in alcoholic solution was similar to that of sucrose.

SPECIES USED

The following plants were used in these experiments: apple, *Pyrus malus* L.; apricot, *Prunus armeniaca* L.; bean, *Phaseolus vulgaris* L.; carrot, *Daucus carota* L. var. *sativa* DC; lilac, *Syringa vulgaris* L.; peach, *Prunus persica* (L.) Stokes.; pineapple, *Ananas comosus* Merr.; potato, *Solanum tuberosum* L.; rhubarb, *Rumex acetosa* L.; sugar beet, *Beta vulgaris* L.; sweet potato, *Ipomoea batatas* Lam.; tobacco, *Nicotiana tabacum* L.; tomato, *Lycopersicon esculentum* Mill.

RESULTS

EFFECT OF ADDITION OF CALCIUM CARBONATE UPON ANALYSES OF PLANT TISSUES FOR SUCROSE

At intervals between November 1931 and June 1932 tests were made

with various kinds of plant tissue as to the differences obtainable in sucrose analyses when samples were prepared with and without the addition of calcium carbonate. The results are shown in Table I. The tissues were chopped into small pieces with a knife or scissors, were mixed thoroughly, portions were weighed out and were dropped into boiling alcohol of the proper volume to cover the tissue and to give a final concentration of 70 per cent alcohol. In the calcium carbonate series 2 grams of $CaCO_3$ were added to the alcohol before the tissue was dropped into it. As shown in Table I two methods of extraction were used. In the first experiments, the sugars were extracted by decanting the alcoholic extract, and again boiling the residue with another quantity of 70 per cent alcohol; after cooling, this

TABLE I

SUCROSE ANALYSES OF TISSUE IN THE PRESENCE AND ABSENCE OF CALCIUM CARBONATE

Extracted by decantation			Extracted in Soxhlet apparatus			
Tissue	Sucrose, % of fresh wt.		Tissue	pH of press-juice	Sucrose, % of fresh wt.	
	Without $CaCO_3$	With $CaCO_3$			Without $CaCO_3$	With $CaCO_3$
Bean, leaves*	0.27	0.29	Pineapple, fruit	3.09	5.63	5.51
Bean, leaves**	0.13	0.11	Pineapple, fruit	3.60	4.50	4.57
Bean, leaves**	0.13	0.14	Pineapple, fruit	3.60	4.91	4.71
Tobacco, leaves	0.20	0.21	Apricot, fruit	3.85	2.66	2.78
Sugar beet, leaves	0.17	0.14	Apricot, fruit	3.43	2.77	2.82
Sugar beet, leaves	0.11	0.09	Peach, fruit	3.43	4.85	4.24
Lilac, leaves	1.55	1.51	Carrot, roots	5.97	3.48	3.62
Tomato, leaves	0.22	0.21	Carrot, roots	6.10	5.43	5.21
Potato, tubers	0.82	0.89	Carrot, roots	6.10	5.40	5.37
Apple, fruits	2.35	2.55	Sweet potato, roots	—	5.12	5.08
Sweet potato, roots	4.27	4.14	Potato, tubers	6.30	1.30	1.32
			Rhubarb, petioles	2.75	0.11	0.14
			Rhubarb, leaves	3.26	0.15	0.16

* Var. Cutshort. ** Var. Black Valentine.

extract was decanted and another quantity of 70 per cent alcohol was added; the number of successive extractions varied from seven to ten, depending upon the rate of leaching as shown by the color of the extract. The values by this method are shown in Table I under the heading "Extracted by decantation." The other method employed for extraction consisted in the use of the Soxhlet apparatus; after the tissues had been dropped into alcohol the alcoholic extract was filtered through paper extraction thimbles, the filtrate being caught in an Erlenmeyer flask; using 70 per cent alcohol the tissue was transferred from the beaker quantitatively to the extraction thimble; the tissue in the thimble was covered with cotton, and the Erlenmeyer flask, fitted with a Soxhlet refluxing apparatus, was placed on an electric hot-plate and the volume of alcohol was adjusted

with 70 per cent alcohol to provide a favorable volume for refluxing. The duration of heating in the Soxhlet apparatus varied in different tests from four to six hours. Results by this method are given in Table I under the heading "Extracted in Soxhlet apparatus." After the alcohol had been removed by evaporation on a water-bath, the aqueous extracts, after they had been cleared by neutral lead acetate and deleaded with potassium oxalate, were analyzed for sucrose, using cold HCl for inversion (3, p. 75) and the permanganate method for determining the cuprous oxide formed upon heating with Fehling's solution (3, p. 78, 80).

The sucrose values with and without $CaCO_3$ are shown in Table I. If the acids in the absence of $CaCO_3$ had produced a measurable hydrolysis of sucrose the values should be lower in the column "without $CaCO_3$"; it is seen, however, that the paired values in the two columns differ from each other only slightly, and that these small differences are not consistent in either direction.

Previously, in experiments with sweet corn, Appleman and Arthur (1), although adding calcium carbonate as a precautionary measure, found that "in the case of sweet corn this is not as important as in the case of many other plant tissues" (1, p. 139). And in a paper which appeared after these experiments had been completed, Archbold (2) reported that the same sucrose values were obtained with apple tissue whether or not $CaCO_3$ was used. The present experiments indicate that this situation is perhaps a general one, and that the necessity of adding $CaCO_3$ to any tissue for the purpose of preventing hydrolysis would have to be proved by experiment.

FAILURE OF $CaCO_3$ TO NEUTRALIZE THE PLANT ACIDS IN ALCOHOLIC SOLUTION

The results in Table I show that the addition of $CaCO_3$ to the tissues had little effect on the sucrose values. Since, as shown in column 5, Table I, many of the tissues were quite acid the question arises why the $CaCO_3$ failed to influence the sucrose values. A partial or perhaps complete explanation is given in Table II which shows the effect of $CaCO_3$ upon the pH of plant juices and organic acids in alcoholic solutions. Samples of 25 cc. of each of the juices were added to boiling alcohol (70 per cent final concentration), in one series with 2 grams of $CaCO_3$ present, and in the other series without $CaCO_3$. Both lots were filtered and the alcohol was evaporated on the steam bath; the residue was taken up with H_2O to the original volume. The pH values are shown in Table II. Addition of $CaCO_3$ did not neutralize the acids under the conditions of the experiment; in fact the pH values were shifted in the alkaline direction by only approximately 0.25 of a pH unit. Apparently, in the tests with the plant tissues this small change in pH did not materially affect the sucrose values.

Table II also shows the results of a similar test in which citric and

malic acid solutions were tested by the same procedure as that described above for plant juices. At pH 3.0 and 4.0 only small changes in the pH values resulted from adding $CaCO_3$. At pH 5.0 the change was marked, but this fact would not be important in connection with these experiments, since it is shown in subsequent paragraphs that at this value the acidity is not sufficient to cause hydrolysis of sucrose in alcoholic solution.

TABLE II

CHANGE IN PH WHEN $CaCO_3$ WAS ADDED TO PLANT JUICES OR ORGANIC ACIDS IN ALCOHOLIC SOLUTION

Solutions used	pH values	
	Without $CaCO_3$	With $CaCO_3$
Orange juice, fruit	3.51	3.58
Pineapple juice, fruit	3.46	3.56
Peach juice, fruit	3.26	3.60
Apricot juice, fruit	3.90	4.37
Rhubarb, petiole	3.04	3.23
Citric acid, 0.2 M	3.09	3.23
Citric acid, 0.2 M	4.10	4.44
Citric acid, 0.2 M	5.12	6.73
Malic acid, 0.2 M	3.09	3.29
Malic acid, 0.2 M	4.02	4.14
Malic acid, 0.2 M	5.12	7.62

These results are confirmatory of those previously reported by Webster (5) who found that samples of plant tissues (the names of which are not given in the article) prepared by adding an excess of $CaCO_3$ in alcohol were not made neutral by this procedure, but were in all cases acid, and required varying amounts of alkali for neutralization. Similarly in the recent article by Archbold (2, p. 411) addition of $CaCO_3$ to apple tissue "only partially neutralized the acid in alcohol solution."

EFFECT OF ORGANIC ACIDS ON THE HYDROLYSIS OF SUCROSE IN ALCOHOLIC SOLUTION

The results described in the previous paragraphs show that the addition of $CaCO_3$ had little effect upon the sucrose values and they indicate that the cause of this lack of influence is related to a failure of the $CaCO_3$ to neutralize the plant acids in alcoholic solution. But these results do not show that no hydrolysis of sucrose took place. Calcium carbonate did not neutralize the acids, but whether this acidity of the tissues was sufficient to invert the sucrose even if not neutralized represents another problem. Since the acidity of plant tissues is due mainly to organic acids, usually citric and malic and to a less extent oxalic and tartaric, it seemed that measurements should be made of the capacity of these organic acids at different pH values to invert sucrose.

In testing the effect of organic acids in inverting sucrose, the experiments were arranged to produce conditions similar to those that are obtained in analyzing plant tissue. The mixed organic acid and sucrose solution was 0.2 M with respect to acid, and contained 5 grams of sucrose per 100 cc. This mixture was added to the proper amount of 95 per cent ethyl alcohol to give a concentration of 70 per cent alcohol by volume. Oxalic and tartaric acid solutions produced precipitates when added to alcohol, and these were filtered through Soxhlet extraction thimbles, the alcholic extract being collected in 300 cc. Erlenmeyer flasks; the Soxhlet extraction procedure similar to that customary in plant tissue analyses was carried out for the periods of one and four hours. At the end of the period of "extraction" the alcoholic solutions were neutralized, and were placed on the steam bath until the alcohol had evaporated; the aqueous solution was then cleared with neutral lead acetate, was deleaded with potassium oxalate, and aliquots were taken for the sugar determination. The results at the different pH values were corrected by deducting the blank values obtained by carrying out the same procedure using samples of the same organic acid solutions which were neutralized to phenolphthalein before the Soxhlet procedure was begun.

TABLE III

HYDROLYSIS OF SUCROSE BY ORGANIC ACIDS IN 70 PER CENT ALCOHOLIC SOLUTION

Acid	Per cent of the sucrose hydrolyzed by refluxing in a Soxhlet apparatus					
	Refluxed 4 hours			Refluxed 1 hour		
	pH 3.0	pH 4.0	pH 5.0	pH 3.0	pH 4.0	pH 5.0
Oxalic	81.8	26.7	0.8	30.8	1.8	0.0
Citric	33.5	2.4	0.0	3.6	0.2	0.0
Tartaric	18.4	2.7	0.3	3.3	0.2	0.0
Malic	12.0	0.7	0.2	2.6	0.3	0.0
	Per cent of the sucrose hydrolyzed by adding sucrose-acid mixture to boiling alcohol					
	Boiled 4 times			Boiled once		
Oxalic	8.9	0.0	0.0	1.3	0.0	0.0
Citric	0.0	0.0	0.0	0.0	0.0	0.0
Tartaric	0.0	0.0	0.0	0.0	0.0	0.0
Malic	0.0	0.0	0.0	0.0	0.0	0.0

The results are shown in the upper half of Table III. The amount of hydrolysis was found to vary with the acid used, the pH value, and the duration of heating. At pH 3.0 and with four hours' heating considerable hydrolysis resulted, but the values were much lower at pH 4.0, and at pH

5.0 no inversion, or at most only a small amount, took place. When the time of heating was reduced to one hour no inversion was obtained at pH 5.0, and the values for citric, tartaric, and malic were so low at pH 4.0 that it seems doubtful whether any hydrolysis at all occurred.

Short periods of heating. Since the time of heating seemed to be an important factor in the amount of hydrolysis, attention was turned to the effect of short periods of boiling in alcohol, such as occurs when tissue is prepared for analysis by dropping it into boiling alcohol, or by successively boiling and decanting. The effect of the different acids at pH 3.0, 4.0, and 5.0 upon sucrose is shown in the lower half of Table III. No hydrolysis occurred with citric, tartaric, or malic acids at any pH value, even when the mixtures of acid and sucrose in 70 per cent alcohol were brought to boiling temperature four times in succession and were cooled after each boiling period. There was measurable inversion only with oxalic acid at pH 3.0.

INVERSION OF SUCROSE BY STORAGE AT ROOM TEMPERATURE IN 70 PER CENT ALCOHOLIC SOLUTION

Acid-sucrose mixtures. Mixtures of organic acids at pH 3.0 and 4.0, at a concentration of 0.2 M, and containing sucrose in the proportion of 5 grams per 100 cc. of acid solution, were added to alcohol to give a final concentration of 70 per cent alcohol. These mixtures were allowed to stand at room temperature for different lengths of time; after the acids were neutralized the reducing sugars were estimated, and a computation was made showing the percentage of the sucrose which had undergone hydrolysis. The results are shown in Table IV. At pH 3.0 there was measurable inversion with oxalic acid especially after four days; a small proportion of the sucrose was inverted by tartaric and malic; but citric acid induced no hydrolysis. At pH 4.0 no hydrolysis was observed with citric, tartaric, or malic in 12 days, and only a small amount by oxalic.

TABLE IV

HYDROLYSIS OF SUCROSE BY ORGANIC ACIDS DURING STORAGE IN 70 PER CENT ALCOHOL

Acid used	Per cent hydrolyzed			
	At pH 3.0		At pH 4.0	
	1 day	4 days	4 days	12 days
Oxalic	0.5	1.7	0.0	1.0
Citric	0.0	0.0	0.0	0.0
Tartaric	0.2	0.6	0.0	0.0
Malic	0.2	0.7	0.0	0.0

Fruit juices. Samples of orange and pineapple juice, previously boiled to destroy enzyme action, were added to cold alcohol to give concentra-

tions of 70 per cent alcohol. The pH values of the juices were 3.65 for orange and 3.60 for pineapple. One sample of each lot was neutralized at the beginning of the experiment, and subsequently other samples were neutralized at the end of one, two, four, and eight weeks. Sucrose deter-

TABLE V

HYDROLYSIS OF SUCROSE IN PLANT JUICES DURING STORAGE IN 70 PER CENT ALCOHOL

Time of storage, weeks	Grams sucrose per 25 cc. of juice	
	Orange	Pineapple
Start	1.07	1.79
1	1.08	1.82
2	1.05	1.83
4	1.07	1.80
8	1.06	1.76

minations were made at the end of the experiment. If the acids of the juices hydrolyzed the sucrose during the storage period, the sucrose values should be high at the start, and should decrease progressively toward the end of the test. The results are shown in Table V from which it is seen that the sucrose values remained practically constant during the storage period of eight weeks.

TITRATION CURVES OF FRUIT JUICES AND OF DIFFERENT CONCENTRATIONS OF ORGANIC ACIDS

In these experiments the concentration of the organic acid solutions was 0.2 M. The question arises whether this concentration is comparable to that encountered in plant juices. In order to get information on this point titration curves were made of different acids at different concentrations, and these were compared with the titration curves of fruit juices. The organic acid solutions were adjusted by the addition of alkali to the same pH as that of the fruit juice, and the titration values were plotted on the same graph as that of the juices. Results of such a comparison are given in Figure 1 in which pH values are plotted against the number of cubic centimeters of 0.25 N NaOH added to 20 cc. of fruit juice or organic acid solution. Curves for orange, pineapple, and peach juice are shown in black lines through the black dots, Figure 1. The titration curves for the organic acids of different concentrations are shown by the symbols described in the legend in the center (right) of the graph. If the organic acid was more concentrated than the juice the symbols for its curve fell below the juice curve, but if the organic acid was too dilute its values appear above the line.

It is seen that 0.1 M citric acid gives a titration curve resembling that of orange juice, at least up to pH 5.0; a slightly lower concentration of

citric would have given values closer to the curve. Pineapple juice and 0.05 M citric have almost identical titration curves. Peach juice and 0.1 M malic acid show curves which are similar.

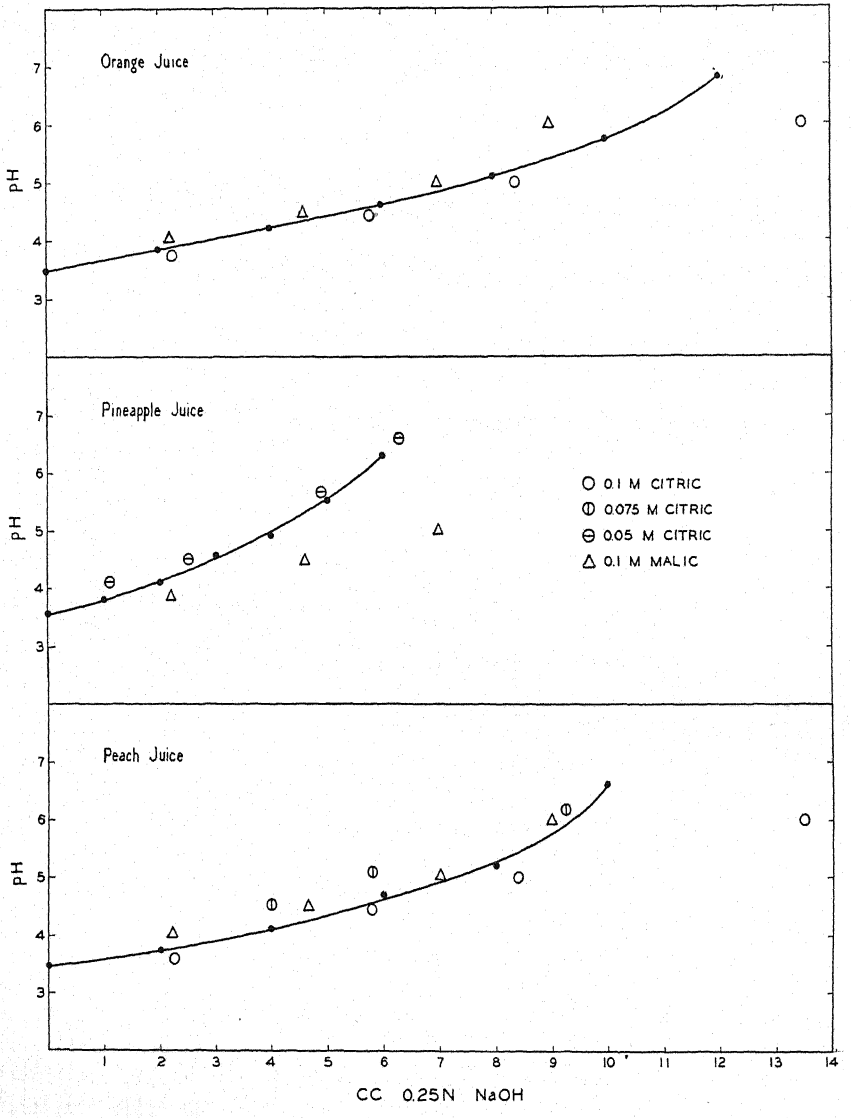


FIGURE 1. Titration curves of orange, pineapple, and peach juices compared with those of organic acid solutions of different concentrations.

No claim is made that the similarity of the curves proves that the buffer system is due to the organic acid used in the comparison. Probably several

substances take part in furnishing the buffer capacity of the juices. But it is believed that the curves offer evidence that the concentration used in the experiments, 0.2 M, was sufficiently similar to those that exist in the tissues to justify its use in testing the effect of organic acids upon the inversion of sucrose.

It will be noted that in preparing the curves only citric and malic were used. There were two reasons for this. In the first place it was found difficult to prepare solutions of tartaric and oxalic that fitted the curves. These two acids have little buffer action between pH 5.0 and 7.0, the curves rising abruptly after passing 5.0. In the second place these acids according to Nelson (4) are not commonly found in fruits, and when present usually occur only in small amounts. This is especially true of oxalic acid which even in rhubarb accounts for only a small proportion of the acidity. Nelson's list shows that citric and malic are by far the most common acid constituents of fruits.

PROCEDURE WITH VERY ACID TISSUES

The experiments with the organic acids showed that at pH 3.0 there was some danger of inverting sucrose especially if the period of heating was prolonged. Some tests were made to modify the procedure in order to reduce the danger of hydrolysis with very acid fruits. An important factor is found in the fact that the extract obtained by the dropping of the tissue into boiling alcohol and decanting contains about two-thirds or three-fourths of the sugar and acid. The residue which remains is then sufficiently low in acid to permit further processes of extraction without danger of hydrolysis. The results are shown in Table VI.

TABLE VI

AMOUNTS OF SUGAR AND ACID REMOVED FROM TISSUE DURING SUCCESSIVE PERIODS OF EXTRACTION

Fruit tissue	Procedure	Per cent of total amount removed	
		Sugar	Acid
Apricot	Tissue dropped into boiling alcohol, cooled, and decanted	78	71
	First hour of extraction with Soxhlet apparatus	18	16
	Second hour in Soxhlet	3	3
Pineapple	Boiled in alcohol and decanted	67	—
	Second boiling and decanting	13	—
	Third boiling and decanting	5	—
	Four hours further extraction in Soxhlet apparatus	15	—

Another possible means of avoiding hydrolysis of sucrose by very acid tissues consists in the use of cold rather than hot alcohol in the first step of the process. This would avoid the use of heat and still further reduce the danger of hydrolysis. In this case, however, we must accept the risk that the tissue may not be killed quickly enough and that enzyme action may continue to act for some time after the tissues are placed in the alcohol. A comparison was made between hot and cold alcohol for the first extraction. The tissue was dropped into alcohol, using cold alcohol for one lot and boiling alcohol for the other; the samples were allowed to stand, and were stirred occasionally during the first few hours; after 20 hours the extracts were decanted and two additional extractions with alcohol were made, in cold alcohol in one case and in hot alcohol in the other; finally the residue of each lot was extracted for four hours in the Soxhlet apparatus.

The extracts from the different tissues were analyzed for sucrose. The results with pineapple, peach, and apricot fruits are shown in Table VII.

TABLE VII

COMPARISON OF COLD AND HOT ALCOHOL FOR THE PRELIMINARY EXTRACTION

Tissue	Sucrose, per cent of fresh weight	
	Cold alcohol method	Hot alcohol method
Pineapple, fruit	7.79	8.01
Peach, fruit	4.85	4.90
Apricot, fruit	4.96	5.04

Slightly lower values were found by the cold alcohol method, but the differences are too small to show conclusively that they were caused by hydrolysis of sucrose. It would require further experiments to furnish a decision on this point, but the preliminary results seem favorable to the view that cold alcohol could be used for the preliminary extraction of the tissue.

COMPARISON OF SUCROSE AND INULIN AS TO EASE OF HYDROLYSIS

A few preliminary tests on the effect of organic acids upon inulin indicated that the pH values for hydrolysis were about the same for inulin as for sucrose, and values similar to those shown in Table III were obtained. Attention was turned to comparing inulin with sucrose as to the facility with which hydrolysis can be accomplished. The results are shown in Table VIII. Solutions of sucrose and inulin containing 0.3 grams per 100 cc. were prepared, being boiled first and cooled. To samples of 50 cc. of each solution were added 10 cc. of a solution of HCl containing different amounts of the concentrated acid as shown in column 1 of Table VIII. After the samples had stood 20 hours at room temperature they were neutralized and the

reducing sugars were estimated. The per cents of the sucrose or inulin hydrolyzed by the different amounts of HCl are shown in columns 2 and 3 in Table VIII. It is seen that the rates of hydrolysis under these conditions are about the same, inulin being hydrolyzed with slightly greater difficulty than sucrose.

TABLE VIII

INVERSION OF SUCROSE AND INULIN BY HYDROCHLORIC ACID

Cc. of conc. HCl in 60 cc. of solution	Per cent hydrolyzed	
	Sucrose	Inulin
8.1	100	100
2.7	100	100
0.9	92	91
0.3	58	53
0.1	27	16

DISCUSSION

The reader should keep in mind that the experiments were carried out in 70 per cent alcoholic solution. No claim is made regarding the effect of calcium carbonate when added to aqueous solutions containing organic acids.

Under the conditions of the experiments, oxalic acid was much more effective in hydrolyzing sucrose than any of the other acids. Consequently if it is known that the acidity of plant tissue taken for analysis is due mainly to oxalic acid, a situation which probably rarely occurs, the precautions suggested in the paragraphs under the heading "Procedure with very acid tissues" should be taken.

The experiments do not show conclusively that hydrolysis could not occur by long standing of very acid tissues in alcoholic solutions. In such cases the tissues can be extracted at once or soon after sampling, the alcoholic extract can be made up to volume and an aliquot taken for sugar analysis; if this aliquot is neutralized at once it could be stored probably for long periods without danger of inversion of sucrose.

In evaporating the alcohol the sugars pass gradually from alcoholic to aqueous solution in which the hydrolyzing powers of the organic acid may be greater. Danger from inversion during this change to aqueous solution can be avoided by neutralizing the alcoholic extract before the evaporation begins.

If a soluble solids determination is to be made, an aliquot can be taken from the alcoholic extract before the acidity is neutralized.

SUMMARY

1. The addition of calcium carbonate in the preparation of plant tissue for analysis has been assumed to be necessary in order to neutralize plant

acids and to prevent the hydrolysis of sucrose. Since the excess carbonate in the plant residues interferes with analyses for various constituents, a satisfactory procedure omitting calcium carbonate would be desirable.

2. In determining the sucrose content of the tissues of 13 different species of plants it was found that similar values were obtained whether or not calcium carbonate was added to the boiling alcohol into which the sample of tissue was dropped in preparing the tissue for analysis.

3. It was found that calcium carbonate did not neutralize the acidity of juices of acid fruits in alcoholic solution, the pH values being displaced only slightly in the alkaline direction. This behavior was also found with citric and malic acid solutions at pH 3.0 and pH 4.0.

4. The hydrolysis of sucrose by the organic acids, oxalic, citric, tartaric, and malic, in 70 per cent alcoholic solution was measured at different pH values and with different periods of heating. With one hour's heating in boiling 70 per cent alcohol, no hydrolysis occurred at pH 5.0, with any of the acids, and even at pH 4.0 inversion by citric, malic, and tartaric was slight, only oxalic being effective under these conditions. At pH 3.0 measurable hydrolysis was found, but when the duration of heating was reduced inversion was avoided with all of the acids except oxalic.

5. Conditions for the preparation of tissues for analysis and for their extraction in the absence of $CaCO_3$, without danger of inverting sucrose, were found.

6. Preliminary experiments with inulin indicated that its behavior towards acids was similar to that of sucrose, and that the danger of hydrolysis of inulin was not greater than that of sucrose.

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SOME FACTORS AFFECTING THE EFFICIENCY OF CONTACT INSECTICIDES. III. FURTHER CHEMICAL AND TOXICOLOGICAL STUDIES OF PYRETHRUM[1]

FRANK WILCOXON AND ALBERT HARTZELL

In recent years many publications have appeared dealing with the chemistry of pyrethrum and various questions connected with its use as an insecticide. Nevertheless there are still certain important points which require investigation. Among these may be mentioned the mode of action on insects, the relative toxicity of pyrethrin I and II, and the need of a simple method for obtaining the pyrethrins in pure form, for the purpose of studying their chemical properties and for standardizing biological tests on insects. The present paper presents a relatively simple method for obtaining small quantities of the pyrethrins freed from the impurities which accompany them in the flowers, together with a study of the penetration of the pyrethrins into the body of certain insects and their effect on nerve tissue.

PURIFICATION OF THE ACTIVE PRINCIPLES

Several investigators have described methods of obtaining the pyrethrins in substantially pure form. Staudinger and Ruzicka (10), who were the first to isolate them and determine their structure, extracted 100 kg. of the flowers with low boiling petroleum ether. The residue after removal of the petroleum ether was repeatedly extracted with methyl alcohol, and the methyl alcohol solutions were freed from resinous material by the addition of water and cooling in a freezing mixture. The aqueous methyl alcohol was removed by means of a vacuum pump, and the remaining material dissolved once more in low boiling petroleum ether. Finally after further purification an oil was obtained which contained about 50 per cent of pyrethrins. In order to obtain the pyrethrins in pure form, they were converted to semicarbazones and the semicarbazones carefully hydrolyzed to give the semicarbazone of the alcoholic part of the esters, pyrethrolone semicarbazone. From the latter pyrethrolone was obtained by shaking with potassium bisulphate solution for a month at a low temperature. The acids of pyrethrin I and II could be obtained from the solution after hydrolysis of the original pyrethrin semicarbazones. Having thus obtained the alcohol pyrethrolone and the two pyrethrin acids, the esters were prepared from them by synthetic methods.

It will be seen that this method is rather troublesome and indirect, since the esters are first decomposed and subsequently regenerated. This method was also used by Tattersfield, Hobson, and Gimingham (12), in

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 56.

their studies on the insecticidal value and analytical estimation of the pyrethrins, although they do not describe the process in detail. Gnadinger and Corl (2) have described the isolation of the pyrethrins, starting with 215 kg. of ground flowers. Their method differed from that of Staudinger in several respects, but particularly in that the semicarbazones of the esters were fractionally crystallized and relatively pure samples of the semicarbazones of pyrethrin I and II were obtained separately. These semicarbazones were converted to the pyrethrins by heating with oxalic acid solution.

In these methods large initial samples are necessary, great volumes of solvents are required, the pyrethrins are made to enter into chemical combination with semicarbazide and must be subsequently regenerated, with possible alteration of their original properties and the yields and final purity of the products are not all that might be desired. For these reasons an attempt was made to isolate the active principles by a purely physical method, not involving the use of semicarbazide, but depending on the differential solubility of the pyrethrins and accompanying impurities in low boiling petroleum ether and 80 per cent methyl alcohol.

Method of purification. The sample of flowers, about 500 g., was extracted with low boiling petroleum ether in a continuous extractor, and the solvent removed at low temperature under the water pump. The crude extract was dissolved in warm methyl alcohol and on cooling the solution for a few minutes, resins separated and were filtered off. The filtrate was treated with norite charcoal, which removed some of the pigments. The methyl alcohol was then removed under the water pump, yielding an oil which contained from 50 to 60 per cent of total pyrethrins. This oil was then submitted to a systematic fractionation between petroleum ether and 80 per cent methyl alcohol according to the scheme illustrated in Figure 1. Each circle in the diagram represents a separatory funnel containing petroleum ether and 80 per cent methyl alcohol. The original sample, after being shaken in funnel No. 1, separates into two layers, which are drawn off. The petroleum ether layer goes to funnel No. 2, where it is shaken with fresh 80 per cent methyl alcohol, while the methyl alcohol layer goes to funnel No. 3, where it is shaken with fresh petroleum ether, and this is continued as shown by the arrows in the diagram. It was found that the impurities tended to concentrate at the ends while the center fraction became richer in the pyrethrins. Since the solubility of the pyrethrins in petroleum ether is considerably greater than in 80 per cent methyl alcohol, about 50 cc. of the latter was used with 25 cc. of petroleum ether. The following experiment is typical of many performed:—

A sample of flowers containing 0.98 per cent of pyrethrin I and II was extracted and submitted to the preliminary purification yielding a product which contained 23.4 per cent of I and 38.3 per cent of II, total 61.7 per

cent. This product was then treated according to the scheme outlined above, and various fractions analyzed. Fraction 16 A and B contained 25.2 per cent I, and 61.9 per cent II, total 87.1 per cent. The analysis of some of the other fractions is shown below.

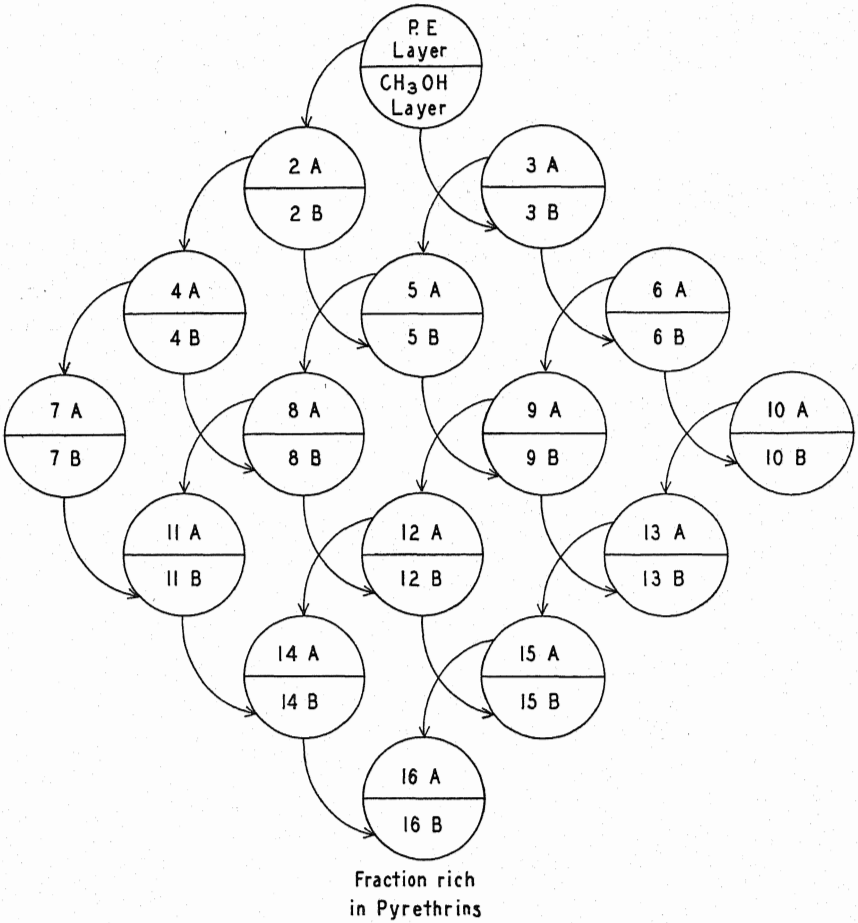


FIGURE 1. Scheme for purification of pyrethrins by use of immiscible solvents.

Fraction	Pyrethrin I	Pyrethrin II	Total
11A	36.9	35.5	72.4
14A	39.0	41.7	80.7
13B	14.3	47.2	61.5
15B	19.7	66.1	85.8

It may be seen that the pyrethrins tend to concentrate in the center of the diagram. The center fractions, 16 A and B, were then submitted to a further

fractionation following the same system as before, yielding a final product which gave on analysis 33.0 per cent of I, and 68.1 per cent of II, total 101.1 per cent, which indicates substantially 100 per cent purity as far as can be determined by the acid titration method.

Another sample which was obtained by this method gave on analysis 44.75 per cent I and 55.3 per cent II. A determination of carbon and hydrogen by microchemical combustion on this sample gave 73.43 per cent C, 8.95 per cent H, while the calculated values for this mixture are 73.4 per cent carbon and 8.49 per cent hydrogen.[2] From these results it appears that small amounts of mixtures of pyrethrin I and II may be obtained substantially free from other impurities.

ATTEMPTED SEPARATION OF PYRETHRIN I AND II

It was observed in the course of this work that the petroleum ether layers were invariably relatively richer in pyrethrin I, while the methyl alcohol layers were richer in pyrethrin II. It was hoped that a separation of the pyrethrins could be effected by a further fractionation of a sample already freed from impurities. This was not entirely successful, chiefly because of the small amount of material available to begin with. A partial separation was, however, effected and fractions obtained which differed widely in ratio of I to II. With these fractions toxicity tests were performed in the manner described previously (3), in order to determine the relative toxicity of the two esters to *Aphis rumicis* L. Authorities are not agreed on this question; Tattersfield has stated that pyrethrin I is many times as toxic as pyrethrin II, while Gnadinger and Corl, working with pyrethrin oil sprays on houseflies concluded that "The difference in toxicity between pyrethrin I and II cannot be great." The question is one of some importance since different samples of flowers may vary considerably in the ratio of I to II, but if the total pyrethrin content were the same those methods of analysis which determine only the sum of the two esters would indicate equal effectiveness. On the other hand if the toxicity of pyrethrin II were negligible compared to that of I, the samples might be evaluated on the basis of their content of pyrethrin I alone, thus shortening the time required for a determination.

RELATIVE TOXICITY OF PYRETHRIN I AND II

In the following experiments samples containing widely differing ratios of I to II were made up to the same total pyrethrin content in acetone. Portions of the acetone solutions were pipetted into water and toxicity

[2] These microcombustions were performed in the Experimental Research Laboratories of the Burroughs Wellcome Company, Tuckahoe, New York, and the authors wish to express their indebtedness to Dr. C. S. Leonard and members of his staff for their kind cooperation.

tests performed as described previously (3), using *Aphis rumicis* on nasturtium (*Tropaeolum minus* L.). The samples were compared in pairs, both members of a given pair being run at the same time. Most of the toxicity tests were performed in duplicate. The results are shown in Table I. It is clear that under these conditions pyrethrin I is much more toxic than pyrethrin II, in agreement with the results reported by Tattersfield.

TABLE I

COMPARATIVE TOXICITY TO APHIS RUMICIS OF PYRETHRUM EXTRACTS, VARYING IN THE RATIO OF PYRETHRIN I TO II

Per cent pyrethrin I	Per cent pyrethrin II	Per cent total pyrethrins	Per cent mortality	
23.1	28.5	51.6	55.5	
9.5	42.1	51.6	25.6	
21.6	24.4	46.0	62.5	53.0
8.3	41.6	49.9	32.0	26.0
43.6	57.1	100.7	22.8	23.5
14.7	76.5	91.2	9.5	10.3
50.6	44.4	95.0	51.1	49.4
2.8	92.6	95.4	17.0	9.1

COMPARISON OF PURIFIED EXTRACTS AND FLOWERS

A further use which may be made of the purified extracts described above lies in the comparison of sprays made from the extracts with sprays made from the flowers to see whether the toxicity of the flowers can be accounted for by their pyrethrin content as shown by analysis. For this purpose samples of analyzed flowers were extracted with petroleum ether, the solvent was removed at low temperature, and the residue dissolved in acetone. Portions of a purified preparation, the analytical results indicating 100.7 per cent total pyrethrins, were dissolved in acetone, and from these spray solutions were made up so that the sprays from the flowers and from the purified extract contained the same content of pyrethrin I. Toxicity experiments were then performed using *Aphis rumicis* on nasturtium in the usual manner. The results are shown in Table II. In every case the flowers gave a higher kill than the extract. The average difference, 13.5 per cent, while significant, is not very great, and it does not appear that any serious error is likely to arise if the flowers are rated on the content of pyrethrin I alone. Several possibilities suggest themselves as the cause of the higher toxicity of the flowers. It may be due to the toxicity of pyrethrin II, of which the content was somewhat higher in the flowers than in the extracts in two of the three cases, or there may be another substance in the flowers which has some slight toxicity in addition to the pyrethrins. Another possibility is that the impurities in the flowers serve as wetting

TABLE II

COMPARATIVE TOXICITY TO APHIS RUMICIS OF PURIFIED PREPARATIONS AND CRUDE EXTRACTS FROM THE FLOWERS, BOTH SAMPLES HAVING THE SAME CONTENT OF PYRETHRIN I

Experiment	Ratio of II to I in extract	Per cent mortality with extract	Ratio of II to I in flowers	Per cent mortality with flowers
1	1.31	64.4 57.5	2.76	72.8 72.7
2	1.24	43.5 42.0	1.88	55.3 53.3
3	2.06	51.5 50.8	1.68	68.6 68.2

agents or emulsifiers, thus making the spray more efficient when made from the flowers. The acid method of analysis has been criticized by Ripert (8) on the ground that acids other than those of the pyrethrins will be included in the final titration, leading to a too large value for the pyrethrin content. However this may be, there is no evidence of such a factor from the figures in Table II. The toxicity on the basis of pyrethrin I content would be slightly but consistently underestimated when compared with the purified esters according to our results.

MODE OF ACTION OF PYRETHRUM AND ITS EFFECTS ON INSECT TISSUES

In a previous publication the writers (14) have shown that aqueous spray solutions do not penetrate the tracheae of insects except when an efficient wetting agent such as soap is present. Such wetting agents markedly increase the effectiveness of nicotine sprays. In the case of pyrethrum sprays a wetting agent has also been found desirable. Nevertheless, with pyrethrum, killing can take place without actual penetration of the tracheae. This is shown by the fact that aqueous emulsions of pyrethrum are quite toxic even without a wetting agent, and also by the fact that concentrated pyrethrum preparations, when placed on portions of the insect far removed from the spiracles and body openings, were able to cause characteristic pyrethrum intoxication and death (3). In an attempt to explain this phenomenon the possible penetration of the material through the integument was studied by means of dyes dissolved in the pyrethrum concentrate. By using oil red, Sudan III, and "oildag," a colloidal graphite, evidence was obtained that pyrethrum could penetrate the integument of insects at least in certain regions.

Tenebrio molitor L. larvae were painted on the dorsal surface with pyrethrum extract, obtained as described under "Method of Purification," colored with oil red or with Sudan III, care being taken that none of the material came in contact with the spiracles. After the insects were dead,

their bodies were sectioned with a freezing microtome and examined immediately under the microscope for the presence of the dye. It was noted that the trichogen and hypodermal cells were stained red even in regions where no tracheae were present when Sudan III and oil red were used (Fig. 2, compare A and B). While it is conceded that the pyrethrins might penetrate to a greater depth than the stain, it would seem improbable that the reverse could be true. Since the setae are located in pores that extend down into the hypodermis it would appear that the pyrethrum extract had entered through the articular membranes into the trichopores and permeated the cells of the hypodermis. Similar membranes are found between the segments of the body and at the attachment of the appendages. These observations suggest that pyrethrum can gain entrance into the body by other means than penetration through the tracheae.

The tendency of pyrethrum preparations to enter through these passages was well illustrated by painting the abdominal portion of exuviae of *Cicada septendecium* L. with pyrethrum extract colored with oil red. It was found that the exuviae take the stain mainly along the conjunctivae while the sclerites were not stained (Fig. 3, B, C, D). When larvae of *Tenebrio molitor* were dipped into pyrethrum extract colored with "oildag" and sectioned with the freezing microtome it was possible to detect the carbon in the hypodermis of the conjunctivae (Fig. 3, A).

Although it has generally been considered that the pyrethrins are insoluble in water, experiments in which preparations of high purity were agitated with distilled water and subsequently carefully filtered, showed that the solubility while small is sufficient to produce a solution somewhat toxic to *Aphis rumicis* and to kill small tropical fish (*Lebistes reticulatus* Peters). It is believed that this is a case of solution rather than emulsification since the resultant mixture was clear and not opaque. It seems probable, therefore, that the pyrethrins would be soluble to a slight extent in the body fluids of insects and in this way be carried to the nerve ganglia where their toxic effects are manifested. We have, then, a plausible explanation for the fact that pyrethrum preparations, although of very low volatility and supposedly insoluble, can exert a toxic action when placed on portions of the integument far removed from the spiracles.

Juillet and others (4) consider that pyrethrum is a neuro-muscular poison and paralytic agent. Saling (9) has shown that the active principle of pyrethrum is a nerve poison as contrasted with a respiratory or blood poison. Krüger (6) has reported morphological changes in the hypodermis, muscles, and nerves of *Corethra* larvae that have been treated with suspensions of pyrethrum flowers in water. The ventral nerve ganglia of treated larvae, he states, showed vacuoles which were not present in the untreated larvae.

In order to study the effects of pyrethrum on the nervous system of

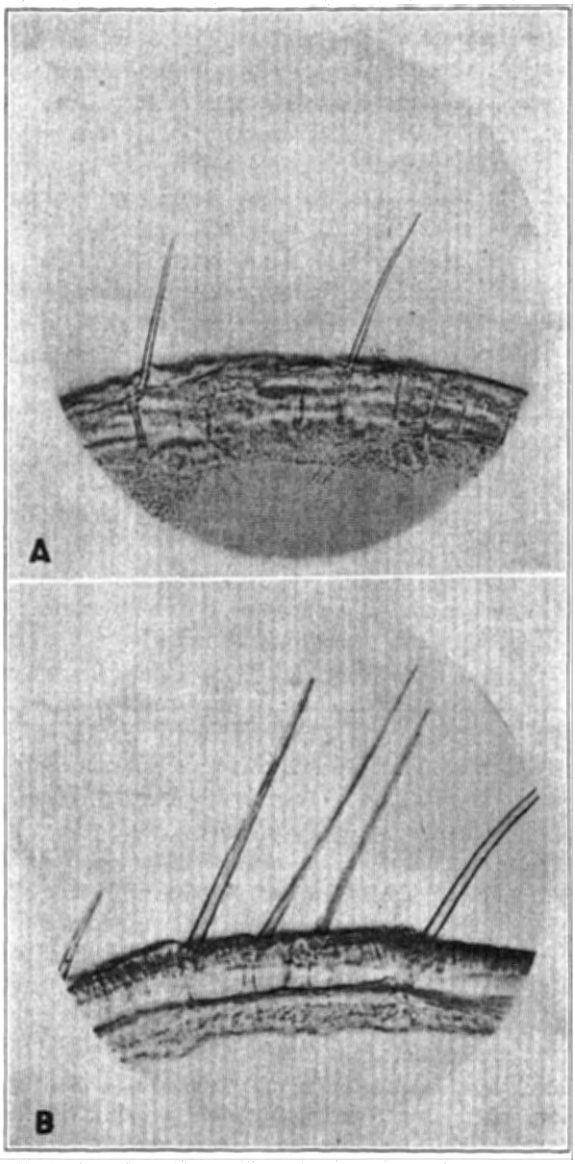


FIGURE 2. Penetration of insect tissue by pyrethrins: (A) Section of the integument of an untreated *Tenebrio molitor* larva. ×200; (B) Section of the integument of a *Tenebrio molitor* larva that had been painted on the dorsal surface with Sudan III dissolved in pyrethrum extract. The hypodermis and trichogen cells at the base of the setae were stained red and appear darker in the photograph. ×200.

insects in the present investigation, larvae of *Tenebrio molitor* were immersed in concentrated pyrethrum extract. After 16 hours they were removed, thoroughly rinsed in acetone solution and dissected under 95 per cent alcohol. Portions of the ventral nerve cord were removed, fixed in 95 per cent alcohol, and stained with toluidine blue according to a method attributed to Nissl by Krause (5, v. 3, p. 651). At the same time a series of larvae killed with concentrated nicotine sulphate and another series killed with lead arsenate were treated in a similar manner. The controls consisted of a series of larvae killed by decapitation and immediately dissected

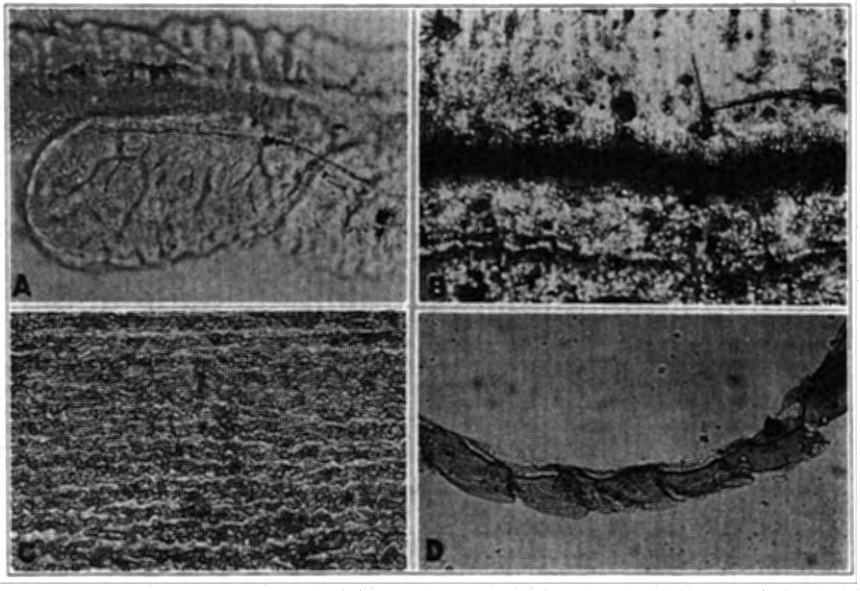


FIGURE 3. Histological studies of penetration of pyrethrum concentrate into insect integument: (A) Section of the integument of *Tenebrio molitor* larva through a conjunctiva. ×130. The larva was killed with "oildag" dissolved in pyrethrum extract. Note the carbon stain in the hypodermis. Portions of an exuvia of *Cicada septendecium* stained with oil red dissolved in pyrethrum extract; (B) Surface view, showing that the cuticula takes the stain mainly along the suture. ×75; (C) An enlargement of B, showing texture of suture. ×130; (D) Cross section through suture. ×130.

in 95 per cent alcohol and of another lot of individuals which were decapitated and the cut ends sealed with paraffin (m.p. 56° C.) for 16 hours prior to dissection. This was done in order to determine whether a period of this length would produce any decomposition effects on the nervous system.

The material was fixed in 95 per cent alcohol for 16 hours, stained for five and one-half hours with 0.1 per cent aqueous toluidine blue, washed with 95 per cent alcohol, dehydrated with absolute alcohol, and imbedded

in paraffin after running through xylol. Because of the fragility of the tissue it was found desirable to make gradual transitions by using combinations of xylol and alcohol, and paraffin and xylol. Sections of 5 μ thickness were made.

Cross sections of the ventral ganglia and nerve cord stained blue throughout in all cases, except in the larvae that had been killed with pyrethrum extract. Scattered among the blue-staining cells, in this case, were areas in which the cells stained violet. In addition there were areas that appeared vacuolated, the margins of which were stained dark blue (Fig. 4, compare A and B). The contrast was so striking that one could distinguish without hesitation between the controls and the larvae treated with pyrethrum extract. Whether this stain is specific for the effects of pyrethrum was not determined although nicotine sulphate, a contact insecticide, and lead arsenate, a stomach poison, did not produce any visible differences from the controls.

The term tigroid has been applied to masses of deeply stained substances in the protoplasm of neurons (Nissl's granules). The chemical nature of these granules is still unknown although they are supposed to be related to the nucleus. Recently Szent-Györgyi (11) has suggested that the tigroid is a reserve polysaccharide of the nerve cells, similar to glycogen, but not identical with it, since it does not stain with iodine.

Examination of the supraoesophageal ganglion of pyrethrum-treated larvae and of nicotine-treated larvae as well as the controls, failed to reveal any violet stained cells or histological changes. The failure to find pathological symptoms in this tissue may be due possibly to the difficulty in making suitable dissections owing to the toughness of the head capsule. We hope that future investigation will settle this point together with the question of the usefulness of toluidine blue in the study of the effect of poisons on insects.

By the use of phosphomolybdic acid, McIndoo (7) was able to precipitate nicotine in the tracheae, suboesophageal ganglion, optic nerves, and cortical layer of the brain of insects killed with nicotine sprays and fumigants. He states that he observed no histological changes in the nervous system that could be attributed to the presence of nicotine. In higher animals histological changes in the brain, spinal cord, and vagus nerve have been noted by several investigators (1, 13) due to the action of nicotine.

In this investigation the attention was centered mainly on a study of the effect of pyrethrum on nerve tissue. As pointed out previously, Krüger (6) has observed morphological changes in the muscles and hypodermis of *Corethra* larvae. As the integument of this insect is considerably more delicate than that of the larvae of *Tenebrio molitor* it would be expected that lesions in the hypodermis would be more marked in the former than in

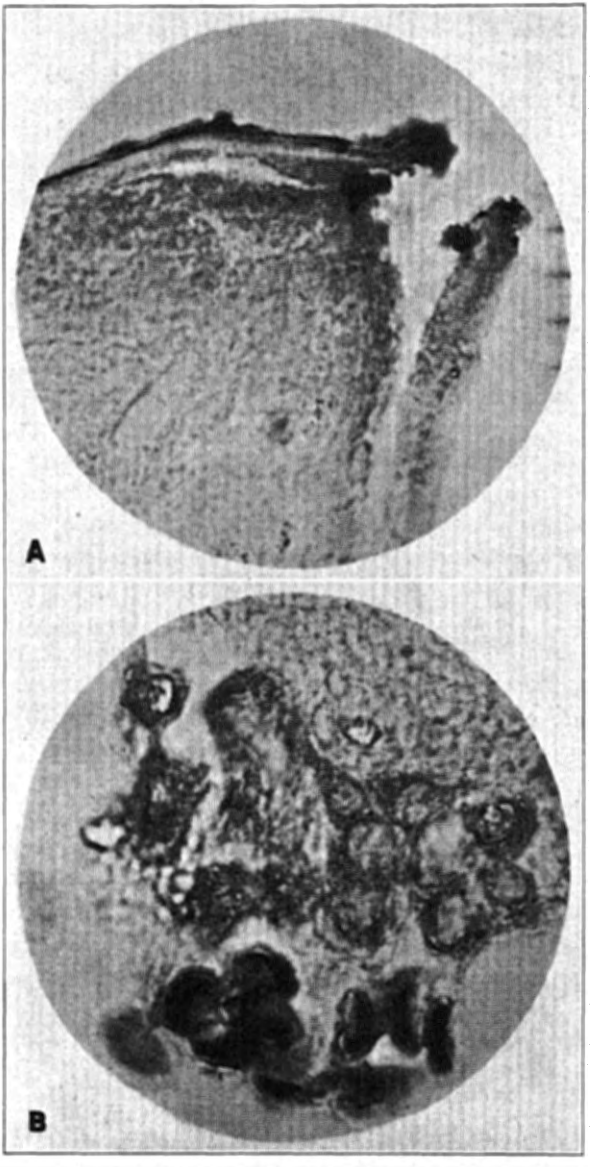


FIGURE 4. Effect of pyrethrum concentrate on nerve ganglia: (A) Cross section of a ventral nerve ganglion of a *Tenebrio molitor* larva killed by decapitation and stained with toluidine blue. ×1350; (B) Cross section of a ventral nerve ganglion of a *Tenebrio molitor* larva that had been killed with pyrethrum extract and stained with toluidine blue. ×1350. Note the vacuolated tissue with dark stained margins; the violet stained areas in the lower part of the photograph appear black.

the last named species. While not wishing to minimize the importance of the effect of pyrethrum on insect muscles and other tissues, it would appear from our studies that the destruction of nerve tissue is a primary factor in bringing about paralysis and death.

SUMMARY

1. A method is described for obtaining samples of the pyrethrins free from accompanying impurities, which is simpler than methods described previously, and which does not require the conversion of the pyrethrins into a derivative, and their subsequent regeneration.

2. In the course of this work samples have been obtained differing widely in the ratio of pyrethrin I to II. Toxicity tests using these samples on *Aphis rumicis* indicated that I is far more toxic that II.

3. Comparative toxicity tests using purified extracts and comparing these with crude extracts of flowers, at the same concentration of pyrethrin I, showed the flowers to be slightly more toxic than the highly purified extracts. Possible reasons for this difference are suggested.

4. The function of wetting agents in promoting the penetration of nicotine and pyrethrum into the tracheal system of insects has been discussed briefly. In the case of pyrethrum it has been found that intoxication and death may ensue from the external application of pyrethrum concentrates, under conditions where no tracheal penetration takes place.

5. Since the pyrethrins have low volatility, the possible penetration through the integument was studied by means of dyes soluble in the pyrethrins. Evidence is presented that the pyrethrins can penetrate the integument, at least in certain regions.

6. It has been shown that the pyrethrins have a low but appreciable solubility in water. These results suggest an explanation as to how the pyrethrum may reach the nerve ganglia when applied externally.

7. Histological changes were detected in nerve ganglia of insects killed by pyrethrum, by means of a staining technique using toluidine blue. These changes were not observed in insects killed by decapitation or by nicotine sulphate or lead arsenate.

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LEAF ENATIONS RESULTING FROM TOBACCO MOSAIC INFECTION IN CERTAIN SPECIES OF NICOTIANA L.

JAMES H. JENSEN

INTRODUCTION

In a report on a study of the mosaic of tobacco in 1903, Iwanowski (4) stated that small leafy outgrowths sometimes occur on the under side of the abnormally narrow leaves of diseased plants. Dickson (1) reported that it is not uncommon for enations to develop on the under side of leaves of tobacco plants infected with tobacco mosaic. Storey (7) described leafy outgrowths on tobacco leaves and presented evidence that they are due to a virus disease. Holmes (3) stated, in describing the symptoms of tobacco mosaic on a number of different hosts, that similar outgrowths occur on leaves of *Nicotiana paniculata* and *N. tomentosa*. No other mention of the occurrence of such malformations resulting from tobacco mosaic infection has been found in the literature. The development of these interesting outgrowths has not been previously studied. The object of this paper is to present further evidence that they may be produced by tobacco mosaic infection and to describe their histological development.

DESCRIPTION OF THE OUTGROWTHS

The enations which develop on leaves of *Nicotiana paniculata* L. and *N. tomentosa* Ruiz & Pav. infected with tobacco mosaic are leaf-like outgrowths which extend downward from the lower surface of the leaf. They vary considerably in size, the larger ones sometimes extending downward as much as one inch from the leaf surface as shown in Figure 1 A. The smaller ones are often so minute that they can be seen only when leaves bearing them are viewed with transmitted light, in which case they appear as dark green lines.

The enations arise as protuberances at the edge of chlorotic areas in the leaf. These chlorotic areas are sometimes circular but more commonly narrow and elongated, although they may be T-shaped, X-shaped, or of other irregular shapes. They are frequently more or less parallel to the larger veins. Occasionally, however, they seem to have no relation to the position of any veins. Enations usually develop equally around the entire border of the chlorotic areas but in rare cases on only a part of it.

Variations in outward extension from the leaf surface, as well as variations in size and shape of the chlorotic areas around which the enations arise, bring about great differences in the morphology of individual outgrowths. Some of the enations have the appearance of small inverted cups. Others appear as double leaf-like protrusions emerging from the surface of the leaf. Figure 1 B shows some of the variations in shape which occur.

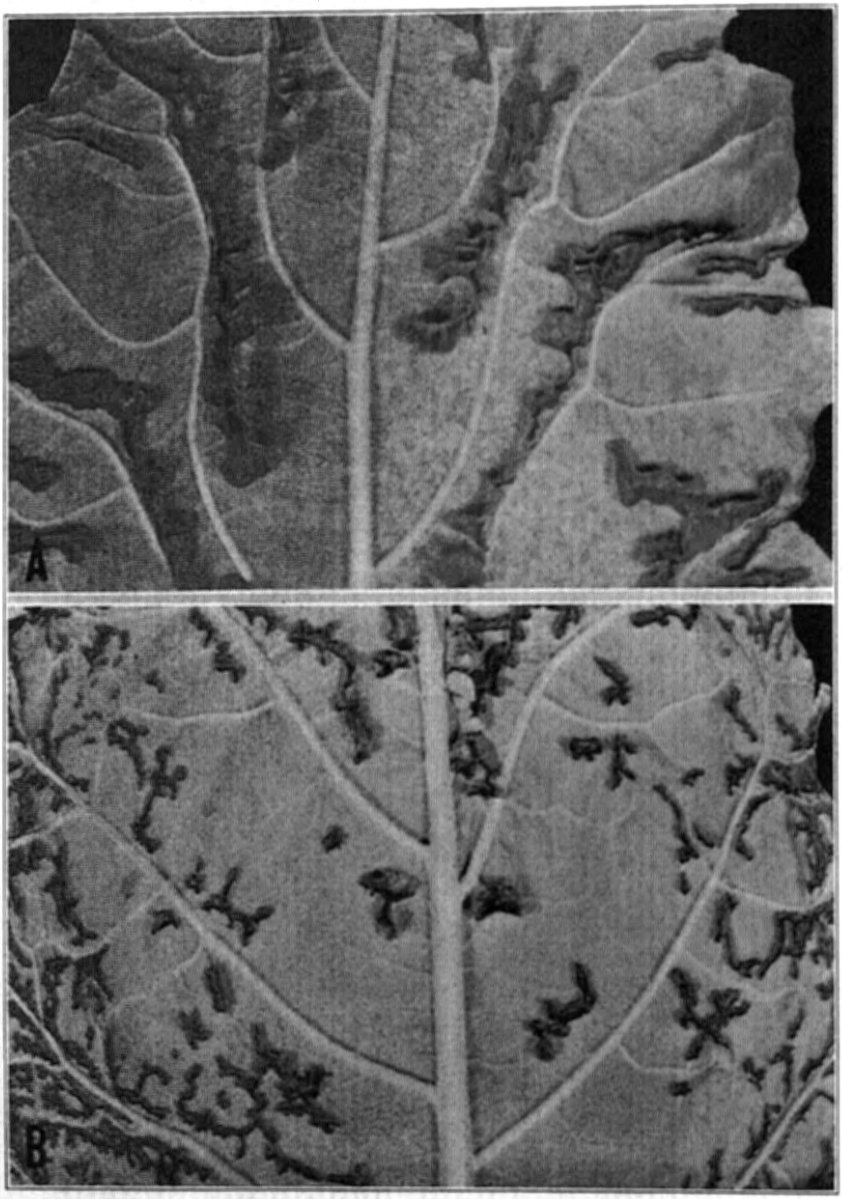


FIGURE 1. Outgrowths on the under side of leaves of *Nicotiana tomentosa* resulting from tobacco mosaic infection: (A) Larger outgrowths some of which extend downward as much as one inch from the surface of the leaf. (B) Smaller outgrowths of various irregular shapes.

The outgrowths are morphologically and structurally similar to the leaves from which they develop. The inner surface of the outgrowth, which faces the chlorotic area around which the protrusion develops, is dark green in color like the upper side of a normal leaf. The outer surface which faces away from the chlorotic area, is greyish like the under side of a normal leaf. The protuberances are usually slightly thinner than healthy leaves. In the large enations, veins are macroscopically visible and arranged in a netted fashion similar to those in the remainder of the leaf. The veins of the outgrowth connect directly with those of the leaf blade. In many instances, as the leaf expands and becomes mottled, similar mottling develops in the outgrowths.

Enations appear only on leaves which develop after systemic symptoms occur, never on inoculated leaves. They usually appear on plants about 20 days after inoculation. Leaves bearing outgrowths are generally distorted and unsymmetrical as well as narrower than healthy leaves.

The number of leaves which develop outgrowths varies with individual plants even when grown under the same conditions. In some cases enations developed on almost all of the leaves produced after systemic infection whereas in others they appeared on only one or two of the leaves. They do not develop around all chlorotic areas in a leaf.

The symptoms of tobacco mosaic in *Nicotiana paniculata* and *N. tomentosa* are generally similar to those found in other species of *Nicotiana* in which the disease produces mottling of the leaves.

OUTGROWTHS DUE TO TOBACCO MOSAIC INFECTION

Although leaf-like outgrowths from the under side of leaves of certain species of *Nicotiana* have been reported to occur on plants infected with tobacco mosaic little experimental evidence has been presented to show that they occur as a result of this disease. Since overgrowths of various kinds are known to be caused by the feeding of certain insects or infection with certain bacteria or fungi, an attempt was made to produce the enations described in this paper in the absence of such gall-producing agents.

Fresh juice from a tobacco plant infected with tobacco mosaic was passed through a Berkefeld filter candle. Samples of the filtrate were then placed in petri dishes containing nutrient agar and held under observation at room temperature for a period of 12 days. Since no colonies of bacteria or fungi appeared on the plates during this period it was concluded that the filtrate was free from such organisms. Samples of the filtrate were used to inoculate 20 healthy young plants of *Nicotiana paniculata*. Twenty similar plants which were not inoculated were used as checks. The plants were held in a greenhouse in which no insects were observed but which nevertheless was fumigated at frequent intervals to insure freedom from insects. All inoculated plants showed the typical symptoms of tobacco mosaic after

the usual period of incubation. Within a month leaf-like outgrowths appeared on the under side of leaves of all the 20 inoculated plants. The check plants remained healthy and no outgrowths developed on the leaves of any of them. This experiment proves that enations may be produced by inoculation with juice containing tobacco mosaic virus free from bacteria or fungi and in the absence of insects.

In 22 other experiments a total of 235 plants of *N. paniculata* and *N. tomentosa* were inoculated with the virus of tobacco mosaic and outgrowths appeared on leaves of all plants which were infected. In one of these experiments 10 plants each of *N. paniculata* and *N. tomentosa* were inoculated with juice from diseased plants and all developed outgrowths. Ten similar plants of each species, inoculated with juice from healthy plants and kept under the same environmental conditions, remained healthy and developed no outgrowths. As many uninoculated check plants as inoculated plants were provided in all other experiments. They were held under the same conditions as the inoculated plants. The checks always remained healthy and free from outgrowths. Plants of both species produced outgrowths in the field as well as under greenhouse conditions. All plants showing mottling and other symptoms of tobacco mosaic produced enations except certain ones which were shaded or inoculated after leaf production had stopped because of approaching maturity. Experiments involving these failures of outgrowth development will be described later. The virus used in most of the experiments was originally obtained from Wisconsin. Samples of virus from California, Kentucky, and New York were also used. The results obtained with samples from these different sources were identical. It is therefore concluded that leaf-like outgrowths result from tobacco mosaic infection and are invariably associated with this disease in plants of *Nicotiana paniculata* and N. *tomentosa* when grown under favorable conditions.

Similar outgrowths are also associated with tobacco mosaic infection in plants of *N. tabacum* L. var. *angustifolia*. They are, however, not an invariable symptom of the disease in this plant. The conditions under which they develop have not been definitely determined. They have been obtained on plants grown from cuttings of diseased plants. They have never been observed on plants grown from seed. It may be that the rapid growth made by plants grown from cuttings is necessary for their development in this variety.

STUDIES OF THE HISTOLOGICAL DEVELOPMENT OF ENATIONS

In this investigation, special consideration was given to a study of the histological development of the outgrowths described above. Examinations were made of fresh and fixed material obtained for the most part from greenhouse plants. Specimens for paraffin sections were fixed in Flem-

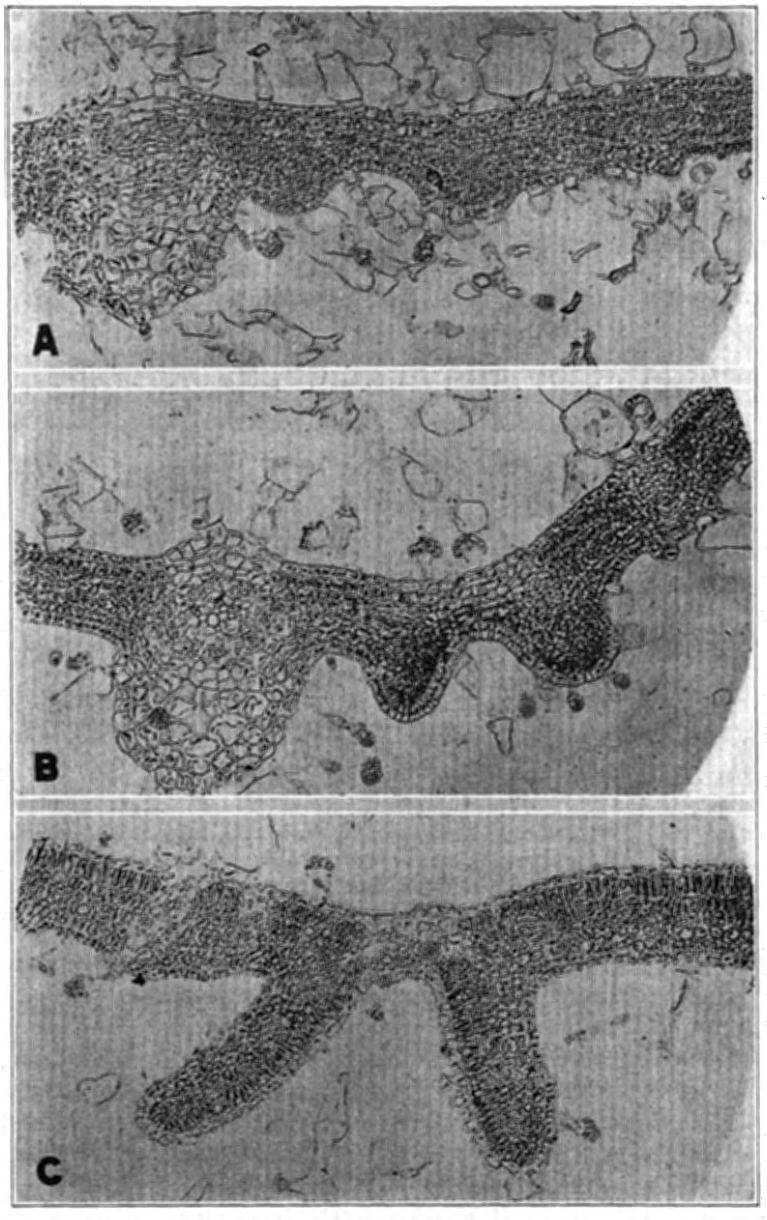


FIGURE 2. Photomicrographs of cross sections showing stages in the histological development of outgrowths. ×130: (A) Early stage showing small ridges on under side of leaf. All interveinal tissue is meristematic. (B) A more advanced stage. (C) Later stage showing the differentiation of palisade in the outgrowth and in the remainder of the leaf.

ming's weak fixative or formol acetic alcohol fixative and stained with Flemming's triple stain.

The first noticeable development in the formation of outgrowths is the appearance of small ridges on the under side of a young leaf. These ridges on the leaf surface are due to an increase in the number of cell layers which result from cell division in the lower three or four layers of tissue. The ridges ordinarily arise in a circular zone around chlorotic areas. A cross section of an early stage shows two protuberances. In some cases the intervening space between them is so short that the two sides of the outgrowth are more or less merged together. In a few cases both sides appear to arise from the same point in the leaf. Cross sections of such enations at an early stage show only one protuberance. Figures 2 A and 6 B present photomicrographs of early stages showing, in cross section, two ridges arising a

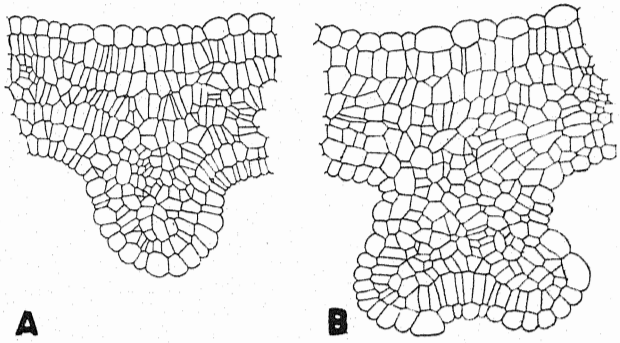


FIGURE 3. Camera lucida drawings of enations which consist of one protuberance in cross section. ×160: (A) An early stage illustrating the increase in number of cell layers due to cell division in the lower layers. (B) A later stage showing early palisade development on the lower side of an outgrowth. Note that the first, second, and third cell layers from the top are continuous across the region above the outgrowth.

short distance apart. Figures 3 A and 6 A illustrate, in cross section, early stages in which but one protuberance developed. In the drawing it is possible to trace all the upper cell layers across the outgrowth. The increase in cell layers is evidently a result of cell division in one or more of the lower three or four layers of tissue. In Figure 2 A it is also possible to trace the upper cell layers across both outgrowths as well as across the intervening area between them. As is shown in both figures illustrating these early stages, all cells are meristematic.

Further development of the outgrowths from such early stages occurs by cell division in the small ridges of tissue. Figure 2 A, B, and C show successive stages in the development of two enations arising at separated points while the drawings in Figure 3 A and B show successive stages in the development of single protuberances.

Mitotic figures were observed in cells of the outgrowth in more than 100 instances. The long axes of the spindles of these figures were usually at right angles to the horizontal plane of the leaf blade. Such figures occurred throughout all parts of the protuberances. Cell division in the malformation and in the remainder of the leaf ceases when the leaf becomes three-fourths to one inch long. These observations demonstrate that enations arise through cell division and growth in the lower cell layers of the leaf.

Tissue which develops in the outgrowth is at first without definite organization and is meristematic like that in the remainder of the leaf. As is shown in the drawing in Figure 4, cells are soon aligned into seven distinct layers like those of a leaf. Cells of these layers and of the corresponding layers of the remainder of the leaf begin differentiation at the same time. The first tissue to differentiate in the outgrowth, as well as in the rest of

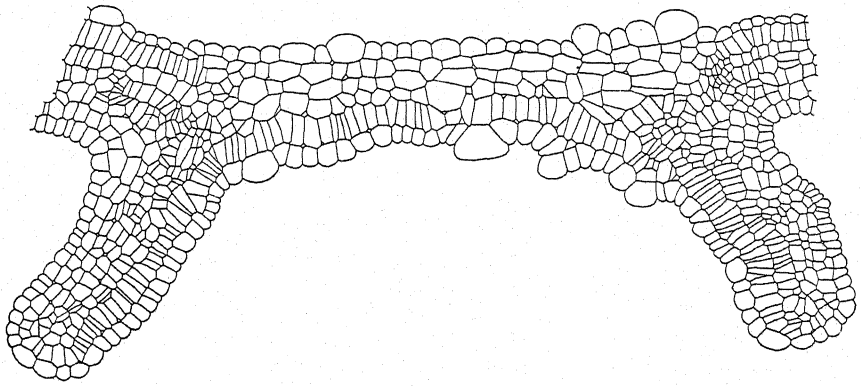


FIGURE 4. Camera lucida drawing showing an outgrowth in cross section at a late stage of development. Note the palisade layer which completely lines the inside of this inverted cup-like enation, while no palisade has developed in the upper layers of the leaf directly above this area. Note also that the sides of the outgrowth have seven cell layers like those of the remainder of the leaf, while only six layers of cells are found in the leaf area between the two sides of the outgrowth. ×160.

the leaf, is the palisade layer, and this differentiation is followed by the appearance of conducting elements and spongy parenchyma. In the early stages the leaf area lying between two sides of a protuberance usually appears in cross section like any other portion of the leaf. Sometimes, however, the development of the cells in this area is arrested and as a result the leaf at that point is thinner. As the outgrowth develops this area becomes markedly chlorotic and does not increase in thickness as do other regions of the leaf. In a fully expanded leaf, these chlorotic areas, which often appear almost translucent when viewed macroscopically, are composed of undifferentiated cells containing small quantities of cytoplasm, very little chlorophyll and poorly developed plastids.

A cross section of an outgrowth on a fully expanded leaf shows that the two sides bear a histological relationship to each other in that the two inner sides are always alike and the two outer sides are always alike in cell layer arrangement (Figs. 4 and 5 B). The most striking feature of the development in this regard is the occurrence of palisade parenchyma in the cell layer below the epidermis of the two inner sides of the enation. In

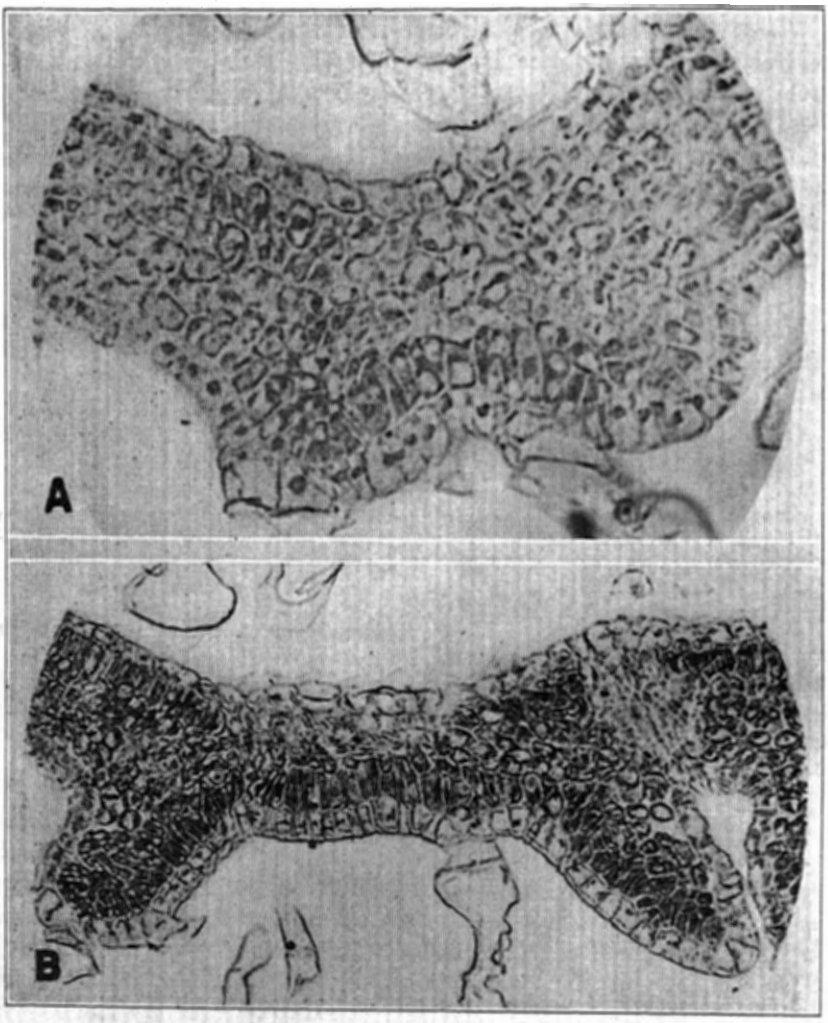


FIGURE 5. Photomicrographs of cross sections of enations: (A) Showing the early development of palisade parenchyma on the under side of the leaf between the two small ridges of the outgrowth. ×440. (B) Showing more mature palisade development on the under side of the leaf between the two sides of the outgrowth. ×225.

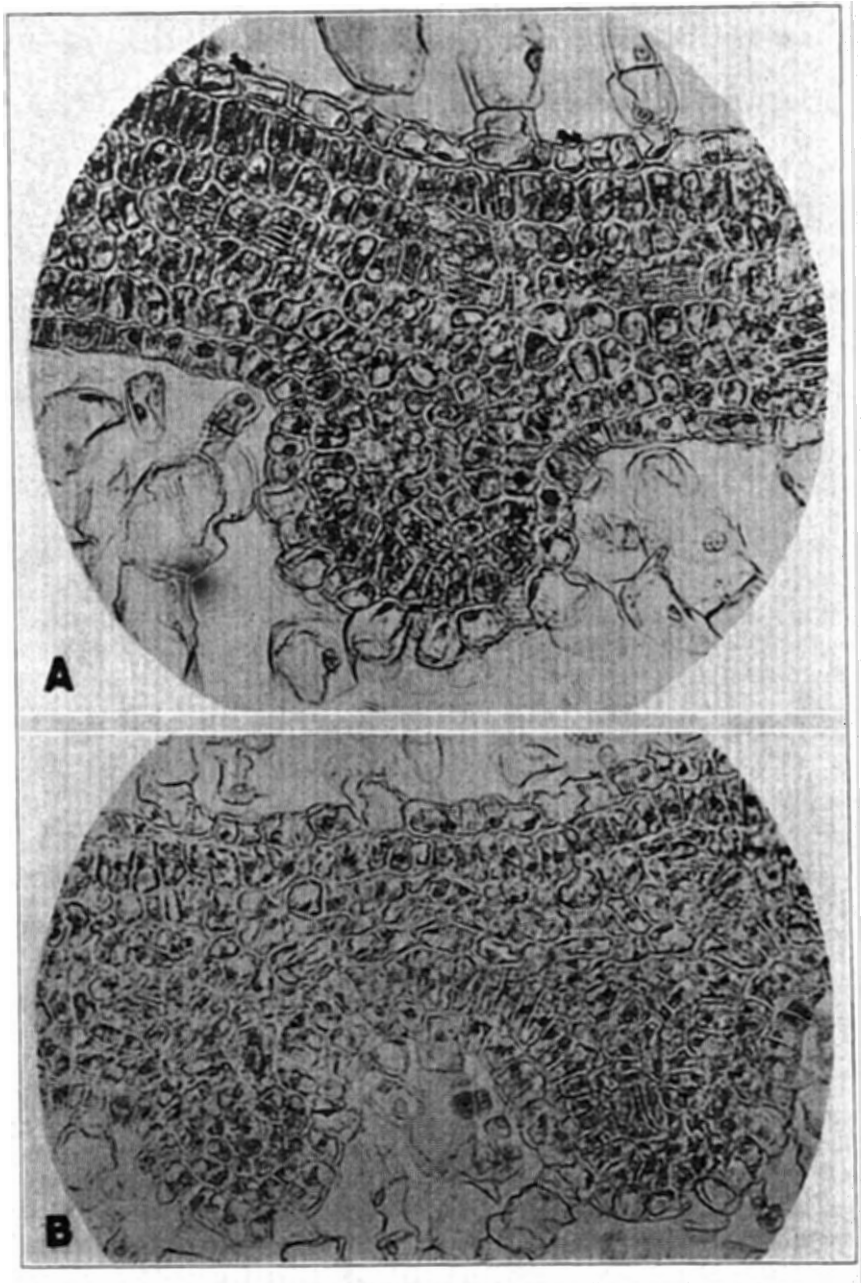


FIGURE 6. Photomicrographs of early stages in the development of enations in cross section: (A) One protuberance. ×350. (B) Two protuberances. ×325.

many instances this palisade layer also extends across the lower side of the intervening area forming a complete layer of palisade cells on the inside of the cup-like or trough-shaped malformations. In other cases the palisade development is limited to the inner sides of the outgrowths. When palisade development occurs in the area forming the bottom of the cup-like out-

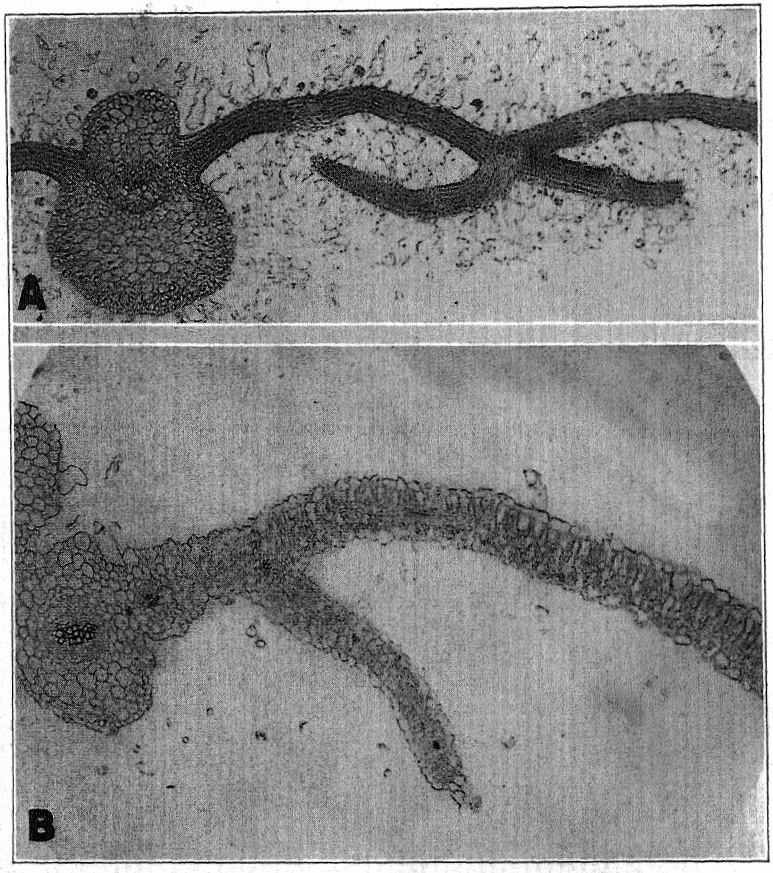


FIGURE 7. Photomicrographs of cross sections of outgrowths. ×45: (A) Showing the development of leaf-like protrusions. The tissue is meristematic with cell division still taking place. (B) Showing the appearance of an outgrowth on a mature leaf. The enations are usually slightly thinner than the remainder of the leaf, as shown in the figure.

growth it is never found on the upper side of the leaf above that area. It is interesting to note, as shown in the illustration in Figure 5 A, that palisade development in the outgrowth occasionally preceded that in the main portion of the leaf.

Tissue similar to that ordinarily found on the lower side of leaves is differentiated in the outer sides of the outgrowth. The cells of the second, third, fourth, and fifth layers from the outer side develop into spongy parenchyma with large intercellular spaces. Thus a portion of a large outgrowth (Fig. 7 A and B) appears in cross section like a portion of the main leaf, containing seven layers of cells which are similar in size, shape, and arrangement to those of the remainder of the leaf.

EFFECT OF AGE AT TIME OF INOCULATION ON OUTGROWTH PRODUCTION

It has been stated previously that enations are formed only on leaves which develop after systemic infection and that they never appear on the inoculated leaves. An experiment was conducted to determine the relation between age of plants at time of inoculation and the production of outgrowths.

Nine plants, potted in large pots to supply sufficient nutrients and soil for growth to maturity, were grouped in three sets of three plants each. The plants were inoculated in sets of three, allowing approximately ten days to elapse between each set of inoculations. In the first set, inoculated about two weeks before blossom buds emerged, a number of new leaves were produced after systemic symptoms appeared and outgrowths developed on several leaves of each of the three plants. In the second set, inoculated just before blossom buds emerged, two plants produced one leaf each and both developed outgrowths. The third set of plants produced no leaves after inoculation. Systemic symptoms appeared about the time that leaf production ceased. On plants of the third set, inoculated several days after flower buds emerged, no outgrowths were formed. This experiment furnished evidence that outgrowths occur only on leaves which develop after systemic infection.

EFFECT OF CERTAIN GROWTH CONDITIONS UPON ENATION PRODUCTION

It has long been known that partial shading of tobacco plants infected with tobacco mosaic tends to reduce the amount of mottling in the leaves and the severity of the symptoms in general (9). Also it has been observed that tobacco plants grown under extremely dry conditions do not exhibit as marked symptoms as those grown under more favorable conditions. An experiment was conducted, therefore, to determine the effect upon outgrowth formation of (a) partial shading of plants and (b) growth under conditions of low soil moisture. In this experiment 12 young *N. paniculata* plants were inoculated and kept in a greenhouse, in full sunlight, and supplied with sufficient moisture. When systemic symptoms appeared about six days after inoculation the 12 plants were divided into three similar groups of four plants each. Four of these plants were placed under a greenhouse bench where they were shaded from direct sunlight but were

kept well watered. Four plants were kept on a greenhouse bench but were watered only once every seven to ten days. They were provided with barely enough water to continue growth. Four plants were kept in full sunlight on a greenhouse bench and were well watered.

Outgrowths appeared within 23 days after inoculation in the plants that were well lighted and well watered. They also appeared on some of the plants grown under dry soil conditions, but not until 30 to 45 days after inoculation; all enations in this set were small. The four plants which were held in the shade of a bench for eight weeks produced no outgrowths alalthough during this period about six new leaves were formed on each of the four plants. On each plant of the other two sets outgrowths had occurred on some of the first six leaves produced after the appearance of systemic symptoms. In order to determine whether or not outgrowths would appear upon these shaded plants if placed under more favorable conditions, two of them were placed on a greenhouse bench where they were in full sunlight. During the next four weeks each of these two plants produced ten new leaves and on each plant outgrowths appeared on some of the new leaves. The other two plants were kept under the bench five months and although new leaves appeared from time to time no outgrowths were produced. Leaves formed in the shade also showed less mottling and distortion than those produced in the sunlight. This experiment demonstrates that by means of partial shading the production of enations may be entirely prevented on plants of *N. paniculata* infected with tobacco mosaic.

DISCUSSION

The experiments reported in this paper demonstrate that enations occur on certain species of *Nicotiana* as a result of tobacco mosaic infection. These enations invariably appear on infected plants kept under favorable conditions of growth but do not appear when such plants are kept shaded from direct sunlight. Outgrowths develop only on leaves produced after systemic infection with the virus. These observations indicate that enations develop only when virus is present in leaves at the time of their early development and when proper physiological conditions prevail.

Histological studies reveal that the malformations are invariably associated with chlorotic areas in the leaf. The underlying factors initiating outgrowth development around such areas are not understood.

The occurrence of palisade on the inner sides and spongy parenchyma on the outer sides of the protuberances might seem to indicate that a folding of the leaf has taken place. However, studies on cross sections of early stages show that folding does not occur but that outgrowths are the result

of multiplication of cells in the lower layers. This point is further emphasized by the fact that it is possible, in every cross section of the outgrowths, to trace the upper cell layers across the region above the two sides of the protuberance and the area between them. Therefore, whatever the initiating factors of outgrowth development may be, it is clear that enations are not the result of folding of the leaf but that they develop as a result of growth, through cell division and enlargement, from the lower cell layers of the leaf.

Outgrowths have been reported to occur on plant leaves as the result of various stimuli. The enations reported by Treviranus (8), Magnus (6), and Hintikka (2) occur on the under side of leaves of *Aristolochia sipho* L'Her. These reports are of especial interest in this connection because they describe enations which resemble those reported in this paper. Illustrations of cross sections of outgrowths which occur on *Aruncus silvester* L., presented by Lingelsheim (5), as well as those on *Prunus avium* L., reported by Young (10), show enations similar to the ones which occur on species of *Nicotiana* infected with tobacco mosaic. The causes of the outgrowths on these plants are unknown. It is possible that they are caused by virus diseases. It is also possible that outgrowths appear on other species of plants as a result of virus diseases. They probably occur on other species of *Nicotiana* and on other varieties of *N. tabacum* than those reported in this paper.

SUMMARY

1. Leaf-like outgrowths on the under side of leaves were found to occur invariably on all plants of *Nicotiana paniculata* L. and *N. tomentosa* Ruiz & Pav. infected with tobacco mosaic except plants shaded from direct sunlight.

2. Similar outgrowths appeared occasionally on plants of *Nicotiana tabacum* L. var. *angustifolia* when grown from cuttings of diseased plants. Enations were never observed on plants of this variety grown from seed.

3. Outgrowths were observed to develop in association with chlorotic areas and only on leaves which were produced after systemic infection.

4. Histological sections of the outgrowths showed that they arise through cell division and enlargement from the lower cell layers of the leaf.

5. Palisade tissue was found, in a number of instances, to develop in the lower cell layers of the leaf between the two sides of outgrowths.

6. Large outgrowths, in cross section, consist of seven cell layers extending downward from the lower surface of the leaf. These cell layers are differentiated into epidermis, palisade, and spongy parenchyma.

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PHYSIOLOGICAL AND CHEMICAL STUDIES OF AFTER-RIPENING OF RHODOTYPOS KERRIOIDES SEEDS

FLORENCE FLEMION

INTRODUCTION

It has long been known that seeds of many rosaceous species are dormant (8) and require a period at low temperature for germination to take place. The conditions optimum for the germination and for the storage of seeds from the viewpoint of their vitality have been thoroughly investigated for a number of species. The literature covering these results has been reviewed in a recent paper by Crocker and Barton (3).

Eckerson (7) was the first worker to study some of the changes taking place in seeds during the after-ripening period at low temperature. She examined seeds of *Crataegus* at weekly intervals while on moist cotton at 5° C. An early change noted was an increase in acidity, which was correlated with a greater water-holding power. Catalase and peroxidase activity increased and sugars appeared toward the end of the after-ripening while fats decreased. Sherman (18) also found that catalase increased during the after-ripening of *Crataegus* seeds. Pack (15, 16) found a decrease in total lipoids and increases in phosphatides, alcohol-soluble material, titrable acids, and sugars during the after-ripening of *Juniperus* seeds.

Recent experiments with seeds of *Sorbus aucuparia* (9) have shown that catalase and peroxidase activities increase greatly during after-ripening while emulsin and amylase activity remain practically unchanged. When seeds were placed at temperatures unfavorable for after-ripening, very little change in catalase and peroxidase activity occurred. Experiments with excised embryos showed that both the seed coat and the embryo are concerned in the dormancy of these seeds. An important characteristic of the dormant seeds was found to be a very limited water-absorbing power and slow water movement.

The present paper presents results obtained with seeds of another rosaceous species, *Rhodotypos kerrioides* Sieb. & Zucc. It was found that these seeds require an after-ripening period of about three months at low temperature. A higher germination results if the seeds have been stored dry for three to nine months at room temperature or if they have been in moist peat moss at 25° or 30° C. for one month prior to the low temperature treatment. Non-after-ripened embryos, under certain conditions, are capable of producing plants which, however, grow abnormally and have the appearance of dwarfs. This is taken up in another paper (10).

The seeds increased in catalase, peroxidase, and lipase activity as after-ripening took place. They also increased in moisture content, titrable acidity, soluble nitrogen, and sucrose, and decreased in fat.

MATERIALS

Most of the seeds used in these experiments were collected from specimens growing in the vicinity of the Boyce Thompson Institute at Yonkers, New York. One lot of seeds was obtained through the courtesy of the New York Botanical Garden, New York City.

EFFECT OF TEMPERATURE ON AFTER-RIPENING

After-ripening and germination at low temperature. In order to determine the optimum temperature for after-ripening, seeds were mixed in moist peat moss[1] (2, p. 116), and placed at various temperatures, and the number of seedlings counted weekly. These experiments were begun in February 1931 with seeds of the 1930 crop. Various constant temperatures from 1° to 30° C. were tried as well as combinations of temperatures in this range, alternated daily and weekly. The data are summarized in Table I which shows the percentage germination resulting during two and one-half, three,

TABLE I

GERMINATION OF SEEDS IN MOIST PEAT MOSS AT VARIOUS CONSTANT AND ALTERNATING LOW TEMPERATURES

Constant temp. °C.	Percentage germ.			Alternating weekly temp. °C.	Percentage germ.			Alternating daily temp. °C.	Percentage germ.		
	2.5 mos.	3 mos.	3.5 mos.		2.5 mos.	3 mos.	3.5 mos.		2.5 mos.	3 mos.	3.5 mos.
1	0	0	0	1– 5	0	1	4	1– 5	0	7	0
5	1	21	25	1–10	0	2	9	1–10	16	62	70
10	0	0	0	1–15	0	0	1	1–15	3	25	48
15	0	0	0	1–20	0	0	0	1–20	0	1	2
20	0	0	0	1–30	0	0	0	1–30	0	0	0
25	0	0	0	5–10	1	4	6	5–10	4	15	25
30	0	0	0	5–15	0	1	2	5–15	2	5	6
				5–20	0	3	3				
				5–30	0	0	1	5–30	0	0	0
				10–30	0	0	0	10–30	0	0	0

and three and one-half months. Lots of 200 seeds were used in each test. The only constant temperature at which any germination resulted was 5° C. After three and one-half months at this temperature 25 per cent had germinated. Seeds alternated daily at 1° and 10° C. germinated 70 per cent, those alternated at 1° and 15° C. germinated 48 per cent, and those alternated at 5° and 10° C. germinated 25 per cent, while in moist peat moss for three and one-half months.

Effect of a previous period at a higher temperature. Seeds from the same lot as that used in the experiments described above were mixed in moist peat moss for periods of one-half, one, one and one-half, two, and three and

[1] Granulated peat moss was obtained from Atkins and Durbrow, New York City.

one-half months at 15°, 20°, 25°, and 30° C. before being transferred to 5° C. As a control the seeds were immediately placed at 5° C. without any previous treatment. The results (Table II) obtained at 5° C. after a previous period of one month at the higher temperatures show that the

TABLE II

EFFECT OF PLACING SEEDS, MIXED IN MOIST PEAT MOSS, AT HIGH TEMPERATURE FOR ONE MONTH UPON SUBSEQUENT GERMINATION WHEN TRANSFERRED FOR VARIOUS PERIODS AT 5°C.

Temp.	Percentage germination at 5°C.		
	2.5 mos.	3 mos.	4 mos.
30°C.	72	86	88
25°C.	5	72	80
20°C.	30	38	40
15°C.	10	14	16
Control	0	16	58

germination resulting is considerably above that of the control which had no high temperature treatment. When seeds were mixed in moist peat moss and placed at 25° or 30° C. prior to the transfer to low temperature, germination took place sooner and a higher percentage was reached than in the case of the control; while when the seeds were placed at 15° or 20° C. prior to the period at low temperature, germination took place earlier but the final germination was not so high as in the control. The results obtained with the seeds kept longer than one month at the higher temperatures were similar except that it was found that when the seeds were kept for three and one-half months at the high temperature some injury resulted as shown by the lower germination obtained. The best results were obtained when the seeds were placed at 30° C. for one month before the period at 5° C. Other experiments showed that the use of temperatures higher than 30° C. did not increase the percentage germination.

Development of a secondary dormancy. In experiments with *Sorbus* seeds (9), it was found that when seeds were partially after-ripened at 1° C. and were then transferred to a higher temperature, a secondary dormancy (1) developed as shown by the fact that such seeds needed a second after-ripening period at 1° C., as long as that required when there was no previous low temperature treatment. Apparently changes took place at the higher temperature which necessitated a further prolonged after-ripening at 1° C.

Similar tests conducted with seeds of *Rhodotypos kerrioides* gave the same results. Extensive experiments were carried out in which seeds were mixed in moist peat moss for various periods at 5° C., then placed for various periods at 20°, 25°, and 30° C. before the completion of the after-ripening at 5° C. The previous period at low temperature did not shorten

the second after-ripening period required when the seeds were at a higher temperature in the interval between the two low temperature treatments.

In these experiments a higher percentage of germination was obtained from the seeds which had the low temperature treatment with subsequent high temperature and low temperature treatment than from the seeds which received low temperature treatment throughout, but no higher than those which received only a period at 30° C. followed by a period at 5° C. The favorable effect of a period at high temperature before the low temperature treatment has been brought out previously in this paper in connection with Table II. The additional period at 5° C., which was applied before the high temperature treatment, did not increase the germination although considerable of the after-ripening processes must have taken place before the seeds were removed from 5° C., for, as will be shown later, many changes occur in the seeds within the first few weeks at low temperature. The period at the higher temperature seems in some way to reverse or nullify these changes so that the seeds again become unresponsive to germinative conditions and require a second after-ripening period.

Effect of dry storage at room temperature. In the fall of 1929, freshly-harvested seeds (lots of 500) were planted in flats and subjected to various constant low temperatures, to various low temperatures alternated weekly, and to 21 ° C. followed by constant 5° C. In August 1930 seeds of this same lot which had been stored dry at room temperature for nine and one-half months were also planted in flats and subjected to the same temperatures as the freshly-harvested seeds. The percentage germination obtained after the flats had been in a greenhouse for one month subsequent to the low temperature treatments is shown in Table III. After three months at 5° C.,

TABLE III

PERCENTAGE OF SEEDLINGS OBTAINED WHEN FRESHLY-HARVESTED SEEDS AND SEEDS STORED FOR NINE AND ONE-HALF MONTHS AT ROOM TEMPERATURE WERE PLANTED IN FLATS KEPT AT VARIOUS TEMPERATURES AND TRANSFERRED TO GREENHOUSE

Treatment	Percentage germination after 1 month in greenhouse	
	Freshly-harvested seeds	Air-dry seeds stored 9.5 months at room temperature
5° C. constant for 3 months	19	68
5° and 10° C. alternated weekly for 3 months	60	73
21° C. for 2 months then transferred to constant 5° C. for 3 months	74	82

only 19 per cent of the fresh seeds germinated while 68 per cent of the stored seeds germinated. It is seen that the stored seeds responded very

well to a temperature which was unfavorable for the fresh seeds. In other words, the stored seeds were not as specific as to the temperature required to bring about a high percentage germination. Other experiments in which seeds were subjected to similar tests in moist peat moss gave similar results as to the superior germinative powers of the seeds which had been stored dry for some time at room temperature.

This is further brought out by the data in Table VI (shown as control) which show the percentage germination resulting after three months in moist peat moss at 5° C. in the case of seeds which had had various periods of room temperature storage previous to the period at 5° C. In the case of freshly-harvested seeds only 6 per cent germinated, while in seeds which had been stored for three months 48 per cent germinated, and in those stored six months and one year 60 and 52 per cent germinated. In this connection it is essential to bear in mind that *Rhodotypos kerrioides* seeds (Table IV) stored dry in open containers gradually lose their vitality after storage for six months; this loss proceeds more rapidly at higher temperatures.

TABLE IV

EFFECT OF STORAGE OF AIR-DRY SEEDS IN OPEN CONTAINERS AND UNDER VACUUM AT VARIOUS TEMPERATURES UPON SUBSEQUENT GERMINATION WHEN IN MOIST PEAT MOSS FOR VARIOUS PERIODS AT 5° C.

Storage temp.	Storage time, years	Percentage germination					
		2.5 months		3 months		4 months	
		Open	Vacuum	Open	Vacuum	Open	Vacuum
−8° C.	½	26	8	29	13	32	23
	1	2	0	4	7	9	14
1° C.	½	9	8	32	19	46	23
	1	6	0	28	7	54	17
	2	0	0	19	2	42	3
5° C.	½	8	4	33	23	48	35
	1	2	0	29	7	67	16
	2	0	0	30	0	38	13
10° C.	½	12	14	48	41	59	48
	1	0	0	10	3	37	32
	2	0	0	17	0	44	28
15° C.	1	0	1	13	12	26	30
20° C.	½	18	1	32	42	42	51
	1	0	0	30	42	53	51
	2	0	0	3	25	20	32

Davis (6) with *Ambrosia trifida* has found that some seeds undergo complete after-ripening when stored dry at room temperature while others remain dormant under these conditions.

VITALITY DURING DRY STORAGE

DRY STORAGE AT VARIOUS TEMPERATURES

In order to test the vitality of seeds under different storage conditions, seeds of the 1929 crop were kept in open containers, in sealed containers (150 cc. bottles corked and sealed with Dekhotinsky cement), and under vacuum (150 cc. Pyrex flasks exhausted with a vacuum pump and then sealed with a flame) for periods of one-half, one, and two years at temperatures of −8°, 1°, 5°, 10°, 15°, 20° C., and at room temperature. At the end of these storage periods, lots of 200 seeds were removed and mixed with moist peat moss and the percentage germination determined while at 5° C. Table IV gives a summary of the data from the open and vacuum storage experiments and Table V shows the percentage germination obtained from seeds which had been stored in open and in sealed containers for one year

TABLE V

EFFECT OF STORAGE OF AIR-DRY SEEDS IN OPEN AND SEALED CONTAINERS AT 5° C. AND ROOM TEMPERATURE FOR ONE YEAR UPON SUBSEQUENT PERCENTAGE GERMINATION WHEN IN MOIST PEAT MOSS FOR VARIOUS PERIODS AT 5° C.

Storage temperature	Percentage germination							
	3 mos.		3.5 mos.		4.5 mos.		5.5 mos.	
	Open	Sealed	Open	Sealed	Open	Sealed	Open	Sealed
5° C.	16	7	35	21	55	47	61	50
Room temp.	0	0	0	3	13	13	19	20

at 5° C. and at room temperature. At −8° to 10° C. the seeds retained their vitality somewhat better when stored open, but at 15° and 20° C. storage under vacuum was as good as or better than the open condition. At the temperatures tested, storage in open containers was superior to storage in sealed containers. The data show that the seeds retain their vitality best in open containers at 1°, 5°, and 10° C.

Under all the storage conditions tested, the seeds gradually lost in vitality as the storage period was prolonged from one-half year to two years. Under the most favorable storage conditions the loss in vitality during two years was not very great, however; the percentage germination obtained after this period was from 10 to 30 per cent below that of the seeds stored one-half a year.

EFFECT OF HUMIDITY ON THE VITALITY OF THE SEEDS

Seeds were stored at room temperature for periods of 3, 6, and 12 months at various humidities in order to determine the effect of humidity on their vitality. The experiments were begun in the fall of 1929 and the seeds were kept in desiccators over such concentrations of sulphuric acid (19) as were required to give the humidity desired. The controls were kept

in desiccators containing only the seeds. At the end of the storage periods lots of 100 seeds were taken from the desiccators and the percentage germination determined during two and one-half and three months in moist peat moss at 5° C.

The results, given in Table VI, show that under no condition tested was the vitality of the seeds any better than that of the controls. The humidities maintained by CaO, concentrated H_2SO_4, and 30 per cent H_2SO_4 (about 75 per cent relative humidity) were definitely injurious with complete loss of the germinative power in one year. Seeds kept over concentrated H_2SO_4 and over CaO were not injured in three months but showed some injury in six months. The high humidity maintained by 30 per cent H_2SO_4 was the most unfavorable of all the conditions tested. Humidities of 25 per cent and 50 per cent gave about the same results as the controls. The work of Heinrich (11) has shown that seeds have a critical moisture content below which the keeping quality is good and above which the keeping quality is poor. This critical moisture content is equal to or a little below the water content of thoroughly air-dry seeds in our climate. Evidently in *Rhodotypos* seeds there is a danger of over-drying.

TABLE VI

EFFECT OF ROOM TEMPERATURE STORAGE OF SEEDS AT VARIOUS HUMIDITIES FOR VARIOUS PERIODS UPON SUBSEQUENT GERMINATION WHEN IN MOIST PEAT MOSS FOR TWO AND ONE-HALF AND THREE MONTHS AT 5°C.

Storage condition	Storage period, months	Percentage germination	
		2.5 months	3 months
Conc. H_2SO_4	3	5	52
	6	0	38
	12	0	0
CaO	3	4	51
	6	0	32
	12	0	0
55.6% H_2SO_4 (about 25% humidity)	3	4	58
	6	47	63
	12	0	34
43.1% H_2SO_4 (about 50% humidity)	3	3	51
	6	28	65
	12	0	15
30.2% H_2SO_4 (about 75% humidity)	3	0	6
	6	0	0
	12	0	0
Control	Freshly-harvested	2	6
	3	1	48
	6	43	60
	12	0	52

SEEDLING PRODUCTION

For the production of seedlings three methods were employed: (1) the seeds were planted in flats after a period of after-ripening at low temperature in some suitable medium; (2) the seeds, planted in flats, were subjected to low temperature, with subsequent transfer to the greenhouse; (3) the seeds, planted in flats, were kept in cold frames out-of-doors where after-ripening took place during the cold winter months.

Planting of after-ripened seeds. When seeds were planted in flats after having undergone after-ripening, the percentage of seedlings obtained was less than that resulting from the other two methods used. Many tests were made under various conditions and with varied media but the percentage of seedlings resulting when the after-ripened seeds were planted in greenhouse flats was only 15 to 60 per cent. The reason for this result is not clear for when the seeds were planted in flats directly and subjected to the after-ripening temperature and then transferred to the greenhouse, about 80 per cent of seedlings resulted.

Subjecting seeds in flats to after-ripening conditions. When seeds were planted in flats in a medium consisting of one-third peat moss, one-third sand, and one-third wood soil and kept under the various after-ripening conditions, a good percentage of seedlings resulted. The data of Table III were obtained in this way and it is seen that under good conditions 82 per cent of seedlings were obtained.

Cold frames. A good seedling production also resulted when the seeds were planted in flats kept out-of-doors in cold frames. Three conditions were used, cold frames without cover, cold frames with a board cover, and

TABLE VII

SEEDLING PRODUCTION OF SEEDS OF THE 1929 CROP WHEN PLANTED OUTSIDE IN COLD FRAMES

Condition of frame	Seedlings counted, date	Percentage seedlings				
		Planted Nov. 13, 1929	Planted Feb. 13, 1930	Planted July 13, 1930	Planted Nov. 13, 1930	Planted Nov. 6, 1931
Mulched	Spring 1930	47	46	—	—	—
	1931	99	53	32	44	—
	1932	99	53	41	50	3
Board-covered	1930	14	50	—	—	—
	1931	94	53	39	20	—
	1932	96	53	46	20	11
Open	1930	0	15	—	—	—
	1931	29	16	16	6	—
	1932	32	18	20	12	0
Greenhouse (21° C.)	1932	0	0	0	0	0

cold frames within which the seeds were mulched (covered with leaves) and then covered with a board cover. Results obtained with the 1929 crop (lots of 1000 seeds) when seeds were planted at various intervals after harvest are shown in Table VII. A high percentage of seedlings was obtained under these conditions except in the open cold frames in which only 30 per cent germinated. Similar results were obtained with seeds from other crops when planted under these conditions.

For the production of seedlings of this species on a large scale, the best method undoubtedly is to plant the seeds soon after harvest in board-covered cold frames. Thus the second spring following the planting, about 95 per cent of seedlings can be obtained.

FACTORS IN DORMANCY

Rôle of Seed Coat

Behavior of excised embryos. That the seed coat plays some rôle in the dormancy of these seeds is shown by experiments with excised embryos. If the outer and inner coats of non-after-ripened seeds are removed and the embryos placed in aerating water (water through which air was continuously passed) or on moist filter paper, results as shown in Figure 1 were obtained. In about 60 per cent of the excised embryos considerable development of the hypocotyl and epicotyl took place in 14 days, especially in the case of embryos kept on moist filter paper. These seedlings grow if transferred to soil but the resultant plants are much less vigorous than plants from after-ripened embryos. The embryos from the aerating water do not produce plants when placed in soil presumably because the roots have not developed root hairs in this medium and the seedlings die because they cannot absorb enough moisture. This is avoided if these embryos are transferred from the aerating water to moist filter paper for a few days before being placed in soil.

That the dormancy of these seeds is not a question of the seed coat alone is shown by the results illustrated in Fig. 2. Seeds were mixed in soil and after-ripened at 5° and 10° C. alternated weekly. The seeds were placed under these conditions at such periods that it was possible to obtain on the same day seeds which had been kept at low temperature two, four, six, eight, and ten weeks. Embryos from such seeds were excised and placed on moist filter paper. Figure 2 illustrates typical embryos from these lots after they had been on moist filter paper for five days. The low temperature after-ripened embryos produced more vigorous seedlings and also produced them more quickly than non-after-ripened embryos. The embryos from the seeds which had been kept at low temperatures for ten weeks developed 100 per cent seedlings when kept five days on moist filter paper while those which had eight weeks of low temperature treatment produced only 75 per cent seedlings in this time. The check embryos and those which had been

kept for two and four weeks at low temperature produced 40, 58, and 60 per cent of seedlings, respectively, after eight days on moist filter paper.

Further evidence as to the rôle of the seed coat is furnished by the experiments with seeds with the outer coats removed. A higher percentage

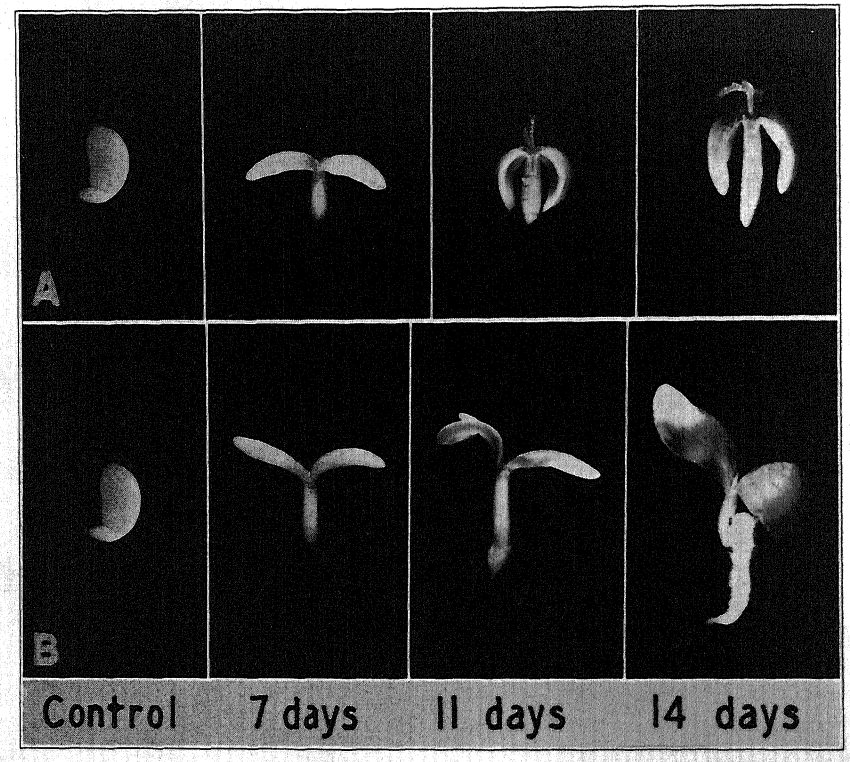


FIGURE 1. The development of excised embryos of non-after-ripened seeds of *Rhodotypos kerrioides* after various intervals in aerating tap water (A), and on moist filter paper (B).

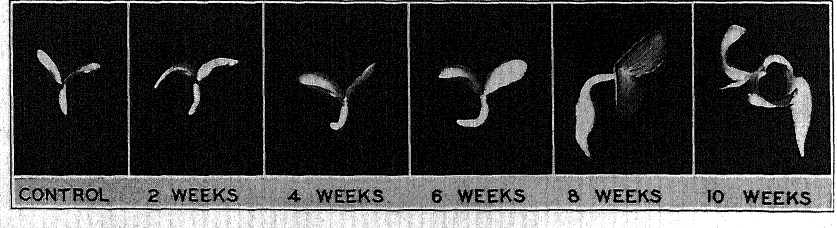


FIGURE 2. After-ripening of the embryo of seeds of *Rhodotypos kerrioides* illustrated by the development after five days on moist filter paper of embryos removed from seeds which had been in moist peat moss for various periods at 5° and 10° C. alternated weekly.

of seedlings results when this coat is removed and germination takes place over a wider temperature range. Thus in one experiment 55, 76, and 46 per cent of seedlings resulted from seeds while in moist peat moss for three and one-quarter months at 1°, 5°, and 10° C. Intact seeds never germinate at 1° C. and germinate very poorly at 10° C., being dependent upon 5° C. or various temperatures, alternated daily or weekly, to produce a good percentage of seedlings. If the outer coats of non-after-ripened seeds are removed and the excised embryos then mixed in moist peat moss and placed at 25° C., about 15 per cent of the embryos show some development of the hypocotyl after about a week. When these are transferred to soil plants which result appear dwarfed. This is described more fully in another paper (10).

These experiments show that both the embryo and the seed coat play important rôles in the dormancy of these seeds.

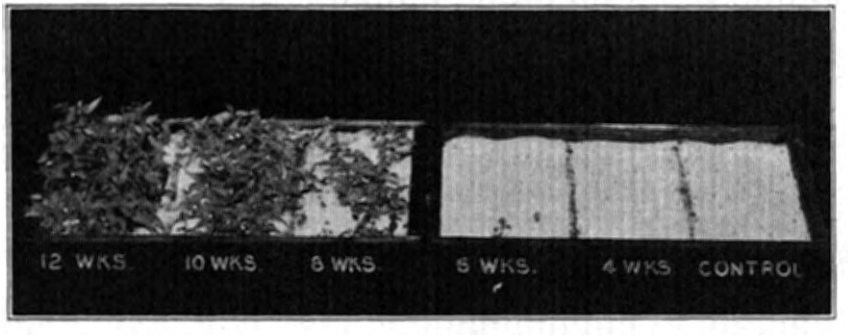


FIGURE 3. Growth resulting three weeks after planting when *Rhodotypos kerrioides* seeds, which had been in moist peat moss for various intervals at 5° and 10° C. alternated weekly, were planted in flats.

CHEMICAL AND ENZYME CHANGES DURING AFTER-RIPENING

In order to learn something about the changes taking place in the embryo during the period at low temperature, seeds of the 1931 crop were mixed in soil and placed at low temperature on March 30, 1932 and at such intervals thereafter that it was possible to obtain on the same day samples of seeds which had been after-ripening for various lengths of time from one week to twelve weeks when after-ripening is complete and germination takes place. The temperature employed was 5° and 10° C. (not rigidly controlled) alternated weekly. The determinations of enzyme activity and the chemical analyses were made on the seed after the outer seed coat had been removed.

The extent to which the after-ripening had taken place after various periods at low temperature in the seeds of these tests is illustrated in Figures 2 and 3. The former, showing the development after five days on

moist filter paper of the excised embryos of seeds which had been kept at low temperature for various intervals, has been discussed in a previous section of the paper. Figure 3 shows the resultant growth of 200 seeds three weeks after planting from lots which had been at low temperature for 12, 10, 8, 6, 4, and 0 weeks. The percentage of seedlings obtained from 12, 10, 8, and 6 week lots was 62.5, 37, 21, and 1 per cent, respectively, while the four week lot and the control produced no seedlings.

CHANGES IN ENZYME ACTIVITY

For the study of the activity of enzymes, samples of 100 embryos were ground up with pure quartz sand in a mortar and washed into 50 cc. volumetric flasks and 5 cc. aliquots drawn for the determinations. When catalase determinations were made, the embryos were ground in the presence of one-half a gram of calcium carbonate. Catalase was determined by measuring the amount of oxygen liberated from hydrogen peroxide by an aliquot of seed extract. The apparatus and procedure described by Davis (5) were used except that the hydrogen peroxide was neutralized with calcium carbonate instead of sodium hydroxide. The activity of catalase in the control seeds was such that an extract of ten seeds liberated 1.3 cc. of oxygen in one-half minute. For the estimation of peroxidase activity, pyrogallol was used as a substrate and the purpurogallin formed determined colorimetrically (13). An extract from ten control seeds formed 10.1 milligrams of purpurogallin under these conditions. The fat-hydrolyzing power of the seeds was determined by allowing the extract prepared from 100 embryos to act on cottonseed oil for 24 hours at 35° C. following the procedure described by Morrow (14).

Results obtained with embryos from seeds which had been kept for 0, 1, 3, 5, 7, 9, and 11 weeks are shown in Figure 4. The activities of the various enzymes are expressed on the basis of the activity of the check expressed as unity. It is seen that as the after-ripening progresses there is an enormous increase in catalase activity until it reaches a value over 18 times that of the check after 11 weeks at low temperature. The increase in peroxidase activity, while not so pronounced as that of catalase, is nevertheless very large. After 11 weeks, the embryos exhibit a peroxidase activity three times that of the control. The results obtained in the determinations of lipase activity were not so uniform as those obtained with catalase and peroxidase but they show very definitely that the lipase activity of the after-ripened seeds is higher than that of the control, the seeds being between three and four times as active as the control after 11 weeks at the low temperature.

Determinations made on seeds which had been kept in moist peat moss at 1° C., which is unfavorable for the after-ripening of these seeds, showed very little change in peroxidase activity, while the catalase activity instead

of increasing showed a definite decrease. Such a decrease, when seeds were placed in moist peat moss at a temperature extremely unfavorable for after-ripening processes, was also found in the case of seeds of *Sorbus aucuparia*.

An increase in the catalase activity of seeds during after-ripening was first found by Eckerson (7) working with *Crataegus* and has since been found by other workers for the seeds of a number of different species (4, 6, 12, 15, 17, 18). An increase in peroxidase activity during the after-ripening period was first found by Eckerson (7) in *Crataegus* seeds and has also been found to take place in seeds of *Sorbus aucuparia* (9).

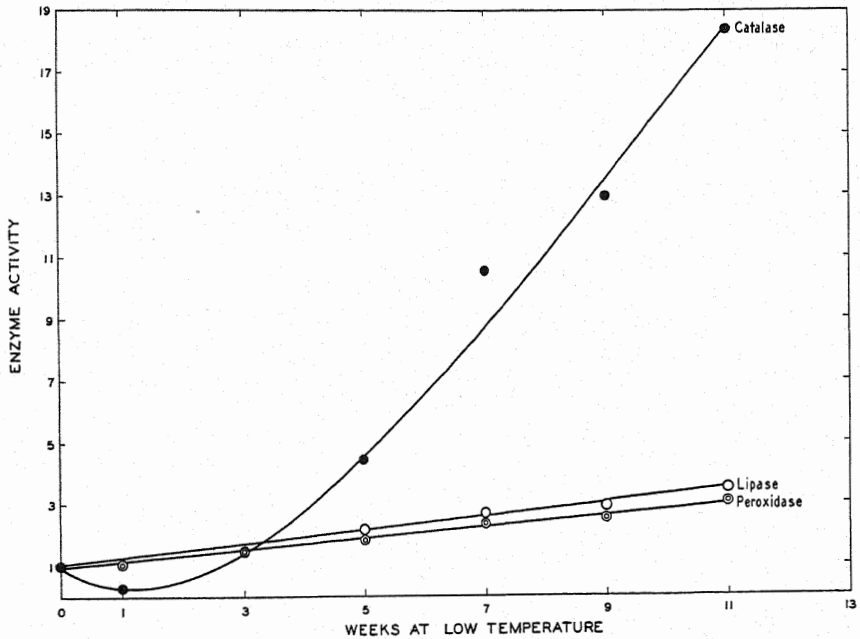


FIGURE 4. Catalase, peroxidase, and lipase activity of embryos of *Rhodotypos kerrioides* after various weeks in moist peat moss at 5° and 10° C. alternated weekly. The activities are calculated on the basis of the activity of the control expressed as one.

CHEMICAL CHANGES

The results of chemical analyses of embryos that have undergone various degrees of after-ripening are summarized in Table VIII. The embryos were dried under vacuum at 65° C. and subsequently extracted with anhydrous ether followed by an extraction with 80 per cent alcohol. Separate samples of embryos were taken for pH determinations and for a determination of the amount of N/50 KOH necessary to neutralize the acids present. The crude fat content slowly decreases as after-ripening progresses

TABLE VIII

CHANGES TAKING PLACE IN THE SEEDS DURING THE AFTER-RIPENING AT LOW TEMPERATURE

Days of stratification	Total dry weight, grams per 100 embryos	Per cent moisture	pH	Cc. N/50 KOH per 25 embryos	Crude fat		Soluble in 80% alcohol				
							Soluble solids		Nitrogen		Sucrose
					Mg. per 100 embryos	Per cent of dry weight	Mg. per 100 embryos	Per cent of dry weight	Mg. per 100 embryos	Per cent of dry weight	Mg. per 100 embryos*
Dry control	2.340	4.84	6.19	3.80	943.2	40.30	182.0	7.78	4.2030	0.1796	45
10 days	2.360	33.35	6.13	3.95	934.0	39.58	179.0	7.59	5.7112	0.2420	39
24 days	2.339	36.62	6.13	4.65	—	—	—	—	—	—	—
38 days	2.293	37.81	6.15	4.50	892.1	38.90	233.0	10.16	7.4919	0.3267	67
52 days	2.322	42.13	6.14	5.00	—	—	—	—	—	—	—
66 days	2.337	45.66	6.14	5.15	861.8	36.88	282.0	12.07	11.2570	0.4817	98
80 days	2.266	50.26	6.07	6.25	822.7	36.30	300.0	13.24	14.8090	0.6534	94
Seedlings**	2.224	—	—	—	693.2	31.13	421.5	18.95	21.3760	0.9611	61

* The amount of free reducing sugars present was so small as to give no copper precipitate in the samples (20 embryos) taken for analysis.

** Hypocotyls 6 to 30 mm. long.

until it is about 10 per cent lower after 80 days at low temperature. The alcohol soluble fraction of the embryos increased from 182 milligrams to 300 milligrams per 100 embryos. The nitrogen soluble in 80 per cent alcohol increased from 4.2 milligrams to 14.8 milligrams per 100 embryos, while the sucrose content (determined after hydrolysis by invertase) doubled during the after-ripening period. There was also a considerable increase in the free acid content of the seeds. Differences in pH were not so evident. All of these results show that during the after-ripening period there is a gradual breaking down of reserve materials into soluble forms which are then available for the growth of the seedlings when germination takes place.

It is important to note the large increase in moisture content during the first ten days of after-ripening. The embryos of the air-dry seeds contained 4.8 per cent moisture and this increased to 33.3 per cent after ten days in moist peat moss. Subsequently further amounts of moisture were gradually taken into the embryos until 50 per cent was present after 80 days. The increase in moisture content after the first sudden rise took place slowly and apparently kept pace with the increase in soluble materials.

The results of the enzyme and chemical changes associated with after-ripening may be summarized as follows: as after-ripening takes place in the seeds of *Rhodotypos kerrioides*, progressive increases in catalase, peroxidase, and lipase activity occur. The final values reached before germination begins are several hundred per cent higher than those for corresponding non-after-ripened seeds. The embryos gradually increase in moisture content, titrable acidity, soluble nitrogen, and sucrose, and decrease in crude fat content.

The results obtained with the seeds of *Rhodotypos kerrioides* are similar to those obtained by Pack (16) with *Juniperus*. He found a decrease in lipoids and increases in the substances soluble in 50 per cent alcohol and in soluble nitrogen as after-ripening took place. Eckerson (7) found an increase in acidity, a decrease in fats, and an appearance of sugars during the after-ripening of *Crataegus* seeds.

SUMMARY

1. The seeds of *Rhodotypos kerrioides* are dormant and require an after-ripening period for germination to take place. This after-ripening occurs in about three months at 5° C. or at 1° and 10° C., 1° and 15° C., or 5° and 10° C. alternated daily or weekly. A much higher percentage germination is obtained if the seeds, mixed in moist peat moss, are kept for one month at 25° or 30° C. before the period at the lower temperatures. A very high seedling production is obtained when seeds are planted in flats and subjected to controlled low temperatures or subjected to the cold winter out-of-doors either mulched or unmulched in board-covered cold frames.

2. When partially after-ripened seeds are transferred to a temperature

unfavorable for after-ripening a secondary dormancy develops, as shown by the fact that when such seeds are subsequently transferred to a temperature favorable for after-ripening, a second after-ripening period which is just as long as if the seeds had not undergone any previous after-ripening is required.

3. Seeds stored dry at room temperature for six to nine months were less specific than freshly-harvested seeds as to the temperature required to bring about germination. Such seeds also produced a higher percentage of seedlings than freshly-harvested seeds. In all cases when seeds were stored dry in open and sealed containers and under vacuum for periods from one-half to two years at temperatures ranging from $-8°$ C. to room temperature, they gradually fell in vitality as the storage period was prolonged. When seeds were stored over various humidities maintained by the proper concentrations of sulphuric acid, no condition was found under which the vitality of the seeds was any better than that of the controls stored air-dry at room temperature. The most favorable storage condition for the seeds was found to be storage in open containers at 1°, 5°, and 10° C.

4. Considerable development of the hypocotyl and epicotyl takes place in 14 days when excised embryos of non-after-ripened seeds are placed on moist filter paper at room temperature. These seedlings grow very slowly when transferred to soil. Tests with excised embryos showed that the embryos are after-ripened after eight weeks at low temperature although germination of intact seeds does not occur until later.

5. Both the embryo and the seed coat play important rôles in the dormancy of these seeds.

6. Analyses of the seeds at intervals of two weeks during the after-ripening period show that the seeds increase in catalase, peroxidase, and lipase activity and also increase in water absorption power, nitrogen soluble in 80 per cent alcohol, titrable acid, and sucrose. The ether-soluble fraction decreases as after-ripening progresses.

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DWARF SEEDLINGS FROM NON-AFTER-RIPENED EMBRYOS OF RHODOTYPOS KERRIOIDES

FLORENCE FLEMION

In connection with the problem of dormancy in seeds Crocker (1) has enumerated several types of dormancy. Among them are those due to the seed coat characters or to an undeveloped or dormant embryo or to a combination of both of these factors. It has been found that in some cases even though dormancy is not primarily due to a hard seed coat, the coat nevertheless plays a rôle (2, 3).

In connection with studies of the dormancy of seeds of *Rhodotypos kerrioides* Sieb. & Zucc., an attempt was made to determine the rôle of these various factors. Experiments were conducted in which the seed coats were removed and the physiological behavior of the excised embryos studied. It was found that both the embryo and the seed coat are important in this connection (4). When the outer seed coats were removed and the embryos placed under proper conditions, seedlings could be obtained. The resultant plants made very slow growth, however, and were quite different from plants obtained from fully after-ripened embryos. This paper is a preliminary report of results obtained thus far.

PRODUCTION OF DWARFS

Seeds of the 1931 crop were used in these experiments. They were collected October 22, and the tests started within ten days after collection. When the outer seed coats were removed and the excised embryos placed in moist peat moss at 25° C., about 15 per cent of the embryos started to grow within a week or so. When the growth of the hypocotyl became evident, the embryos were planted in soil and placed in the greenhouse.

Seedlings obtained in this way did not grow in a normal manner but developed an appearance characteristic of dwarfs. This is evidenced by short, stocky hypocotyls and internodes and small, dark green leaves. Such plants are illustrated in Figure 1. The top row shows four plants about four months after being transferred to soil. The seed coats were removed and the embryos placed in moist acid peat at 25° C. on November 2, 1931. The two shown on the left were planted in soil on November 24 and the two on the right on November 10, 1931. The bottom row shows these same plants five weeks later. It can be seen that the plants were very slow in growing.

Figure 2 shows a plant developed from seed which had been after-ripened for three months at 5° C. and planted in soil on March 12, 1932. The same plant was photographed 19, 52, and 117 days after being trans-

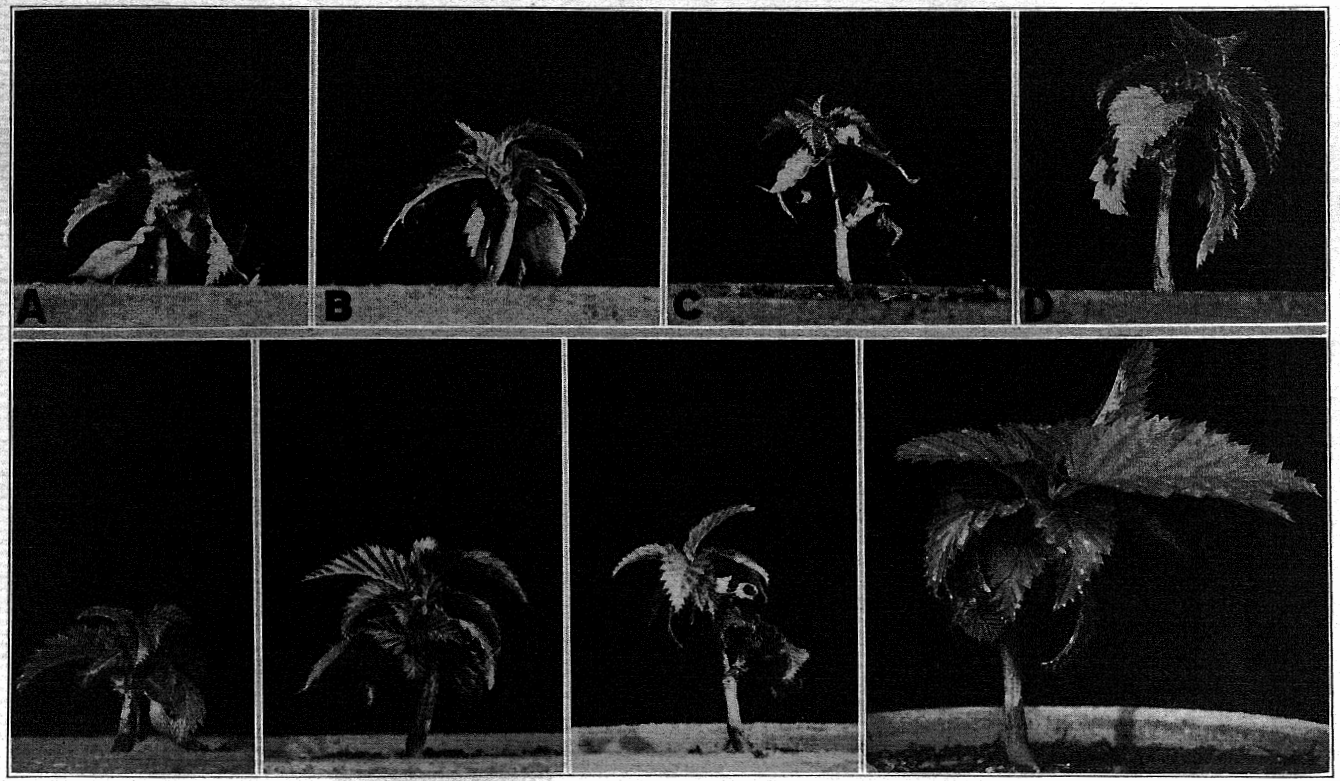


FIGURE 1. Upper row, four plants from non-after-ripened embryos of seeds of *Rhodotypos kerrioides* photographed 122 days (A & B) and 136 days (C & D) after planting. The lower row shows the same plants five weeks later (natural size).

ferred to soil. These photographs show the plant one-sixth natural size while the plants in Figure 1 are natural size. All plants in Figure 1 are older than the oldest plant shown in Figure 2.

SUBSEQUENT GROWTH OF DWARFS

The plants obtained in this way did not remain dwarfed but started to grow quite normally about the middle of May. These plants were then about seven months old in the case of those started in November and about four months old in the case of those started in January. Growth began



FIGURE 2. A plant typical of those resulting from after-ripened embryos of seeds of *Rhodotypos kerrioides* photographed 19, 32, and 117 days after planting (one-sixth natural size).

about the same time in both lots. An examination of the plant in the lower right of Figure 1 shows that in this plant the terminal bud has started to grow. The photograph was taken on May 3, 1932. A further illustration of this result is shown in Figure 3. The two photographs on the left show a dwarf plant when four and five and one-half months old, while the photograph on the right shows the same plant when seven and one-half months old. The terminal bud started to grow in early May and made rapid growth in the two months' interval between the two photographs.

Non-after-ripened embryos started later than February did not pro-

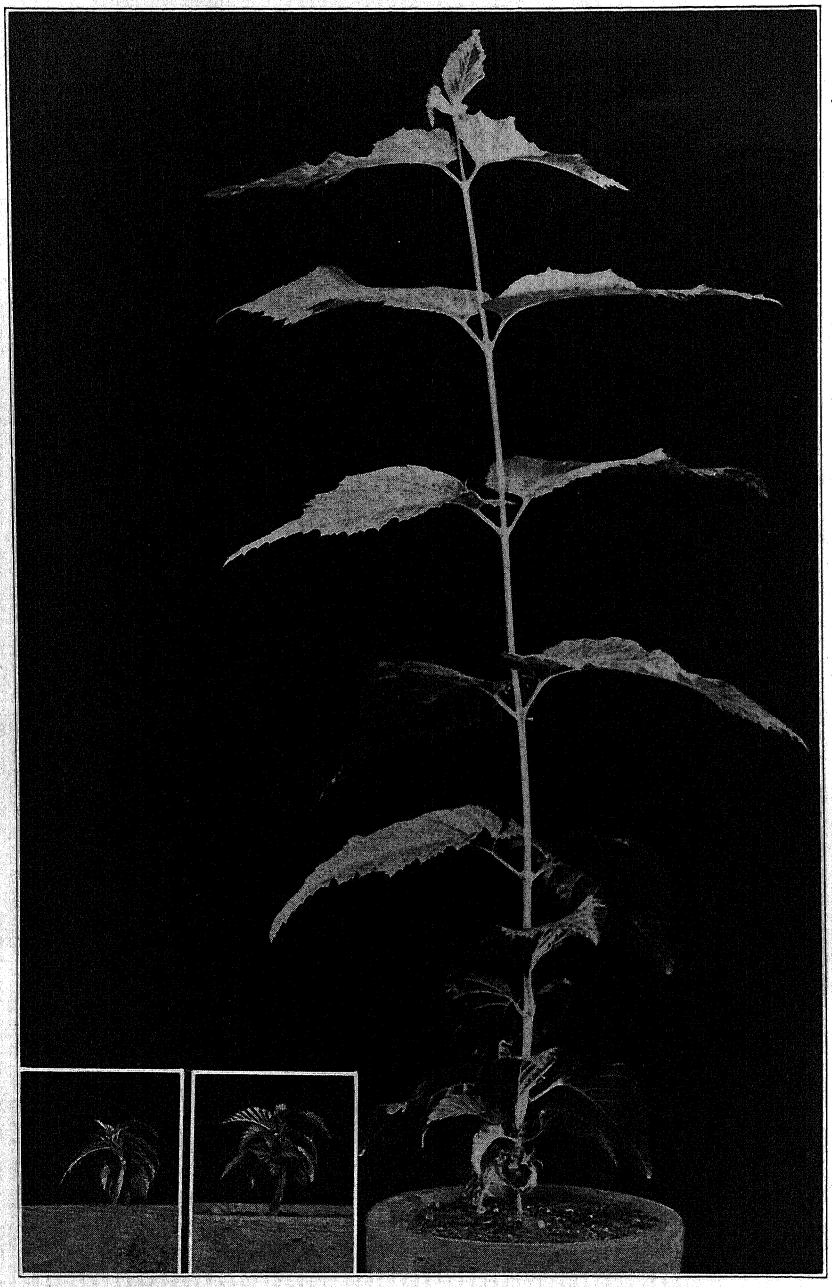


FIGURE 3. A plant from a non-after-ripened embryo of *Rhodotypos kerrioides* photographed 122, 161, and 226 days after planting. This illustrates the normal growth of the terminal bud which took place during the interval between the last two photographs (one-half natural size).

duce dwarf plants. A study of the dormant period of these seeds has shown that the seeds stored air-dry at room temperatures undergo some changes which alter the required low temperature period (4, p. 146). It is possible that by March the seeds had changed sufficiently so that normal growth resulted when the excised embryos were planted.

The causes for the initiation of growth in dwarf plants at this time of year are not known but the possibility that the increased length of day in the spring was a factor is being investigated.

It is important to note that these dwarf seedlings produced normal plants although neither the seeds nor the young seedlings had been subjected to low temperature.

It is not possible to state at this time why dwarf seedlings are produced when these non-after-ripened embryos are induced to grow. This problem is being investigated further along various lines. It seems that after-ripening at low temperature in a germinator or even at room temperature in dry storage brings about some change or changes which are necessary for normal growth. Knowledge of what these changes are would mark a considerable advance in our knowledge of dormancy.

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COTTON FIBERS. III. CELL DIVISIONS IN THE EPIDERMAL LAYER OF THE OVULE SUBSEQUENT TO FERTILIZATION

WANDA K. FARR[1]

Cells in the process of division were observed by Gulati (4) in the epidermal layer of the ovules of *Gossypium sanguineum*×*G. cernuum* on the first, fourth, seventh, and tenth days following fertilization. From a study of the same tissue in *G. hirsutum*, *G. indicum*, and *G. neglectum*, Singh (5) also concluded that the number of cells increased during the development of the fertilized ovule. Mitotic divisions in the epidermal layer of the ovule of *G. hirsutum* from the date of flowering to the twelfth day following were reported by Farr (3). These findings are not in agreement with those of Barritt (1) who would account for the extension of the epidermal tissue of the ovules in Egyptian cotton through enlargement of the non-fiber-forming cells. In a very recent communication (2) he has reported the absence of cell division at any stage of development of the epidermal layer following fertilization.

The question of the presence of cell divisions in this fiber-forming layer during at least a portion of the period of fiber initiation and elongation seems to await confirmatory evidence in the form of photomicrographs of typical division stages. It is also important to consider such evidence as may be available concerning the development of fibers from the daughter cells of these divisions.

MATERIAL AND METHODS

Ovules of every daily stage of development were selected from a single pure bred strain of *Gossypium hirsutum* L. The bolls were collected at approximately the same hour in the early morning in order to insure turgidity of the tissues. Microscopic examinations had indicated that fibers of any age attached to the ovule wall could be well preserved in 1.4 per cent formalin in 70 per cent alcohol. The bolls were dissected in the field and the ovules transferred immediately to the killing and fixing solution. Ovules over 14 days of age were split longitudinally in order to facilitate penetration of the fixing solution. During the examination of the fibers so preserved, the other tissues of the ovules were found to be equally free from distortion. Portions of the material from every daily stage were therefore dehydrated, imbedded in paraffin, and sectioned to thicknesses varying from 4 to 10 microns. Staining with iron haematoxylin and counter-

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FIGURE 1. See legend on opposite page.

staining with safranin and gold-orange revealed the various stages of karyokinesis and cytokinesis indicated in camera lucida sketches (3, p. 454, Fig. 4). The photomicrographs here presented supplement these previously published illustrations with a more complete representation of the protoplasmic changes in the cells concerned.

The technique employed has been surprisingly successful from the cytological standpoint. It is possible, however, that other methods especially designed to bring out details of both chromatic and achromatic figures may produce an even clearer and more complete series of stages. The final description of the process will be deferred therefore until results with various other methods have been obtained.

CELL DIVISIONS

In order to provide some basis for a conception of the large numbers of epidermal cells which may be engaged in division at a given time, the illustrations in both Figure 1 and Figure 2 were chosen from median longitudinal sections of an ovule collected four days after fertilization. Observations of many longitudinal and cross sections of ovules of approximately the same age have indicated that the number of dividing cells in the central region is roughly representative of the average number over the entire surface of the ovule. From the proximal to the distal end of the ovule, at this early period of development, there appears to be an increasing gradient with respect to the number of cell divisions as well as the number of cells engaged in fiber growth initiation. As the ovule increases in age and size, a reversal in the direction of this gradient is brought about and after ten days from the time of fertilization the larger numbers of both dividing and young fiber-forming cells may be found at the proximal end of the ovule.

In Figure 1 A, *b* and *c* show the contrast in appearance of a cell in an early stage of fiber formation and one whose nucleus is in an early stage of division. In Figure 1 A *b* the nucleus is compact and stains deeply. The bulging of the outer wall is accompanied by a drawing away of the more dense protoplasm from the inner wall. Strands of material extend from the central region of the cell in various directions toward the cell wall presenting an appearance suggestive of a loose suspension of the nucleus in the cytoplasmic matrix. These same phenomena, shown also in Figure 2 F *e*, characterize the earliest stage of fiber formation as observed in living material. In the adjacent cell, Figure 1 A *c*, the nucleus is in a very early

FIGURE 1. Nuclear and cytoplasmic behavior in dividing and fiber-forming cells. A *a*, an anaphase; A *b*, young fiber showing dense nucleus and basal vacuole; A *c*, early prophase showing enlarged nucleus; B *a*, prophase in which chromosomes are dispersed in direction of long axis of the cell; B *b*, a metaphase; C *a*, late telophase; C *b*, early telophase showing formation of cell plate; C *c*, late prophase. ×1170.

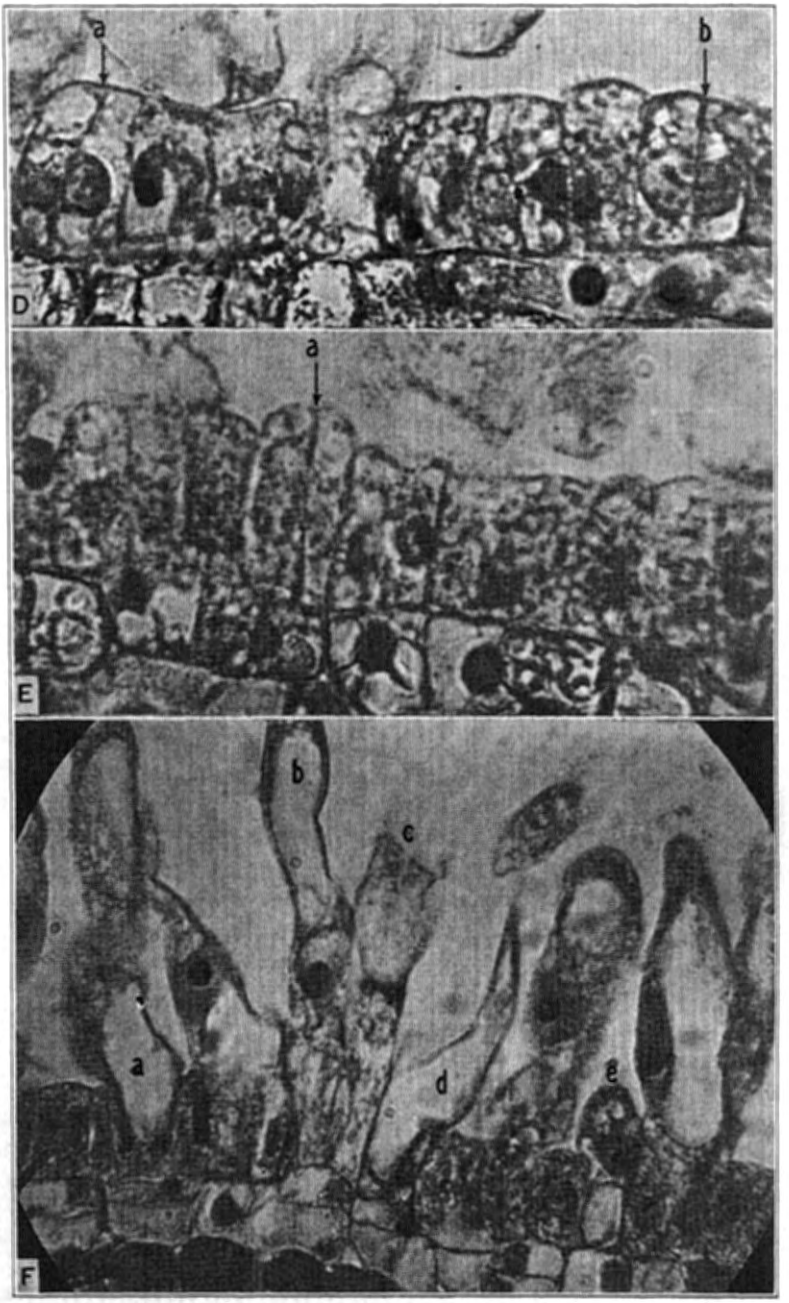


FIGURE 2. See legend on opposite page.

prophase as evidenced by its enlargement. The nuclear membrane and nucleolus have not yet disappeared. The chromatin reticulum may be seen although it is not particularly sharp at this focus.

Figure 1 C *c* represents a later prophase in which the nuclear membrane and the nucleolus have disappeared. The separate chromosomes resulting from the breaking up of the spireme appear to be held in a more or less spherical nucleoplasmic matrix in the central part of the cell. In a still later prophase, Figure 1 B *a*, the chromosome mass is dispersed in the direction of the long axis of the cell. The metaphase shown in poorer focus, Figure 1 B *b*, follows. One stage of anaphase may be seen in Figure 1 A *a*.

A telophase, during the process of cell-plate formation, is shown in Figure 1 C *b*. Figure 2 D *b* illustrates a later telophase in which the cell-plate formation is complete and in which nuclear reorganization is well advanced. A still later telophase as indicated by the nuclear size, shape, and density is shown in Figure 2 D *a*.

FIBER FORMATION

In the variety upon which these observations have been made it seems to be quite certain that initiation of fiber growth continues over a period of 10 or 12 days following fertilization. Available information will not permit, at present, a final statement concerning the formation of these new fibers from daughter cells of divisions which have taken place after the date of flowering. It has not been possible to follow through a process of cell division and fiber growth initiation in living material. If such a sequence were observed, however, one could not be certain that it was representative of the normal procedure within the boll and had not resulted from the abnormal conditions to which the tissue had been subjected.

There are, however, in the paraffin material, large numbers of fiber-forming cells in which the appearance of the protoplasm suggests that they may have been the daughter cells of a recent division. Such an impression is strengthened by the frequent appearance of two cells in a very early stage of fiber formation as shown in Figure 2 E *a*. The nuclei in these cells appear to be in a late telophase, comparable to that shown in Figure 2 D *b*, but not so late as that shown in Figure 2 D *a*. The partition between the two cells has the characteristics of one that is newly formed. The cells have remained attached along this line of separation so that a continuous curve is maintained by the tips of the two young fibers. From such in-

FIGURE 2. Late stages in cell division and certain appearances in early stages of fiber formation. D *a*, late stage of nuclear reorganization; D *b*, early stages of nuclear reorganization; E *a*, early stage of fiber formation in two adjacent cells whose nuclei appear to be incompletely reorganized; F *a*, *b*, *c*, and *d*, lateral wall relationships in the basal portions of fiber-forming cells; F *e*, young fiber showing dense nucleus and basal vacuole. D and E ×1170, F ×660.

stances as this it would seem that the process of cell differentiation may begin even before the process of cell division has been completed. In more advanced stages of fiber formation a similar attachment may be observed for even greater distances as shown in Figure 2 F, *b* and *c*. This appearance is in sharp contrast to that shown in the basal regions of the fibers *a* and *d*, Figure 2 F. These illustrations merely suggest the possibility that the pairs of cells concerned may be sister cells of a very recent division. It is hoped that future study may lead to a correct interpretation of the relationships existing between both fiber-forming and non-fiber-forming cells of the epidermal tissue.

SUMMARY

Typical stages of nuclear and cell division in the epidermal layer of the cotton ovule subsequent to the date of flowering are presented in the form of photomicrographs.

Additional illustrations suggest the possibility that daughter cells of such divisions may take part in the formation of cotton-seed fibers.

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THE FORM OF THE TOXICITY SURFACE FOR COPPER SULPHATE AND FOR SULPHUR, IN RELATION TO CONIDIA OF SCLEROTINIA AMERICANA[1]

S. E. A. McCallan and Frank Wilcoxon

When a toxic agent is permitted to act on a group of individuals, and the percentage of the total number showing some particular response is determined after varying intervals of time, the results may be shown by a toxicity curve in which the percentage response is plotted against time, other variables supposedly being held constant. An alternative method is to observe the percentage response at some fixed time but using various concentrations of the toxic agent. A third method consists in finding the relation between time and concentration for a fixed value of percentage response, for example 50 per cent. Many investigators have studied the curves obtained by these three methods, usually for the purpose of improving the accuracy of biological assays, or in an attempt to deduce the mechanism of toxic action, from the form of the curves obtained. Papers dealing with the error of toxicity determinations have been published by Trevan (14), by Durham, Gaddum, and Marchal (3), and by Burn (2). Theoretical discussions of the form of toxicity curves are given by Powers (9), who considered the time-concentration curve for a fixed per cent mortality, and by Rahn (10), who dealt with mortality-time curves, at a fixed concentration. The subject has also been reviewed by Brooks (1) who points out that the form of toxicity curves has usually been explained either by analogy with the course of a chemical reaction, or by a variation in resistance to the toxic agent among the individuals used in the experiment. It was by this latter hypothesis that Smith (12) interpreted the curves obtained by him for the killing of *Botrytis* spores by phenol. Recently there has been a tendency to include both these factors as simultaneously operating to influence the form of toxicity curves (8, 10).

The relation between the three most important variables, time, concentration, and percentage response, can be best exhibited by a solid model in three dimensions, which defines the *toxicity surface*. A theoretical treatment of toxicity should be capable of explaining the change in the form of this surface in different regions, while a knowledge of this change is also necessary in order to locate the region in which toxicity experiments may be most accurately performed.

The present paper contains the results of a study of the germination of conidia of *Sclerotinia americana*, in relation to time and to the concentration of copper sulphate, this compound being chosen as an example of a

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 58.

soluble toxic substance. A similar study is included in which the toxic agent was sulphur dust, as an example of a relatively insoluble substance which, after reduction by the spores, gives rise to hydrogen sulphide which is believed to be the actual toxic agent.

METHODS

The general method of determining the toxicity of chemicals to fungous spores by means of the moist chamber tests, previously described in detail (5, 16), has been employed. The test fungus was *Sclerotinia americana* (Worm.) Nort. & Ezek., conidia 7 days old being germinated at a temperature of 22° to 25° C. The fungi were cultured at 20° C. prior to the germination tests.

In studying the toxicity of copper sulphate, the spores were placed directly in the solutions, allowance being made for the resulting dilution. The concentrations of sulphur dust were determined by an actual count of particles in the field under observation as described in a former article (16).

To obtain the time curves, germination counts were made at intervals of about 3 hours for the first 15 hours, after which they were taken at longer intervals, the last count being made in all cases after at least 48 hours had elapsed and in some cases as late as 100 hours. For each concentration and each interval of time, 20 microscopic fields with a total of about 600 spores were counted. Each toxicity surface as illustrated is thus the result of observations on 5 concentrations of the toxic agent and 7 to 8 time intervals, or a total of 35 to 40 points.

The solid models were constructed by shaping the germination-time curves for each concentration of toxic agent out of cardboard or thin metal sheets, standing these sheets apart at the required intervals on the concentration axis, and filling in between with plaster of Paris to give the smooth toxicity surface. Each model is based on the results of an individual experiment. Repetitions of these experiments were performed and always gave fundamentally the same results.

TOXICITY SURFACE FOR COPPER SULPHATE

The toxicity surface for copper sulphate is illustrated in Figure 1. It will be seen that the germination-time curves constitute a family of sigmoid curves, the time of most rapid germination occurring later and at a lower germination percentage, the greater the concentration of the toxic agent. The final germination percentage is lower the greater the concentration of toxic agent, and this final value is reached in about 12 hours. These curves resemble the type (a) curves illustrated by Martin (7), and seem to be the type most commonly obtained in toxicity studies. The authors have never encountered the types labelled (b) and (c) by Martin,

where the germination continually increases in the presence of the toxic agent, finally equalling that of the check. It should be remembered, however, that our experiments deal only with *percentage of spore germination* in drops of water with no added nutrient, and that the toxic agent was present throughout the experiment. The germination-concentration curves also constitute a family of sigmoid curves, the point of most rapid change of germination occurring at a lower concentration, the earlier the time. There is very little change in the form of the curves after 12 hours.

The curves showing the relation between time and concentration for a fixed germination percentage are not sigmoid but resemble hyperbolas.

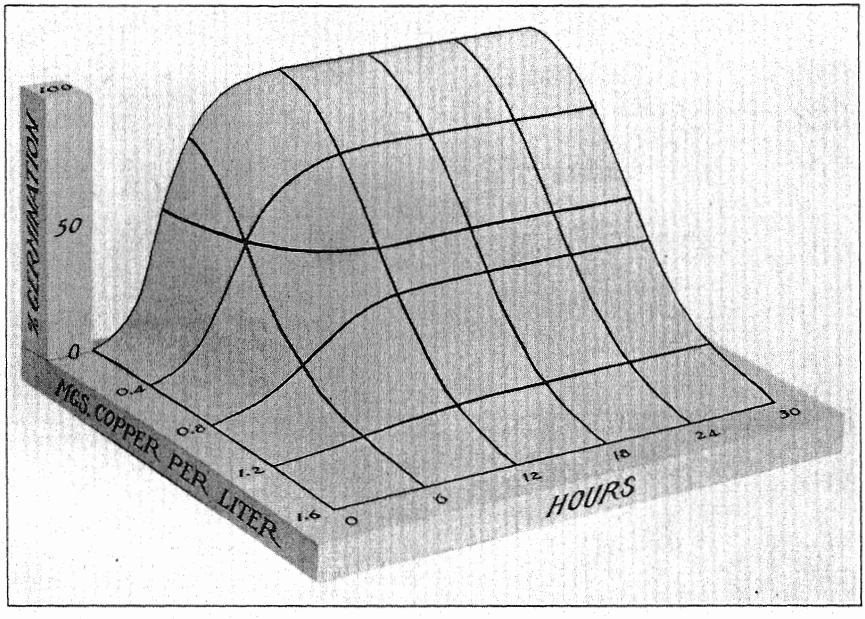


FIGURE 1. The toxicity surface for the action of copper sulphate on conidia of *Sclerotinia americana*.

They indicate such a relation between time and concentration that the same effect can be produced by a low concentration in a short period of time, or by a high concentration in a long period of time.

We may consider these curves to be quite typical since similar ones have been obtained by investigators in other fields. Trevan (14) and also Burn (2) give mortality-concentration curves for animals treated with various drugs which correspond to our germination-concentration curves. Mortality-time curves having the general characteristics of our germination-time curves have been found by several investigators in the case of insects treated with fumigants. Finally the time-concentration curves re-

semble those obtained by Powers (9) and by Gersdorff (4) in the case of goldfish treated with various toxic agents.

There is therefore nothing in the form of this toxicity surface which is peculiar to the germination of fungous spores, but it illustrates the manner in which soluble toxic agents in general affect the response of a large number of living organisms. Most attempts which have been made to explain the form of toxicity curves have dealt with only one of the three related sets of curves. A comprehensive theory should, however, be capable of explaining not only the form of one of the curves of a set, but also the form of the toxicity surface as a whole.

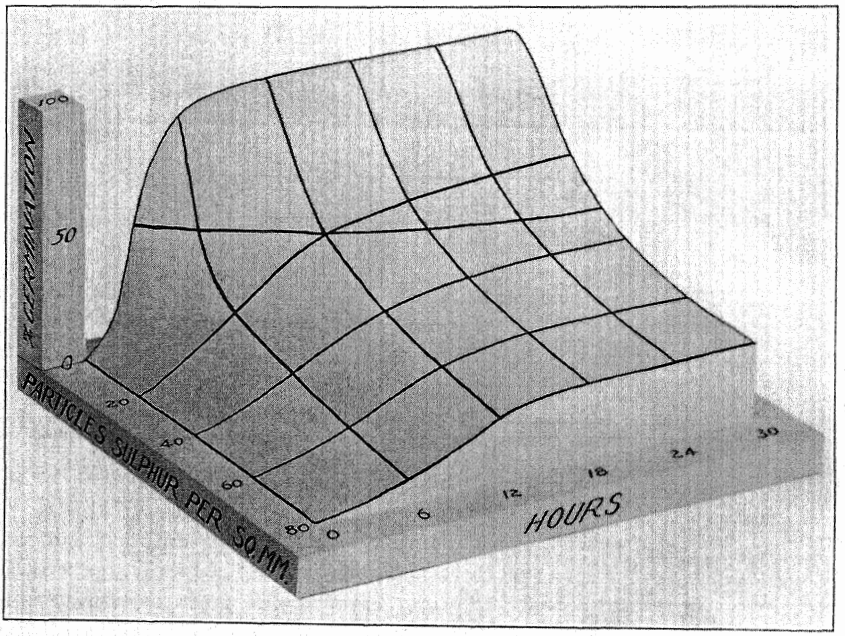


FIGURE 2. The toxicity surface for the action of sulphur dust on conidia of *Sclerotinia americana*.

TOXICITY SURFACE FOR SULPHUR DUST

In the case of sulphur dust toxicity surface (Fig. 2) the germination-time curves resemble those for copper sulphate, but the germination-concentration curves are much less symmetrical. The effect of small concentrations of dust is very marked, but as the concentration increases the effect becomes less and less until finally a nearly constant germination is reached. This leads also to a difference in the form of the time-concentration curves which vary in shape at different germination levels. Evidence

has been obtained that the sulphur itself is not the actual toxic agent (5) and this factor may give rise to the differences in the surfaces.

PRECISION OF TOXICITY DETERMINATIONS

Various investigators have stressed the importance of working at certain points on a toxicity curve in order to obtain the most accurate results with the minimum number of individuals. Tattersfield and Morris (13) state that most accurate results are obtained by working near 50 per cent mortality with mortality-time curves. Burn (2) has also suggested that the point of 50 per cent mortality is the most accurate region when dealing with mortality-concentration curves. Powers (9) has studied the time-concentration curves for a fixed mortality, and has derived an expression for toxicity based on the form of this curve within certain limits of time and concentration. Richardson (11) has compared the accuracy of working with mortality-concentration curves and the curves when 50 per cent of the house flies were paralyzed at various times and concentrations, and states that the latter method is capable of greater precision than the former, in the cases observed by him.

These various methods can be best compared by considering the properties of the toxicity surface. If we know the characteristic surface for the action of a given toxic substance on a given species, the error of a toxicity determination expressed in terms of concentration may be estimated for any region of the surface. This error depends on the standard deviation of the binomial series, that is $\sqrt{Npq}$, where N is the number of individuals counted, p and q represent the fraction of the spores germinated and ungerminated respectively; it depends also on the slope of the germination-concentration curve by which the standard deviation must be divided in order to find the error in estimating the concentration, which arises from this standard deviation. For example, suppose we observe that in a certain copper sulphate solution the germination percentage is 36 after 30 hours, 100 spores being counted. This corresponds to a concentration of 0.8 mg. copper per liter. What is the error in estimating this concentration? The standard deviation of the percentage germination, assuming the theoretical binomial error which has been shown to be justified in these experiments (6), is $\sqrt{100 \times .36 \times .64}$ or 4.8. The slope at this point is 1.02 as estimated with a protractor; therefore the error in estimating the concentration is $\frac{4.8}{1.02 \times 100}$, or 0.047 mg., the factor 100 occurring in the denominator because the value of one unit of germination is one hundred times that of one unit of concentration in the model as constructed. If we wish to express the error as a percentage, we must divide the error in concentration by the concentration itself which is 0.8 mg., giving $\frac{0.047 \times 100}{0.8}$

or 5.9 per cent for the percentage error in concentration arising from the uncertainty in estimating the germination percentage. Two such error curves for copper sulphate and for sulphur, derived from the solid models mentioned previously, are illustrated in Figure 3. It is evident that the accuracy of an experiment may vary widely depending on the material used and the particular germination percentage at which an experiment is performed. In general extremely high or low germination percentages should be avoided, but the most accurate region is not necessarily at 50 per cent germination as implied by Wardle (15, p. 145) and others (2, 11, 13).

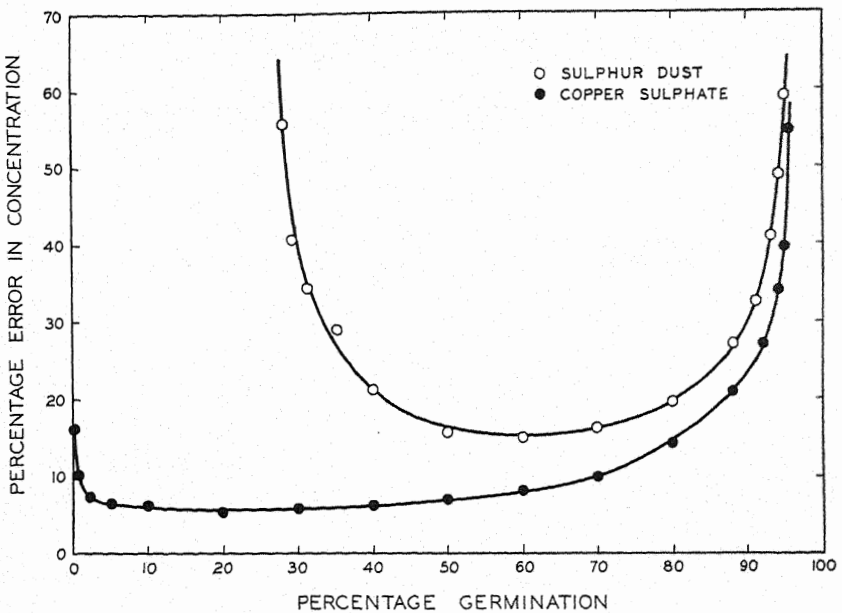


FIGURE 3. The relation between the percentage error of the experiment expressed in terms of concentration and the per cent germination obtained, when conidia of *Sclerotinia americana* are exposed to the toxic agents, sulphur dust and copper sulphate solution. These curves are derived from the solid models shown in Figures 1 and 2, assuming the use of 100 spores for each point.

The toxicity experiment might be performed in another manner. The time required to permit 50 per cent germination might be determined and by reference to the horizontal time-concentration curve for 50 per cent germination the concentration might be estimated. In order to estimate the error in this case the standard deviation, $\sqrt{100 \times .5 \times .5}$, or 5 must be divided by the slope of the germination-time curve at this point, and then by the slope of the time-concentration curve in order to obtain the

corresponding error in concentration. This gives exactly the same result as dividing 5 by the slope of the germination-concentration curve at this point directly, because the slope $\frac{\text{germination}}{\text{concentration}} = \text{slope}\frac{\text{germination}}{\text{time}} \times \text{slope}\frac{\text{time}}{\text{concentration}}$ at any given point on the surface.

Hence at a given point of the surface it is immaterial whether we compare two toxic agents by finding the time required in each case for a fixed percentage germination, or the percentage germination at a definite time, insofar as the accuracy of the result is concerned. However, in toxicity experiments in different fields one method may be more convenient than the other.

Another point to be considered when comparing the toxicity of one substance with another is the fact that the toxicity surface for one substance may intersect that for another, so that in one region A may be more toxic than B while in another region the reverse may be true. Only in case the toxicity surface for substance A lies wholly within that for substance B can we make the general statement that A is more toxic than B.

SUMMARY

1. The various types of curves encountered in toxicity studies are discussed and the relation between them is shown by means of a solid model, or toxicity surface in which the three coordinates are time, concentration of toxic agent, and percentage of organisms exhibiting a certain response.

2. The form of the toxicity surface has been determined for the germination of conidia of *Sclerotinia americana* in the presence of copper sulphate, as an example of a soluble toxic agent, and of sulphur dust, as an example of an insoluble one.

3. It is shown that the precision of a toxicity experiment depends not only on the number of organisms used but also on the form of the toxicity surface, and a knowledge of the latter makes it possible to decide in what region to work in order to obtain the greatest precision. It is found that high precision is impossible if the percentage germination is very high or very low, but the point of minimum error will vary depending on the form of the toxicity surface.

4. Whether comparisons of toxic agents are made on the basis of the times required for an equal percentage response, or on the basis of percentage responding in equal times, is largely a matter of convenience, for at a given point on the toxicity surface both methods are capable of equal precision.

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BASES FOR CALCULATIONS IN MEASURING CHANGES IN LEAVES DURING THE NIGHT

F. E. DENNY

Previous papers (2, 3) have dealt with the importance of a suitable basis for the computation of results when measurements of the diurnal changes which occur in leaves are being made. Emphasis has been placed upon the unsuitability of dry weight or fresh weight as a basis for these calculations. Favorable results were obtained by the use of the twin-leaf and the residual-dry-weight methods.

This is a report of additional experiments on the same problem. Not only were samples obtained showing the total change from night until the next morning, but samples were collected also at approximately 9:00 p.m. and 2:00 a.m., thus permitting a more complete story of the changes during the night.

The results corroborate the previous reports in showing that the twin-leaf and residual-dry-weight methods can be depended upon to furnish a correct measure of the changes in various constituents. It is shown, also, that in the present experiments the total nitrogen could serve as a basis for computation, and that the values computed on this basis agreed well with those found by the other methods.

METHODS

Two varieties of beans were used, *Phaseolus vulgaris* L., varieties Henderson's New Stringless and Cutshort, and one variety of peanut, *Arachis hypogaea* L. variety Virginia Bunch. The plants were full grown at the time of sampling; some of the pods were nearly fully formed and others were in various earlier stages of development. Samples of leaves were taken at intervals from about 6:00 p.m. September 9, 1931, to about 6:00 a.m. the following morning. In the afternoon of the 9th of September, 300 leaves of each variety were marked with tags having identification characters to show that 100 represented samples to be taken at the beginning and at the end of the period from approximately 6:00 p.m. to 9:30 p.m., 100 to be taken for the interval from 6:00 p.m. to 2:00 a.m., and 100 for the interval from 6:00 p.m. to 6:00 a.m. Since it was not possible to gather the samples at the exact time periods shown above, sampling began at 5:40 p.m. and ended at 6:40 a.m., the approximate time for each sample being shown in the various tables of results.

In selecting the leaves, care was taken to choose only those that had opposite leaflets which appeared to be equal in size and to be fully grown. In the case of beans one lateral leaflet was taken from each trifoliate leaf at the beginning of the sampling period and the other lateral was left for

the sample at the end of the period; thus, there were 100 leaflets in each sample, and there were six samples of each bean variety. In the case of peanut three leaflets were taken from each leaf at the beginning, and the opposite three at the end; thus, there were 300 peanut leaflets in each sample and six samples in all. Care was also taken to equalize any difference in the leaflets due to a previous position with respect to the sun, by alternately taking right and left leaflets in the first sample for each period. Leaf-blades only were used, the petioles being discarded.

Fresh weights of the samples were obtained for the early evening samples, but by 9:30 p.m. and especially by 2:00 and 6:00 a.m. the leaves were so wet by dew or guttation liquid that fresh weights became meaningless. Within a few minutes after removal from the plants the leaf samples were dropped into tared beakers containing boiling 95 per cent ethyl alcohol. Subsequently, the alcohol was evaporated off slowly on a steam bath, and after drying in an electric vacuum oven regulated at 70° the dry weight was obtained. The tissue was then ground with a mortar and pestle until it would pass through a 60-mesh sieve. After again being dried, portions of this leaf powder were weighed out for the analysis.

The weighed portions of leaf tissue were extracted seven times successively with hot 70 per cent ethyl alcohol (by volume) in a 100 cc. Pyrex test tube, being centrifuged and decanted after each extraction. The alcoholic solutions from the various extractions of each sample of leaf powder were combined and aliquots were taken for the soluble nitrogen and for total sugar. For the soluble nitrogen the method of Pucher, Leavenworth, and Vickery (5), which includes nitrate nitrogen, was used. For the sugar determinations neutral lead acetate was added to the aqueous solution obtained after evaporation of the alcohol and the excess lead was removed with potassium oxalate. Total sugar was determined on this liquid after inversion with hydrochloric acid in the cold (1, p. 95). For the sugar determinations the Munson and Walker method was used (1, p. 78) and the cuprous oxide was titrated with a potassium permanganate solution which had been standardized with a sugar solution of known concentration. The total sugar was expressed as invert sugar and the values include all reducing substances which are soluble in 70 per cent alcohol and in water. The residues remaining after extraction with 70 per cent alcohol were used for the insoluble nitrogen and polysaccharide determinations. Insoluble nitrogen was determined by the Gunning method (1, p. 7). The polysaccharides were estimated by the acid hydrolysis method (1, p. 95) and represent the substances insoluble in 70 per cent alcohol and hydrolyzable by acid; they were calculated as starch, and although starch was the principal constituent in this fraction, other substances also were present. Total carbohydrate in this paper represents the sum of the polysaccharides and the sugar, and the residual-dry-weight value was

obtained by subtracting the total carbohydrate from the dry weight. Total nitrogen was obtained by adding the values for insoluble and soluble nitrogen.

EXPERIMENTAL RESULTS

ANALYSES OF POWDERED LEAVES

The results of the analyses of the powdered leaves are shown in Table I, in which the composition is expressed as the percentage of the dry weight. These values *cannot* be used to obtain a dependable measure of the changes that occurred in the leaves during the time intervals shown in

TABLE I

ANALYSES OF POWDERED LEAVES

Plant	Time	Per cent of the dry weight			
		Polysacch.	Total sugars	Insol. N	Sol. N
Stringless bean	5:40 P.M.	14.76	2.79	4.01	0.38
	9:20 P.M.	11.62	2.47	4.17	0.46
	6:30 P.M.	13.81	2.45	4.06	0.40
	2:00 A.M.	7.73	2.28	4.32	0.48
	7:20 P.M.	13.61	2.19	3.98	0.42
	5:45 A.M.	5.67	1.72	4.29	0.48
Cutshort bean	5:50 P.M.	18.18	2.52	4.19	0.24
	9:30 P.M.	14.07	2.02	4.29	0.30
	6:40 P.M.	16.79	2.21	4.27	0.31
	2:20 A.M.	8.50	1.94	4.73	0.37
	7:30 P.M.	15.06	1.80	4.32	0.39
	6:00 A.M.	6.60	1.09	4.76	0.46
Peanut	6:00 P.M.	24.06	2.96	3.22	0.42
	9:40 P.M.	23.17	2.60	3.23	0.44
	6:50 P.M.	26.45	2.48	2.94	0.34
	2:40 A.M.	23.92	2.20	3.03	0.35
	7:40 P.M.	25.55	2.63	2.92	0.34
	6:40 A.M.	22.02	2.31	3.09	0.33

Note: The paired horizontal lines represent results from paired leaflets, e.g., the sample at 5:40 P.M. was obtained by taking one leaflet and the one at 9:20 P.M. the opposite leaflet from each pair of leaflets.

column 2. It is true that columns 3 and 4 indicate that the polysaccharides and sugars decreased during each one of the intervals; but such data cannot show how much change occurred; that is, they cannot give a correct estimate of the percentage loss during the interval. Thus, column 4, if used to calculate the percentage change of sugars in Stringless bean indicates that the change from 5:40 p.m. to 9:20 p.m. was from 2.79 to 2.47, which would be a percentage loss of 11.5 per cent, likewise the value from

6:30 p.m. to 2:00 a.m. would be 6.9 per cent, and from 7:20 p.m. to 5:45 a.m. it would be 21.4 per cent. But the values calculated on the twin-leaf, residual-dry-weight, and total nitrogen bases, as shown in later paragraphs, give values of about 15, 14, and 30 per cent for the losses in sugar during these same intervals. This error in the calculated losses by the use of the dry weight basis need not be regarded as serious in this particular case, but the nitrogen values in columns 5 and 6 show how erroneous the conclusion could become if only the dry weight basis were used in the calculation. The dry weight percentages indicate gains in nitrogen during nearly all of the time intervals. But when the nitrogen is calculated upon more dependable bases, such as twin-leaf and residual-dry-weight, no changes in nitrogen, or at least only slight and inconclusive changes, were found. In such cases, therefore, the dry weight basis would give a result which would be neither quantitatively nor even qualitatively correct. The errors in using the dry weight as a basis arise from the fact that the dry weight itself is continuously changing during the night, and hence it cannot be used as a dependable denominator in determining the ratios representing the values which we list as percentage changes.

TWIN-LEAF BASIS FOR COMPUTING CHANGES

Since the dry weight of the entire sample of leaves at each sampling period was obtained, the percentage values in Table I can be used to determine the total weight of the various constituents in all the leaves of each sample. And since in taking the samples paired leaflets were used we can determine the total amount at the beginning and at the end of each period; these values will then be directly comparable, and the percentage loss or gain can be computed at once. The values for the total amounts of constituents in the leaflets of each sample are shown in Table II. For example, the dry weight of the 100 leaves of Stringless bean of the 5:40 p.m. sample was 19.928 g.; the twin-leaves which were left on until 9:20 p.m. and then removed had a dry weight of 19.108 g.; the loss during the interval was, therefore, 0.820 g., which is 4.1 per cent of the amount at the beginning of the period. This will show how the values in Table II may be used to obtain a dependable estimate of the rate of change of the various constituents in the three species used in the experiment. More complete data regarding the changes found in the leaves by the twin-leaf method are shown in Table V, columns 3 to 5. But a mere inspection of Table II shows that losses in dry weight, sugar, and polysaccharides occurred, and that these losses were greater for beans than for peanuts; this inspection also shows that the nitrogen values were nearly the same at the beginning and at the end of each sampling period.

The values in Table II may be used to determine whether the residual-dry-weight of the twin-leaf samples was a constant. The residual-dry-

weight, first proposed by Mason and Maskell (4) as a basis for computing analytical results, is the value obtained by subtracting the total carbohydrate from the dry weight. Let us apply this procedure to the values in Table II. Starting with the Stringless bean sample at 5:40 p.m., the residual-dry-weight is $19.928 - 3.497 = 16.431$, and for the corresponding twin-leaves taken at 9:20 p.m. it is $19.108 - 2.692 = 16.416$. In like manner the residual-dry-weights may be determined for all the paired samples. The values obtained in this way are shown in Table III. In column 4 is

TABLE II
DATA FOR COMPUTING ON TWIN-LEAF BASIS

Plant	Time	Amount in entire sample						
		Dry wt., g.	Poly-sacch., g.	Total sugar, mg.	Total carbo-hydr., g.	Insol. N, mg.	Sol. N, mg.	Total N, mg.
Stringless bean	5:40 P.M.	19.928	2.941	556	3.497	799	76	875
	9:20 P.M.	19.108	2.220	472	2.692	797	88	885
	6:30 P.M.	19.674	2.717	482	3.199	799	79	878
	2:00 A.M.	18.110	1.399	413	1.812	782	87	869
	7:20 P.M.	19.885	2.706	435	3.141	791	84	875
	5:45 A.M.	17.667	1.001	304	1.305	758	85	843
Cutshort bean	5:50 P.M.	11.265	2.048	284	2.332	472	27	499
	9:30 P.M.	10.903	1.534	220	1.754	468	33	501
	6:40 P.M.	11.504	1.931	254	2.185	491	36	527
	2:20 A.M.	10.510	0.893	204	1.097	497	39	536
	7:30 P.M.	11.570	1.742	208	1.950	500	45	545
	6:00 A.M.	10.583	0.698	115	0.813	504	49	553
Peanut	6:00 P.M.	12.157	2.925	360	3.285	391	51	442
	9:40 P.M.	11.942	2.767	310	3.077	386	53	439
	6:50 P.M.	12.962	3.428	321	3.749	381	44	425
	2:40 A.M.	12.568	3.006	276	3.282	381	44	425
	7:40 P.M.	11.299	2.887	297	3.184	330	38	368
	6:40 A.M.	10.840	2.387	250	2.637	335	36	371

shown the percentage total deviation between the two values. The average error is about 1.2 per cent. The correspondence of values is good enough to justify the conclusion that the residual-dry-weight is a constant; it also increases the confidence we can have that the paired leaflets selected for the twin-leaf samples were sufficiently identical to form a good basis for a direct comparison of the total amounts of constituents found in them.

RESIDUAL-DRY-WEIGHT BASIS FOR COMPUTING CHANGES

Although it would be possible to recalculate the data on the basis of the residual-dry-weights obtained in the manner described in the preceding paragraph, we can obtain a figure for residual-dry-weight which is independent of the twin-leaf totals for dry weight, if we obtain it from the original analysis of the leaf powder. For example, referring again to Table I, columns 3 and 4, we find that for each gram of dry weight (Stringless bean at 5:40 p.m.) there was 0.1476 g. of polysaccharide and 0.0279 g. of

TABLE III

CONSTANCY OF RESIDUAL-DRY-WEIGHTS

Plant	Time	Residual-dry-weight	% Deviation
Stringless bean	5:40 P.M.	16.431	0.1
	9:20 P.M.	16.416	
	6:30 P.M.	16.475	1.1
	2:00 A.M.	16.298	
	7:20 P.M.	16.744	2.3
	5:45 A.M.	16.362	
Cutshort bean	5:50 P.M.	8.933	2.4
	9:30 P.M.	9.149	
	6:40 P.M.	9.319	1.0
	2.20 A.M.	9.413	
	7:30 P.M.	9.620	1.6
	6:00 A.M.	9.770	
Peanut	6:00 P.M.	8.872	0.1
	9:40 A.M.	8.865	
	6:50 P.M.	9.213	0.8
	2:40 A.M.	9.286	
	7:40 P.M.	8.115	1.1
	6:40 A.M.	8.203	

total sugar; this gives 0.1755 g. of total carbohydrate, and consequently the residual-dry-weight per gram of dry weight is $1.000-0.1755=0.8245$; therefore the dry weight corresponding to one gram of residual-dry-weight is $1\div 0.8245=1.213$. It is in this way that the values in Table IV were obtained. All the constituents have been brought to a standard basis for comparison, i.e., to the amount of constituent associated with one gram of residual-dry-weight. And since it has been shown in the previous section of this paper, and also in a previous paper (3), that the residual-dry-weight is constant, the values in Table IV, showing at various sampling periods the amounts of constituents associated with one gram of residual-dry-weight, are at once directly comparable. We may proceed to take the differences between the values of the comparable samples and calculate

this difference as a percentage of the amount present at the beginning of the period of observation. For example, for Stringless bean at 5:40 p.m. the dry weight was 1.213 g., and at 9:20 p.m. it was 1.164 g. per gram of residual-dry-weight. The loss during the period was, therefore, 0.049 g. which is 4.0 per cent of 1.213. Changes in various constituents may be measured in this way. Several calculations have been made using the values in Table IV and these are shown in Table V, columns 6 to 8. Further discussion of these values will be postponed until Table V is reached.

TABLE IV

DATA FOR COMPUTING ON RESIDUAL-DRY-WEIGHT BASIS

Plant	Time	Amount per gram of residual-dry-weight						
		Dry wt., g.	Poly-sacch., mg.	Total sugar, mg.	Total carbo-hydr., mg.	Insol. N, mg.	Sol. N, mg.	Total N, mg.
Stringless bean	5:40 P.M.	1.213	179.0	33.8	212.8	48.63	4.61	53.24
	9:20 P.M.	1.164	135.3	28.7	164.0	48.53	5.35	53.88
	6:30 P.M.	1.194	164.9	29.3	194.2	48.48	4.78	53.26
	2:00 A.M.	1.111	85.9	25.3	111.2	48.00	5.33	53.33
	7:20 P.M.	1.188	161.6	26.0	187.6	47.27	4.99	52.26
	5:45 A.M.	1.080	61.2	18.6	79.8	46.32	5.18	51.50
Cutshort bean	5:50 P.M.	1.261	229.2	31.8	261.0	52.84	3.03	55.87
	9:30 P.M.	1.192	167.7	24.1	191.8	51.13	3.58	54.71
	6:40 P.M.	1.235	207.3	27.3	234.6	52.72	3.83	56.55
	2:20 A.M.	1.117	94.9	21.7	116.6	52.81	4.13	56.94
	7:30 P.M.	1.203	181.1	21.7	202.8	51.96	4.69	56.65
	6:00 A.M.	1.083	71.5	11.8	83.3	51.57	4.98	56.55
Peanut	6:00 P.M.	1.370	329.7	40.6	370.3	44.12	5.76	49.88
	9:40 P.M.	1.347	312.1	35.0	347.1	43.51	5.93	49.44
	6:50 P.M.	1.407	372.2	34.9	407.1	41.37	4.78	46.15
	2:40 A.M.	1.354	323.8	29.8	353.6	41.01	4.74	45.75
	7:40 P.M.	1.392	355.8	36.6	392.4	40.66	4.73	45.39
	6:40 A.M.	1.322	291.0	30.5	321.5	40.84	4.36	45.20

TOTAL NITROGEN BASIS FOR COMPUTING CHANGES

From both Tables II and IV it is seen that the total nitrogen content has not changed significantly. If this is the case nitrogen should form a good basis for computing the changes in the various constituents, and should give essentially the same result as that on either the twin-leaf or residual-dry-weight basis. In order to compute the various constituents on the total nitrogen basis we turn again to Table I. At 5:40 p.m. there were $4.01+0.38=4.39$ grams of total nitrogen associated with 100 grams of dry

weight, 14.76 g. of polysaccharide, 2.79 g. of total sugar, and 17.55 g. of total carbohydrate. Thus, at 5:40 p.m. there were $17.55 \div 4.39 = 3.996$ g. of total carbohydrate per gram of total nitrogen; and at 9:20 p.m. there were $(11.62+2.47) \div (4.17+0.46) = 3.042$ g. of total carbohydrate per gram of total nitrogen. The loss during the period was 0.954 g. of total carbohydrate, which is 23.9 per cent of the amount present at the beginning of the period. In this way the percentage changes in dry weight, total

TABLE V

COMPARISON OF DIFFERENT METHODS OF COMPUTING CHANGES

Plant	Period	Per cent loss								
		Twin-leaf basis			Residual-dry-weight basis			Total nitrogen basis		
		Dry wt.	Total carbohydr.	Total sugar	Dry wt.	Total carbohydr.	Total sugar	Dry wt.	Total carbohydr.	Total sugar
Stringless bean	5:40 P.M. to 9:20 P.M.	4.1	23.0	15.1	4.0	22.9	15.1	5.2	23.9	16.1
	6:30 P.M. to 2:00 A.M.	8.0	43.3	14.3	6.9	42.7	13.8	7.1	42.8	13.6
	7:20 P.M. to 5:45 A.M.	11.1	58.3	30.2	9.1	57.5	28.5	7.8	56.9	27.6
Cutshort bean	5:50 P.M. to 9:30 P.M.	3.2	24.8	22.5	5.5	26.6	24.2	3.4	24.3	21.6
	6:40 P.M. to 2:20 A.M.	8.6	49.7	19.6	9.5	50.3	20.5	10.0	50.7	21.2
	7:30 P.M. to 6:00 A.M.	8.5	57.2	44.6	10.0	58.8	45.6	9.8	58.8	45.4
Peanut	6:00 P.M. to 9:40 P.M.	1.8	6.3	13.9	1.7	6.3	13.7	0.8	5.6	12.9
	6:50 P.M. to 2:40 A.M.	3.0	12.4	14.0	3.8	13.1	14.6	3.0	12.7	14.0
	7:40 P.M. to 6:40 A.M.	4.1	17.2	15.8	5.0	18.0	16.7	4.7	18.0	16.3

carbohydrate, and total sugar have been determined using total nitrogen as a basis, and the values are shown in Table V, columns 9 to 11.

COMPARISON OF DIFFERENT METHODS OF COMPUTING CHANGES

Three different bases (twin-leaf, residual-dry-weight, and total nitrogen) were used in computing changes in leaves, and Table V brings these results together for comparison. The percentage losses in dry weight, total carbohydrate, and total sugar by each method of computing are shown for

each time interval. It is seen that good agreement was obtained. This could occur only under the three following conditions: (a) that the twin-leaf samples were closely agreeing pairs at the time of their selection as pairs; (b) that the residual-dry-weight was a constant; (c) that the total nitrogen did not change during the period of sampling. If any one of these conditions had not been fulfilled the fact would have been disclosed by non-agreement of the values by this method with those of the other methods.

It may seem to the reader that since the computations by these three methods are derived from the same analytical values, the methods, in one sense, are not independent of each other and that consequently the three methods might be expected to give essentially the same results provided no arithmetical errors are made.

It is true that all computations start with the analyses of the leaf powders, but from that point onward the three methods become independent. The twin-leaf method takes into account the total amounts in all of the leaves of each sample, while the residual-dry-weight method is independent of these total amounts and deals only with the weight of the aliquots of the leaf powder taken for the analyses. Hence, the error which might arise in the twin-leaf method, by inequality of the paired leaves at the start of the sampling period, is entirely avoided by the residual-dry-weight method, at least so far as the present experiments are concerned.

It is clear that the nitrogen values are entirely independent of both, since neither the total amount of dry weight in the sample of leaves, nor the residual-dry-weight of the aliquot of powder taken for analysis play any part in the computation of the amount of any constituent on the total nitrogen basis.

PROGRESSIVE CHANGES DURING THE NIGHT

Changes in polysaccharides are shown in Figure 1 which has been prepared by using the data in Tables I, II, and IV, calculating the percentage changes by the twin-leaf, residual-dry-weight, and total nitrogen methods, and plotting the value obtained by averaging the results from the three methods. It is seen that a rather steady fall in polysaccharides occurred in the three tissues, being, however, much more rapid in the beans than in the peanut. About one-fourth of the polysaccharides that were present in bean leaves at 6:00 p.m. was lost by about 9:30 p.m., and by 6:00 a.m. the loss was about 60 per cent of the evening values.

In the preceding paragraphs the changes have been expressed as percentage losses from the beginning to the end of the various sampling periods. We may obtain curves for the entire period from night to morning, showing the amount of any constituent on any one of the three bases (twin-leaf, residual-dry-weight, or total nitrogen) by plotting the values for any constituent at the different periods during the night. This has been

done in Figure 2 for total carbohydrate and in Figure 3 for total sugar. The residual-dry-weight values were taken from Table II and the total nitrogen values from Table I. The values on the residual-dry-weight and total nitrogen bases are plotted on the same scale, and it will be seen that the agreement between them is very good. For total carbohydrate a smooth curve showing, in the case of the bean leaves, a continuous fall in the amount from night to morning was obtained. The curve for peanut is less satisfactory and the agreement between the two bases was not as good. The total carbohydrate curve for peanut shows a rise from 6:00 to 7:00 p.m., but this depends upon the accuracy of the 6:00 p.m. sample, and until the experiment is repeated too much reliance need not be placed upon it.

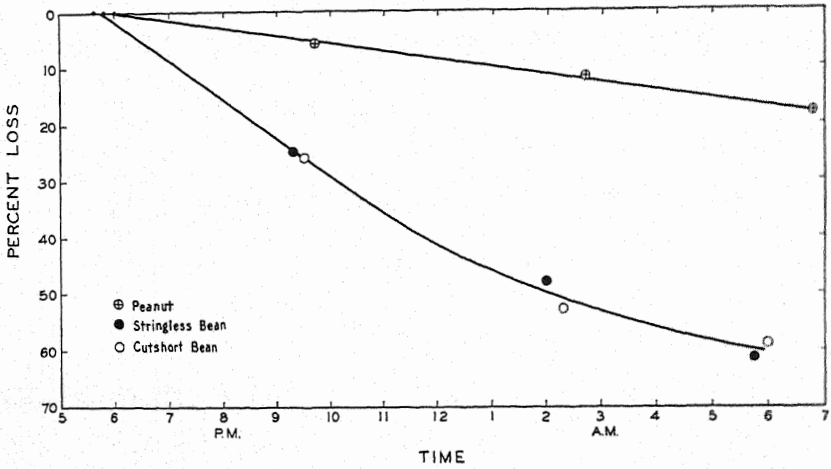


FIGURE 1. Per cent loss of polysaccharides (alcohol insoluble acid hydrolyzable substances, including starch) of peanuts and beans at intervals from night to morning; percentage computed on the basis of the amount present in the initial sample taken in the evening.

The curves showing the total sugar values are given in Figure 3. They indicate that there was an actual increase in the amount of sugar in the leaves in the early portion of the evening. This increase occurred at about 7:30 to 9:30 p.m. for the beans and at a slightly earlier period for peanut. Possibly these rather small changes in the shapes of the curves are not really significant. But the shapes of the curves are so similar for the three types of tissue, and the rise in the sugar curve occurs at so nearly the same time, that it seems justifiable to call attention to the possibility that the curves represent an actual occurrence. The amount of sugar that is present at any time is a resultant of three factors acting simultaneously: (a) rate of formation of sugar either by synthesis or by hydrolysis of stored starch; (b) rate of respiration; (c) rate of translocation. It is not difficult to inter-

pret the sugar curves on the following basis: that rapid loss of sugar occurred in the relatively warm hours of the early evening because of high respiration and because starch hydrolysis did not keep pace with sugar loss, that in the later and cooler hours the hydrolysis of starch furnished sufficient sugar to more than offset the rate of loss by respiration and translocation. This would have caused a rise in the sugar curve. However, this is merely an interpretation, and it is offered to show that the shape of the sugar curves represents a condition that could have occurred.

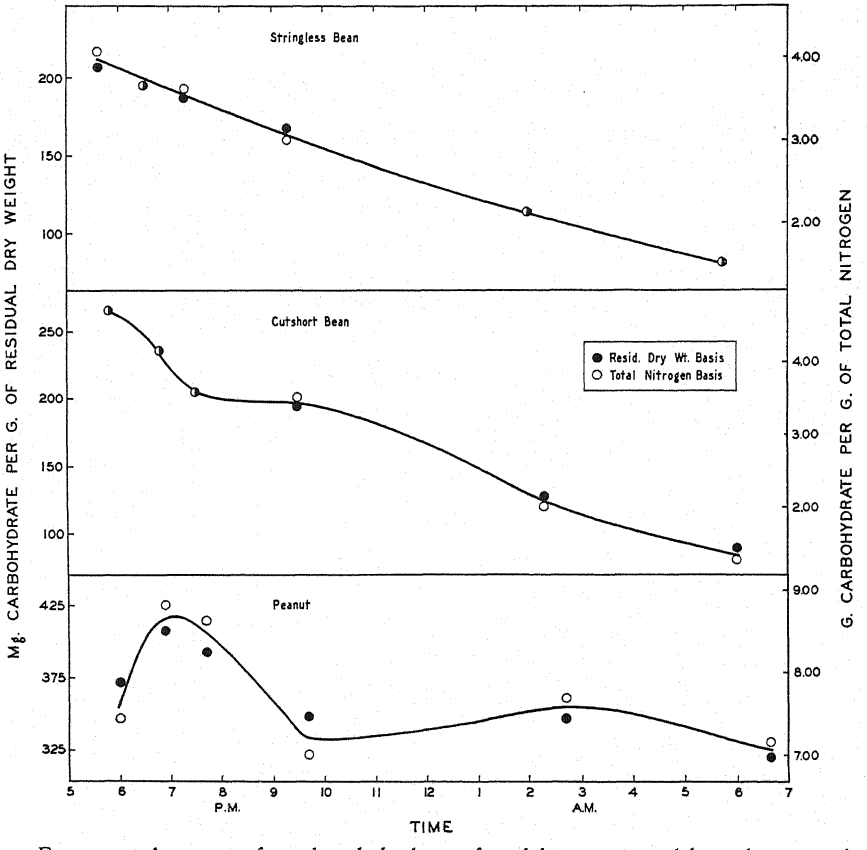


FIGURE 2. Amounts of total carbohydrates found in peanut and bean leaves at intervals during the night. Comparison of residual-dry-weight and total nitrogen as bases for calculating the change in total carbohydrates.

DISCUSSION

It should be emphasized that these results were obtained with fully grown leaves. The measurement of the changes in young leaves which are in the phases of enlargement and differentiation represents a different

problem. Probably neither the residual-dry-weight nor the total nitrogen would be a suitable basis for calculation in such a case, but it seems likely that the twin-leaf basis could be used satisfactorily.

It is not suggested that the total nitrogen could be used generally in all experiments as a basis for computing changes in other constituents. It could be used only in cases in which it was known that the nitrogen did not change during the course of the experiment. In the present experiments

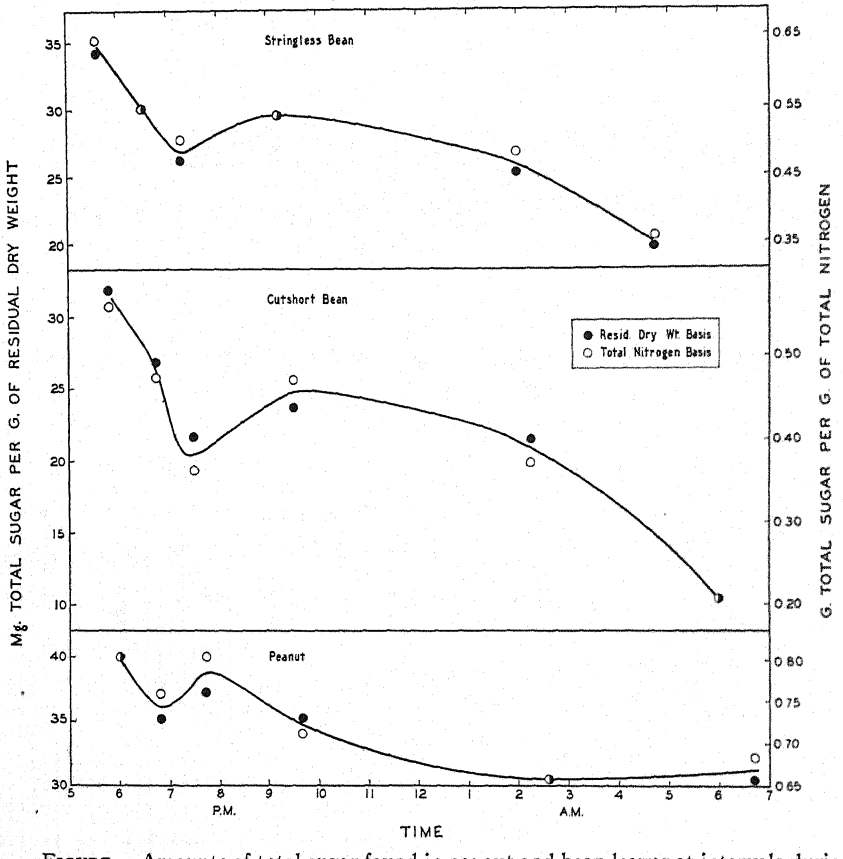


FIGURE 3. Amounts of total sugar found in peanut and bean leaves at intervals during the night. Comparison of residual-dry-weight and total nitrogen as bases for calculating the change in total sugars.

it was shown by both the twin-leaf and residual-dry-weight methods that the total nitrogen did not change materially during the period of sampling, and it is only because of this uniformity in the amount of this constituent that it was justifiable to use the total nitrogen as a basis. But in any experiment under conditions in which the nitrogen does not remain constant it could not be used as a basis. Thus, if the tissues are in active growth so

that new tissue is being formed, or, if conditions are found under which nitrogen is being transported from the leaf in appreciable amounts to other portions of the plant, in such cases the nitrogen would be as unsuitable as dry weight, fresh weight, or any other changing fraction in serving as a basis for computation.

Consequently, in order to use total nitrogen as a basis for expressing the amounts of other constituents, enough preliminary work with the particular tissue used in the experiment would need to be carried out in order to be certain that the amount of this fraction was remaining essentially constant. When this fact is once established, it is likely that total nitrogen would be found to be a convenient basis; it can be more accurately determined than the residual-dry-weight, and is not subject to the possible non-uniformity in pairs of leaves which operates in the twin-leaf method.

Although it has been emphasized that the residual-dry-weight method had an important advantage over the twin-leaf method in these experiments because it was independent of any error that may have been introduced in selecting twin-leaves that were not sufficiently uniform, it should not be assumed that a sampling error could not occur in using the residual-dry-weight method. If uniformity in the age and position of leaves in the different samples is not obtained then the sampling error could become important. In the present experiments this was not a large factor because the twin-leaf method of selecting leaves insured uniformity as to age and position of the leaves in the sample.

SUMMARY

1. Samples of leaf-blades of fully grown leaves of two varieties of bean and one variety of peanut were taken at approximately 6:00 p.m., 9:30 p.m., 2:00 a.m., and 6:00 a.m. and the dry weight and the amounts of polysaccharides, sugars, and nitrogen of the tissue were determined.

2. In order to permit measurements by the twin-leaf method the leaflets were selected in pairs, one leaf of each pair being taken at the beginning of the period and the other at the end. For beans 100 such pairs were taken for each period and for peanuts 300 pairs. The difference between total amounts of constituents in the entire sample at the beginning and at the end of the period expressed as a percentage of the amount at the beginning was taken as a measure of the change during the period.

3. The residual-dry-weight, which was obtained by subtracting the total carbohydrates from the dry weight, was also used as a basis for computing the change in constituent.

4. Finally, since it was found that the total nitrogen of the samples was not changing during the period of sampling it was used as a basis for calculation.

5. The twin-leaf, residual-dry-weight, and total nitrogen bases gave essentially the same values for the percentage change in constituents during the experimental periods.

6. Computations on the dry weight basis gave results that were not quantitatively, and in some cases not even qualitatively, correct. The fresh weight basis could not be used because the occurrence of dew made the determination of the fresh weight impossible.

7. Curves showing the changes in the polysaccharides, total carbohydrates, and total sugars in the leaves during the night are given.

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THE EFFECT OF CARBON MONOXIDE ON PLANTS

P. W. ZIMMERMAN, WILLIAM CROCKER, AND A. E. HITCHCOCK

In an earlier paper (5) the authors reported that carbon monoxide induced adventitious roots to form on young stem tissues and stimulated latent root primordia into growth. The carbon monoxide was specific for root formation since it did not induce new shoots to form. While the experiments were conducted to determine especially the effects of carbon monoxide on root formation and growth, many other responses were noted. Also, additional experiments have been performed since the first paper was published. The purpose of the present paper is, therefore, to extend the earlier report and include results of experiments to date.

MATERIALS AND METHODS

Carbon monoxide gas used in the experiments was made by heating 150 grams of oxalic acid with 300 cc. concentrated sulphuric acid and collecting the gas over water. The gas comes over in equal parts of carbon dioxide and carbon monoxide. In the early experiments this mixture was used, but in later experiments the gas was scrubbed with soda lime and potassium hydroxide, until it was approximately 96 per cent pure carbon monoxide gas. In a number of cases the gas was further scrubbed with bromine in order to remove any traces of unsaturated hydrocarbons. The effect on plants was the same whether the carbon monoxide was washed with bromine or not. The efficiency of bromine scrubbing was shown by the fact than an ethylene-air mixture was so completely freed from the unsaturated hydrocarbon that the purified air did not cause epinasty of tomatoes. Unless otherwise stated, the plants were exposed to one per cent of the gas under bell jars or in Wardian cases. Check plants were kept in comparable conditions without the gas. Concentrations used varied from 0.01 per cent to approximately 50 per cent by volume. When bell jars were used, gas was sometimes left throughout the experiment without being replenished, but where Wardian cases were used they were aired out frequently and a new gas supply introduced. This practice was thought advisable because Wardian cases are apt to allow some gas to escape. The plants used in the experiments were pot grown and usually included a variety of sizes for each of the different species.

RESULTS

During the course of the experiments 108 species of plants were exposed to carbon monoxide gas. Though there was considerable variation in susceptibility of the different species, the following types of response to carbon monoxide were commonly produced: (a) epinastic or hyponastic

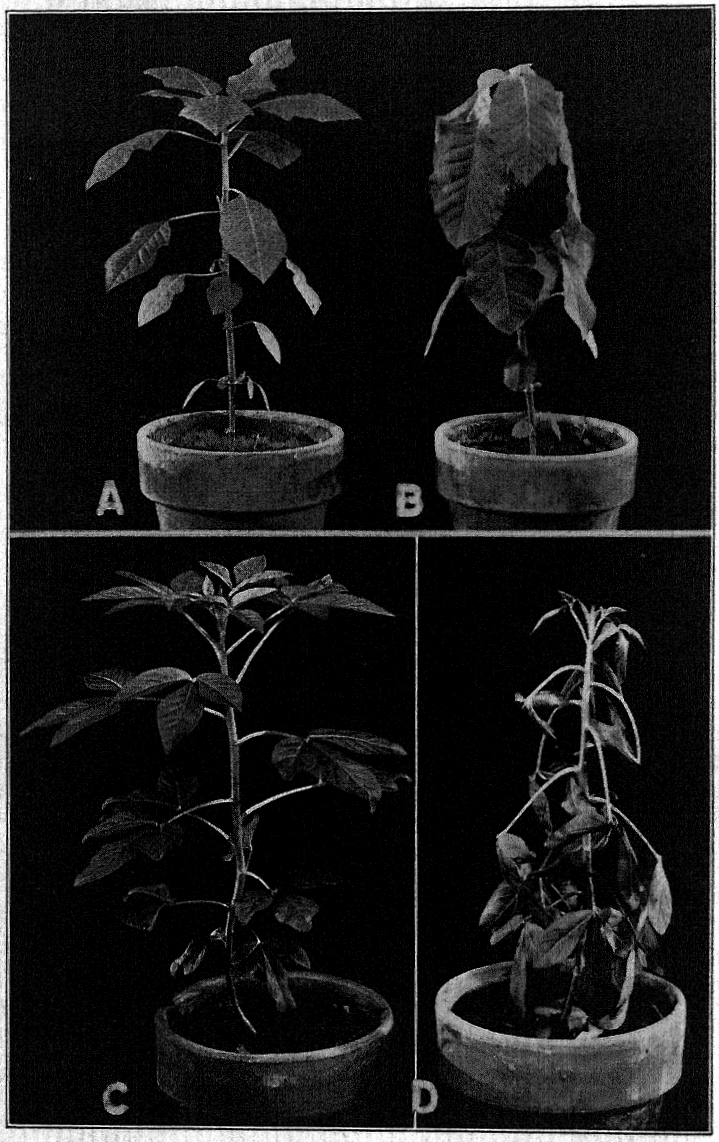


FIGURE 1. Carbon monoxide-induced epinasty of leaves. A. *Euphorbia heterophylla* control. B. *Euphorbia heterophylla* treated with 0.1 per cent carbon monoxide for 48 hours. C. *Cleome spinosa* control. D. *Cleome spinosa* treated with 0.8 per cent carbon monoxide for 48 hours.

growth of leaves, (b) aging of oldest leaves, (c) abscission of leaves, flowers, and fruits, (d) abnormal development of lenticular tissue, (e) modification in the rate of growth, (f) abnormal size of new organs produced while plants were in the gas, and (g) growth of adventitious roots on stems. These responses were not the same for all the species. Some, for example, did not show epinastic growth of leaves but exhibited other responses. A few species did not appear to be affected in any way by the gas.

EPINASTY AND HYPONASTY OF LEAVES

The first noticeable response of many plants to carbon monoxide was epinastic growth of leaves (Fig. 1). This occurred on some species within six hours after exposure to the gas. Several types of epinasty were evident as described for ethylene (1). In fact, epinasty caused by carbon monoxide was indistinguishable from that caused by ethylene. The difference was in the concentration of each gas required to cause the response. Ethylene, for example, was 5000 times as effective as carbon monoxide.

Of the 108 species exposed to carbon monoxide, the following 45 showed epinastic response: *Abutilon hybridum* Voss, *Amaranthus retroflexus* L., *Brassica oleracea* L. var. *botrytis* L., *Bryophyllum pinnatum* Kurz., *Capsicum frutescens* L. var. Ruby King, *Celosia argentea* var. *cristata* Kuntze, *Chenopodium album* L., *Chrysanthemum coronarium* L., *Cleome spinosa* L., *Coleus blumei* Benth., *Coreopsis drummondii* Torr. & Gray, *Cosmos bipinnatus* Cav., *C. sulphureus* Cav., *Dahlia juarezii* Hort., *D. pinnati* Cav., *Bougainvillea* sp., *Diospyros virginiana* L., *Euphorbia heterophylla* L., *E. pulcherrima* Willd., *Fagopyrum esculentum* Moench, *Fuchsia hybrida* Voss, *Galinsoga parviflora* Cav., *Gossypium hirsutum* L., *Helianthus debilis* Nutt., *Heliotropium peruvianum* L., *Hydrangea macrophylla* DC., *Lycopersicon esculentum* Mill. var. Marglobe, *Medicago sativa* L., *Melissa officinalis* L., *Mimosa pudica* L., *Nicotiana langsdorfi* Schrank, *N. rustica* L., *Petunia hybrida* Vilm., *Pilea microphylla* Liebm., *Potentilla reptans* L., *Ricinus communis* L., *Salvia splendens* Ker., *Solanum melongena* L. var. *esculentum* Nees, *S. pseudocapsicum* L., *S. tuberosum* L., *Tagetes erecta* L., *T. patula* L., *Torenia fournieri* Lind., and *Zebrina pendula* Schnizl.

The following species did not show epinasty of leaves when treated with carbon monoxide gas: *Acer palmatum* Thunb., *Ailanthus glandulosa* Desf., *Aloe arborescens* Mill., *Araucaria excelsa* R. Br., *Artemisia sacrorum* Lebed., *Asparagus sprengeri* Regel, *Begonia semperflorens* Link & Otto var. Vernon, *Beta vulgaris* L. var. *cicla* L., *Brachycome iberidifolia* Benth., *Cucurbita maxima* Duchesne var. Delicious, *C. maxima* Duchesne var. Hubbard, *C. pepo* L., *Daucus carota* L. var. *sativa* DC., *Dianthus* sp., *Euonymus japonica* L.f., *Forsythia viridissima* Lindl., *Gardenia jasminoides* Ellis, *Hedera helix* L., *Hyacinthus orientalis* L. var. Bismarck, *H. orientalis* L.

var. Marconi, *Ilex aquifolium* L., *I. cassine* L., *I. crenata* Thunb., *I. opaca* Ait., *I. vomitoria* Ait., *Impatiens balsamina* L., *Ipomoea batatas* Lam., *Lactuca sativa* L. var. *longifolia* Lam., *Laelia purpurata* Lindl. & Paxt., *Laeliocattleya* sp., *Lilium speciosum* Thunb. var. Rubrum, *Lunaria annua* L., *Magnolia virginiana* L., *Myrica carolinensis* L., *Narcissus odorus* L. var. Campernelle, *Nephrolepis exaltata* Schott var. *bostoniensis* Davenp., *Nicotiana alata* Link & Otto, *N. glauca* Graham, *N. suaveolens* Lehm., *N. sylvestris* Spegaz. & Comes., *N. tabacum* (seven varieties), *N. tomentosa* Ruiz. & Pav., *Oncidium apliatum* Lindl., *O. splendidum* A. Rich., *Oxalis corniculata* L. var. *repens* Zucc., *Panicum* sp., *Rhododendron obtusum* Planch. var. Hinodigiri, *Ribes grossularia* L., *R. nigrum* L., *R. sativum* Syme., *Rosa chinensis* Jacq. var. *manetti* Dipp., *Rosa* sp., *Salix babylonica* L., *Solanum dulcamara* L., *Sorbus aucuparia* L., *Stellaria media* (L.) Cyrill., *Taxus cuspidata* Sieb. & Zucc., *Thunbergia alata* Bojer, *Tropaeolum majus* L. var. *nanum* Hort., *Vaccinium corymbosum* L. var. Adams, *Viburnum tomentosum* Thunb., *Vinca major* L. var. *elegantissima* Hort., and *Zea mays* L. var. *everta* Bailey.

Several species exhibited hyponastic growth, causing the leaves to move upward or the leaf blade to curl up. The latter response was especially marked on young leaves of *Solanum dulcamara*, *Bougainvillea* sp., *Fagopyrum esculentum*, and *Dianthus*. Open flowers of *Dianthus* closed when treated and buds failed to open when in gas.

The leaves of *Tagetes erecta* (marigold) all showed marked epinasty during the first forty-eight hours of exposure to carbon monoxide but thereafter the young leaves rose to their original position and finally exhibited considerable hyponasty. Also young leaves produced while the plant was in gas assumed a position above the normal equilibrium position of leaves on control plants.

In contrast to this type of response *Helianthus debilis* and *Ricinus communis* showed epinasty of all leaves throughout continuous exposure for 13 days (Fig. 2 A and B).

ENTRANCE OF CARBON MONOXIDE

Carbon monoxide gas entered the plants and then diffused readily through the internal tissues. This is well demonstrated by Figure 3. A flask containing carbon monoxide was sealed over a leaf. The gas entered the leaf and then spread throughout all the tissues, causing epinasty as if the whole plant had been surrounded by carbon monoxide. This type of experiment was first described in an earlier paper (6) for movement of ethylene and illuminating gas through plants. In unpublished data it has been shown that acetylene and propylene act similarly.

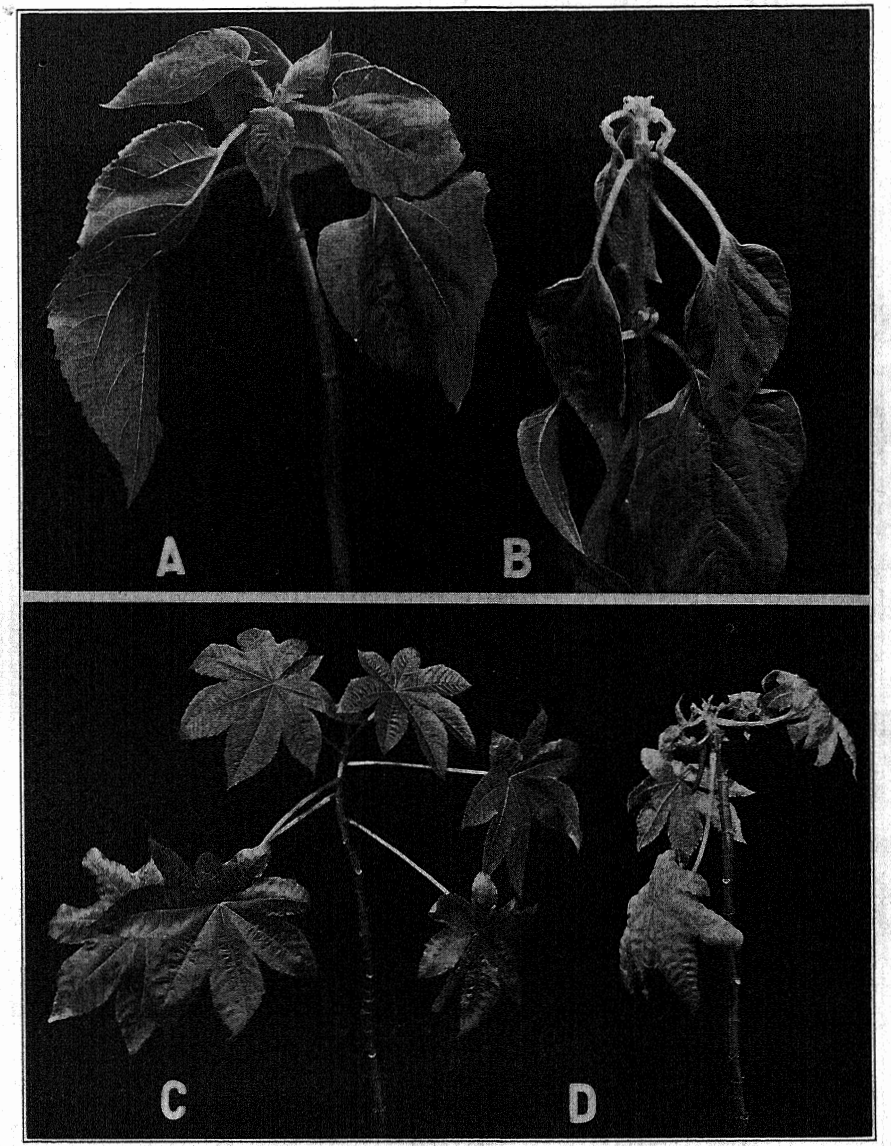


FIGURE 2. Abnormally small leaves and epinasty developed while the plants were being exposed to gas. A. *Helianthus debilis* control. B. *Helianthus debilis* exposed to one per cent carbon monoxide for 13 days. C. *Ricinus communis* control. D. *Ricinus communis* exposed to one per cent carbon monoxide for 13 days.

RETARDATION OF GROWTH

Stems. Carbon monoxide gas retarded elongation of stems but was much less effective than ethylene and illuminating gas where equal concentrations were used (2, 4, 7). Injury increased with the concentration and consequently stunting was greater with the higher concentrations of gas. Practically no retardation was noted with 0.01 per cent carbon monoxide but there was considerable with some plants when one per cent was used as shown in Table I. Richards and MacDougal (4) found that germi-

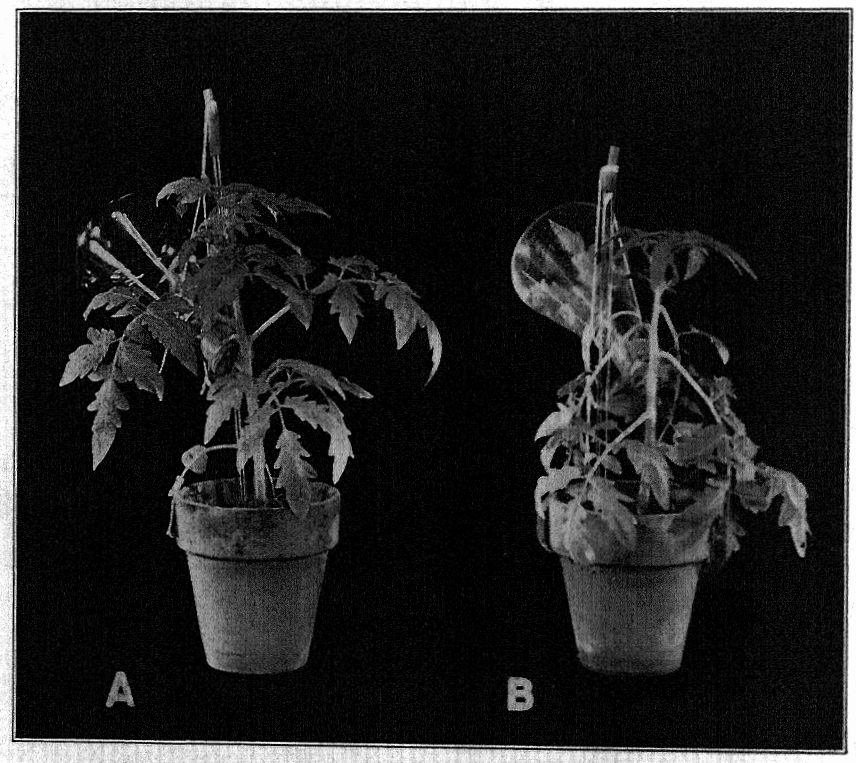


FIGURE 3. Movement of carbon monoxide gas into and through the plants. A. Tomato plant with a flask containing air sealed over a leaf. B. Tomato plant with a flask containing carbon monoxide and air mixture sealed over a leaf. The gas entered the leaf and diffused throughout the plant, causing epinasty of leaves.

nating seeds were retarded in growth when treated with high concentrations (probably 70 per cent) of carbon monoxide. Their growth figures show a ratio of 25 to 15 for elongation of control and treated vetch stems. For corn their measurements from the seed to the tip of the longest leaf showed 21 for the controls to 11 for the treated; for the main axis there was a ratio of 20 to 15. Our results recorded in Table I show that there

was considerable variation among the different species of plants, tobacco being only slightly retarded while others were greatly retarded.

Abnormal leaves produced while the plants were exposed to carbon monoxide. As shown in Table I, growth continued while the plants were being exposed to carbon monoxide gas. New leaves were produced but they were, as a rule, abnormal in size and shape. The most evident response was the modification in size of the leaf (Figs. 2 and 4). This is well exemplified by *Nicotiana tabacum*, *Solanum melongena*, and *Helianthus debilis* (Figs.

TABLE I

RETARDATION IN GROWTH OF PLANTS EXPOSED TO ONE PER CENT CARBON MONOXIDE GAS

Plants used	Period of treatment	Original length of stem in inches	Final length of stem in inches	Gain in inches	Percentage gain
Celosia	Control	6.25	19.50	13.25	210
	21 days	6.25	13.00	6.75	108
Chrysanthemum	Control	6.00	13.50	7.50	125
	23 days	6.50	10.25	3.75	57
Cleome	Control	5.50	19.50	14.00	254
	21 days	6.25	9.50	3.25	52
Coreopsis	Control	7.75	27.75	20.00	258
	23 days	17.75	34.75	17.00	95
Coreopsis	Control	4.00	13.00	9.00	225
	4 days	4.50	13.25	8.75	194
Dahlia	Control	5.25	14.50	9.25	176
	14 days	4.00	10.50	6.50	162
Euphorbia	Control	17.75	19.75	2.00	11
	4 days	24.25	25.50	1.25	5
Forsythia	Control	9.50	13.75	4.25	46
	11 days	10.50	11.25	0.75	7
Lycopersicon	Control	9.00	14.25	5.25	57
(Average of 9)	12 days	9.00	13.50	4.50	47
Lycopersicon	Control	12.25	17.75	5.50	43
(Average of 6)	9 days	12.50	16.50	3.50	26
Lycopersicon	Control	23.50	28.50	5.00	20
(Average of 2)	7 days	21.50	25.25	3.50	15
Lycopersicon	Control	20.50	24.50	4.00	19
(Average of 2)	5 days	19.25	22.00	3.00	14
Lycopersicon	Control	9.25	11.50	2.25	24
	4 days	7.75	9.25	1.50	19
Lycopersicon	Control	9.25	11.50	2.25	24
	5 days	9.50	11.50	2.00	21

TABLE I (*continued*)

Plants used	Period of treatment in days	Original length of stem in inches	Final leugth of stem in inches	Gain in inches	Percentage gain
Nicotiana (Turkish)	Control	11.25	21.00	9.75	86
	14 days	12.75	23.25	10.50	82
Nicotiana (Turkish)	Control	13.25	22.00	7.75	58
	23 days	14.75	23.25	8.50	57
Solanum	Control	7.50	11.00	3.50	46
	4 days	6.75	8.25	1.50	22
Tagetes	Control	4.25	5.00	0.75	17
	8 days	4.50	5.50	1.00	20
Tagetes	Control	17.50	23.50	6.00	34
	7 days	19.25	23.50	4.00	20
Tagetes	Control	17.00	24.50	7.50	44
	10 days	16.50	20.50	4.00	24
Tagetes	Control	22.00	24.00	2.00	9
	4 days	26.00	26.75	0.75	2
Tagetes	Control	10.50	17.25	6.75	64
	13 days	11.00	17.75	7.75	70

2 and 4). The new leaves failed to grow as large as was normal for the species. *Solanum melongena* developed abnormally small leaves, the blades of which had a tendency to curl down at the edge (Fig. 4 D). *Helianthus* leaves came smaller and smaller with continuous exposure until they appeared rudimentary. In most cases the leaves were complete and normal in shape but abnormally small.

It might be assumed that lack of photosynthesis caused small leaves but iodine tests indicated the presence of a normal supply of starch in treated leaves. Padoa (3) found that carbon monoxide interfered with the normal production of enzymes in germinating seeds. If this were true also for growing plants, then it could be assumed that synthesis of food and assimilation might easily be disturbed. As yet, however, no factors have been found to account for the abnormally small leaves produced while the plants are in carbon monoxide gas.

INJURY AND ABSCISSION

Injury and abscission of leaves varied with the species and the concentration of carbon monoxide gas. Generally the leaves abscissed in order of their age from old toward the young, but in the case of *Bougainvillea* sp. and *Ilex vomitoria* the young leaves fell first. The young leaves of *Bougainvillea* abscissed within 48 hours after exposure to a one per cent concen-

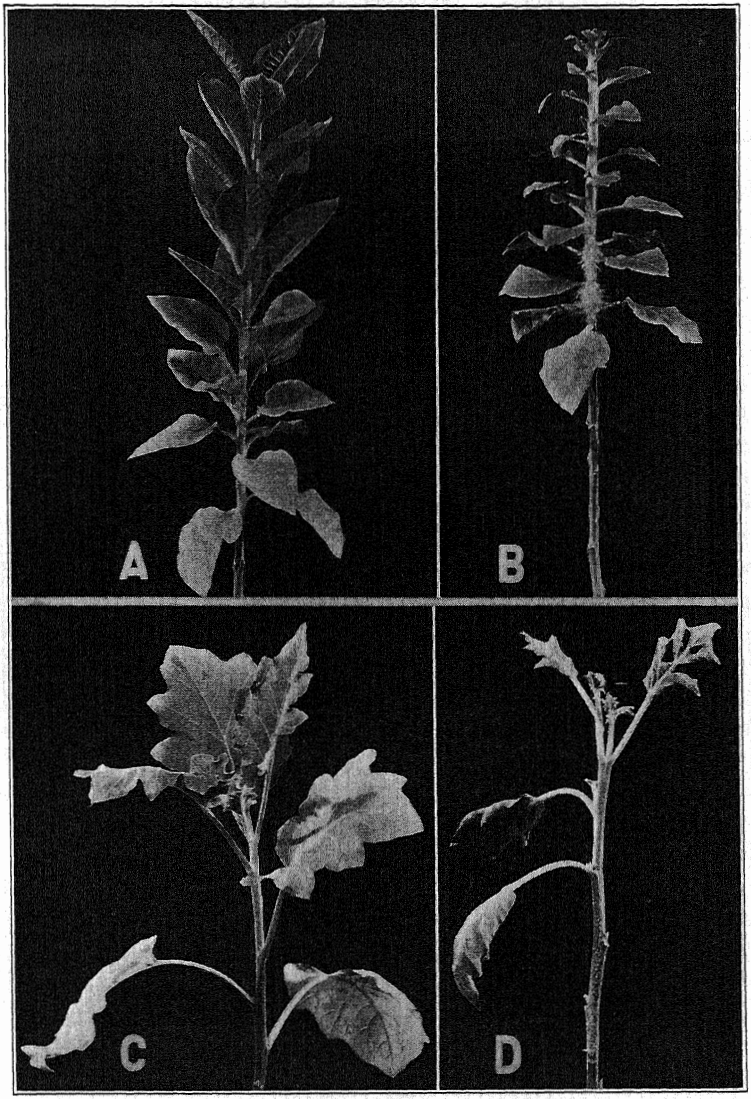


FIGURE 4. Abnormal leaves developed while the plants were being exposed to carbon monoxide gas. A. *Nicotiana tabacum* control. B. *Nicotiana tabacum* treated with 0.8 per cent carbon monoxide for 23 days. Note the comparatively large diameter of the stem tip, the abnormally small leaves, and roots from a zone which was near the tip when the treatment began. C. *Solanum melongena* control. D. *Solanum melongena* treated for 16 days with 0.8 per cent carbon monoxide. Note abnormally small leaves with deep lobes and holes and epinasty of leaf blades.

tration. All of the leaves fell in four days. *Euonymus japonica* (variegated) lost all of its leaves in seven days but in the reverse order from *Bougainvillea*. As a rule stem tips remained alive and grew while in the gas. The higher concentrations caused rapid yellowing and abscission for many species. *Nephrolepis* was unaffected by exposure to ten per cent carbon monoxide gas. This species was reported (1) as resistant to high concentrations of ethylene and illuminating gas. *Mimosa pudica* in one per cent carbon monoxide showed yellowing and abscission of leaflets in 48 hours. Later petioles abscissed and in 72 hours all of the leaves had fallen. The plants were then removed from the gas and within four days new shoots appeared from a large number of lateral buds and the stem tips resumed growth. Flower buds of most species were caused to turn yellow and absciss when treated with one per cent carbon monoxide. *Solanum pseudocapsicum* flower buds started abscissing within 24 hours after the treatment began. At the end of the fourth day all of the buds, flowers, and ripe fruits had fallen. Green fruits were resistant and clung to the stem after all of the leaves had fallen.

The injury caused by carbon monoxide was in no sense like that from toxic gases such as sulphur dioxide or ammonia. It was more like that of stimulants which increase metabolism and, therefore, cause aging. Ethylene and carbon monoxide caused similar injury and abscission, but to produce a given response required a concentration of carbon monoxide 5000 times that of ethylene.

Stem tissues and shoot buds were very resistant to carbon monoxide, not being injured with a one per cent concentration during long exposures. When plants were removed to air they recovered quickly and frequently produced an abnormally large number of side shoots. When the exposures were of 10 to 30 days' duration, new shoots frequently arose while in the gas. This was true particularly of *Solanum melongena* and several *Nicotiana* species. *Hydrangea macrophylla* produced many new shoots from buds along the stem after a 30-day treatment in a one per cent concentration of carbon monoxide gas. *Myrica carolinensis*, *Ilex vomitoria*, and *Ilex cassine* produced many new shoots near the soil surface from latent buds not clearly visible at the time they were treated (Fig. 5).

Bark tissues frequently became spotted with hypertrophied lenticels. This condition usually was most evident on exposed roots or the basal part of stems as in *Euphorbia pulcherrima* and *Solanum pseudocapsicum*, but frequently the entire stem became spotted as shown in Figure 4 for *Solanum melongena* and Figure 5 for *Myrica*. *Heliotropium* produced hypertrophied tissue on both sides of the base of the petioles where they united with the stem (Fig. 6). This tissue appeared to be associated with an abscission layer which did not extend entirely across the petiole. Microscopic sections of fresh material showed that only a few layers of cells beneath

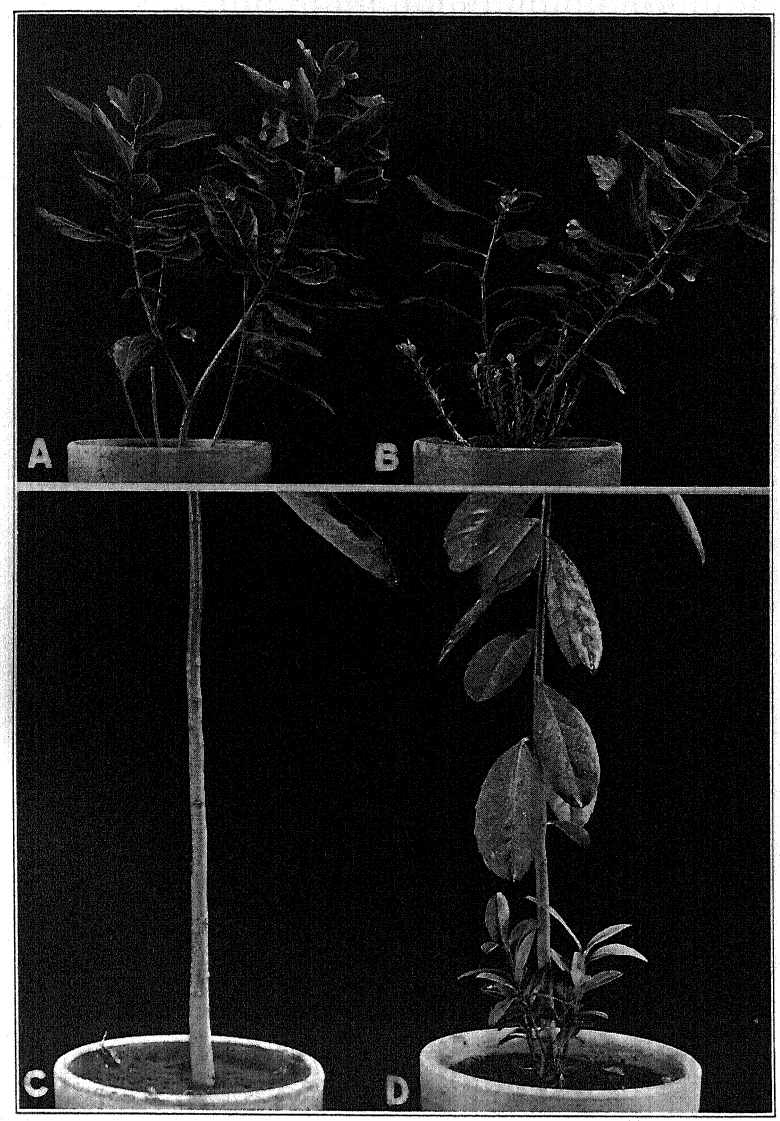


FIGURE 5. Production of new shoots near the soil surface from latent buds not clearly visible. A. *Myrica carolinensis* control. B. *Myrica carolinensis* treated with one per cent carbon monoxide at irregular intervals over a period of 40 days. C. *Ilex cassine* control. D. *Ilex cassine* treated as described under "B."

the epidermis became hypertrophied. The leaves did not absciss as was the case for most types. When badly injured by the gas they turned yellow and died and then rotted away, frequently leaving stubs of the petioles remaining attached to the stem.

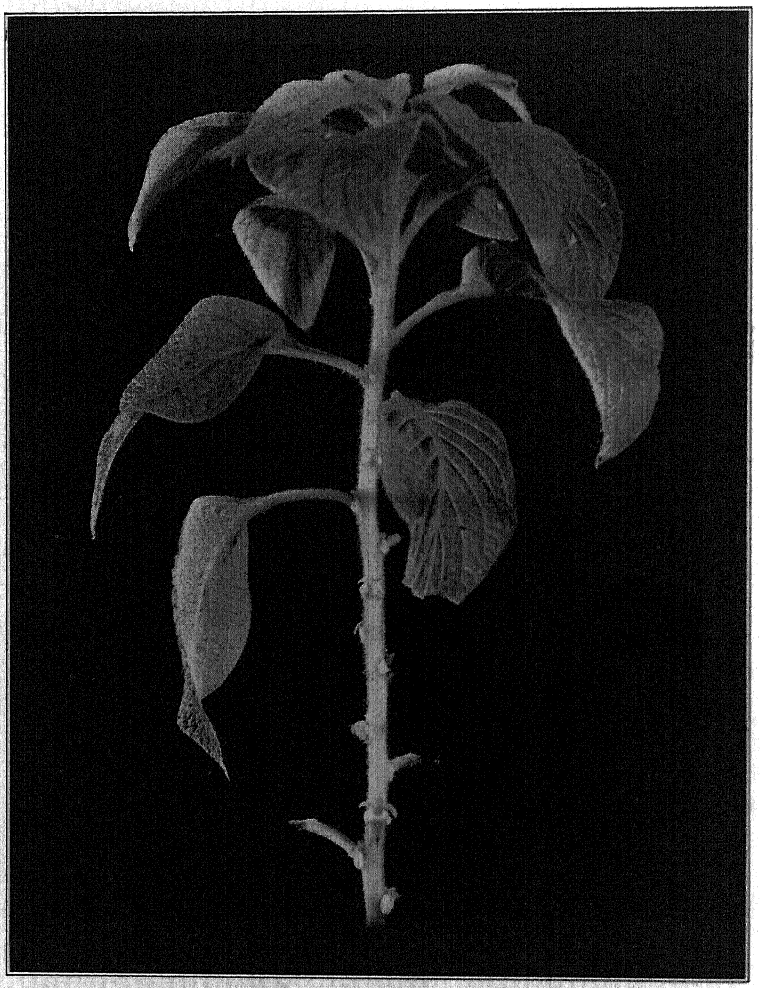


FIGURE 6. *Heliotropium* treated for 5 days with one per cent carbon monoxide. Some of the leaves were removed to show hypertrophied tissues at the base of petioles.

CARBON MONOXIDE AS A STIMULANT AND AN ANAESTHETIC

Crocker, Zimmerman, and Hitchcock (1) showed that the same concentration of ethylene acted as an anaesthetic on the growing portion of the plant and as a stimulus initiating growth on the upper face of petioles.

Table I shows that carbon monoxide retarded growth of stems and Figure 2 shows that it initiates growth on the upper face of the petiole the same as ethylene. In addition carbon monoxide initiates roots and stimulates existing root and shoot primordia and parts of other organs (5). Ethylene is known to be a good anaesthetic for mammals but to them carbon monoxide is highly toxic. Its toxicity is due to the fact that carbon monoxide displaces oxygen from the haemoglobin molecule, thereby suffocating the animal. Were it not for this reaction with haemoglobin carbon monoxide might be a good anaesthetic for mammals. In order to test this theory the following arthropoda were used: centipedes (*Scutigera* sp.), sow bugs (*Cylisticus* sp.), meal worms (*Tenebrio molitor* L.), and slugs (*Limax* sp.) were exposed to 80 per cent carbon monoxide and found to become completely anaesthetized in less than five minutes. When removed from the gas they were limp and appeared to be dead but in two minutes the centipedes showed signs of life by moving their antennae and legs. In five minutes they were crawling around as if nothing had happened to them. The sow bugs and meal worms were slower to recover but they appeared entirely normal in ten minutes. The slugs started moving about slowly in seven minutes after being removed from the gas. Centipedes exposed to a mixture of 50 per cent carbon monoxide, 25 per cent oxygen, and nitrogen showed no ill effects during or after a 90-minute exposure. With a 71 per cent carbon monoxide, oxygen, and air mixture, centipedes appeared somewhat sluggish or partially anaesthetized after 30 minutes, but continued able to crawl slowly even at the end of the 90-minute treatment. After removal from the gas the animals recovered in a short time and became quite as active as usual.

It seems logical, therefore, to conclude that carbon monoxide may properly be classed with ethylene as an anaesthetic and that it would anaesthetize mammals were it not for its reaction with the haemoglobin molecule causing death.

The concentration of gas required to anaesthetize animals is vastly greater than that required for plants. In practice a concentration of 80 to 85 per cent of ethylene is used by physicians to anaesthetize their patients preparatory to operating. Plants are anaesthetized with 0.00001 per cent ethylene or 0.05 per cent carbon monoxide.

ANAESTHESIA AND MOVEMENTS OF PLANTS DURING EXPOSURE TO CARBON MONOXIDE

Control plants of Mimosa pudica. The leaves of *Mimosa pudica*, in light, normally assume an expanded position with leaflets in approximately the same plane (Fig. 7 A). Overlapping occurs at different levels, but there is usually no contact of one leaf with another. When assuming a night position or when responding to external stimuli, the leaflets of the

second order fold up forming an acute angle over the midribs of the leaflets of the first order, and the petioles move downward. Motion pictures showed that the leaves of control plants moved about considerably but they did not rub against or interfere with each other.

Mimosa pudica in carbon monoxide gas. Figure 7 B shows a picture of *Mimosa pudica* after an 18-hour exposure to one per cent carbon monoxide. The plant was unable to maintain its orderly arrangement of its leaves while in the gas (Fig. 7 A). Most of the secondary leaflets moved downward, a few moved upward, while still others were approximately horizontal.

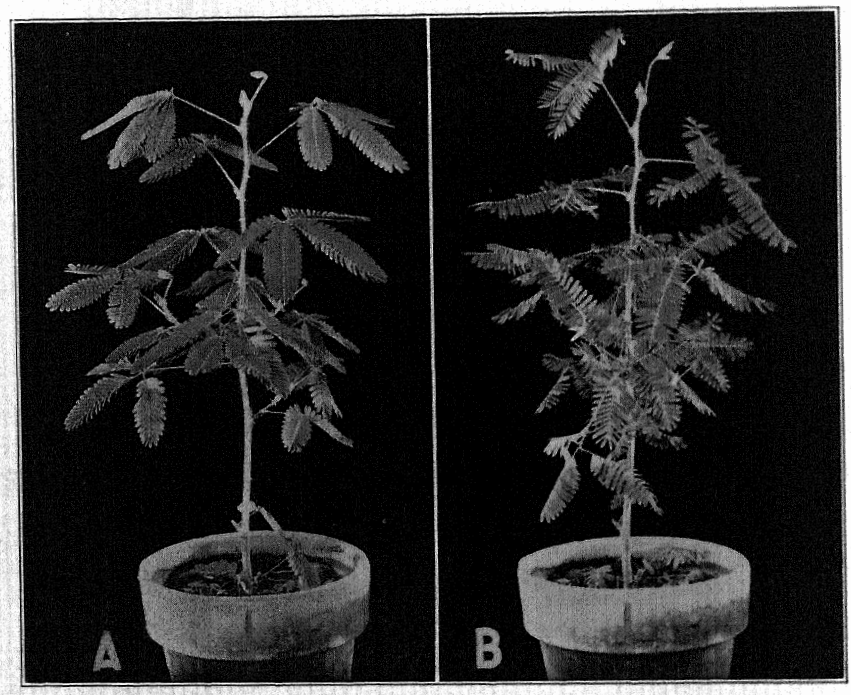


FIGURE 7. The anaesthetic effect of carbon monoxide on plants. A. *Mimosa pudica* control. B. The same plant as "A" after exposure to one per cent carbon monoxide for 18 hours. Note the change in the normal equilibrium position of the leaves and the disturbance of normal correlation.

The petioles assumed various angles from vertical to below the horizontal position. After 18 hours in one per cent carbon monoxide, the plant was removed and found to be insensitive to light, heat, or contact stimuli. Ten hours later, however, the leaves resumed their normal equilibrium position and again responded to external stimuli. This temporary loss of sensitiveness to external stimuli was due to carbon monoxide acting as an anaesthet-

ic and the condition induced in the plant was similar to a state of induced anaesthesia in animals. Another set of *Mimosa* plants exposed to 0.5 per cent carbon monoxide for 18 hours lost their orderly arrangement of leaves and became insensitive to external stimuli. Three hours after removal from this low concentration of the gas the leaves resumed their normal positions and were again sensitive to light and contact stimuli. Plants treated with two per cent carbon monoxide for 20 hours lost some of their secondary leaflets and were insensitive to heat and contact stimuli for 18 hours after removal from the gas. Twenty-four hours later, however, they showed a fair response. Even young leaves which had lost many of their secondary leaflets continued to grow and from appearances were otherwise normal. Mimosas exposed for 48 hours to one per cent carbon monoxide lost all of their leaves. The plants, however, were not killed, for when removed to a greenhouse the terminal bud continued to grow and many new shoots arose from lateral buds. These results show that injury and recovery vary with the concentration and length of exposure to the gas.

Motion pictures of Mimosa pudica. In order to understand better what happens while plants are in carbon monoxide gas, photographic exposures were made of control and treated plants at the rate of 96 per hour for motion pictures. When the films were run in the projector the pictures on the screen showed the movements 600 times as fast as normal. This enables one to see in a few minutes what actually happens over a long period of time. The results were that within two hours after the experiment was started, the leaves of the plant in carbon monoxide started changing their normal equilibrium position to gravity. After six hours in the gas they assumed a ruffled appearance and moved about without correlation and more rapidly than those of control plants. Frequently neighboring leaves became entangled and later separated with a jerk. The movements of control leaves were orderly, causing no interference whatever; they moved up and down and from one side to the other, but the range was comparatively narrow. In contrast to normal movements, leaves of treated plants swept widely from side to side and moved up and down through at least 180°. There was no apparent epinasty such as shown by leaves of tomato plants treated with carbon monoxide gas. The shifting in position of the leaves must have been due to turgor changes affecting the pulvinal tissues situated at the base of leaflets and petioles. The plant had apparently become anaesthetized and lost control of the mechanism governing turgor conditions in pulvinal cells. The rate of movements increased up to 27 hours when the experiment was stopped. At that time the plant was insensitive to external stimuli and a few of the secondary leaflets had fallen. However, after 24 hours in a greenhouse the plant again resumed its normal equilibrium position to gravity and was sensitive to external stimuli.

ADVENTITIOUS ROOTS

Since the first report on initiation of roots (5) additional facts concerning the effect of carbon monoxide have been gathered. It was stated that leafy tomato cuttings exposed to carbon monoxide gas rooted better than those without leaves. The indication was that light was an important factor for root production. Additional tests have been made with tomato plants exposed to gas while kept in complete darkness as well as comparable sets in daylight. The result was that young plants exposed to carbon monoxide and kept continuously in dark did not produce adventitious roots while comparable sets exposed in light produce numerous adventitious roots along the stem. Tomato plants that had reached the flowering stage rooted while in darkness when exposed to carbon monoxide gas but not as well as comparable sets exposed in light. These results show that the presence of carbon monoxide does not prevent the manufacture of food materials when the plant is in light. They further show that to react to the carbon monoxide young plants must be in a position to manufacture food material. The older stems have a food storage supply and may be induced by carbon monoxide to produce roots in darkness, but even old plants root better if exposed to the gas while in light.

In general better root growth was obtained during the long summer months than in fall and winter. Long periods of dark, cloudy weather also interfered with rooting response when the plants were exposed to carbon monoxide.

SUMMARY

1. Of the 108 species of plants treated with carbon monoxide, 45 showed epinastic growth of leaves. Several species showed hyponasty which caused upward curling of leaves.

2. Stem elongation of most species was retarded, varying with the concentration of the gas.

3. New leaves produced while the plants were in carbon monoxide were normal in shape but abnormally small.

4. Injury was evidenced by abnormal yellowing of the leaves, beginning with the oldest.

5. Abscission of leaves was usually associated with yellowing, though in a few cases abscission of young leaves occurred without yellowing.

6. Carbon monoxide gas caused hypertrophied tissues on stems and roots of most species.

7. During recovery an abnormally large number of side shoots arose from latent buds of many species.

8. Motion pictures of *Mimosa pudica* showed that carbon monoxide gas caused the leaves to lose their correlation and change their normal equilibrium position to gravity. They lost their sensitiveness to contact or

heat stimuli but the leaves moved about more rapidly than those of controls. Plants exposed for only 24 hours were not badly injured and recovered to approximately normal condition within a day.

9. Since carbon monoxide causes growth rigor and loss of sensitiveness to external stimuli, it is here considered as an anaesthetic. This conclusion was supported by the fact that animals which do not have haemoglobin were readily anaesthetized by an 80 per cent carbon monoxide air mixture of the gas.

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EFFECT OF VARIOUS CHEMICALS ON THE SUGAR CONTENT, RESPIRATORY RATE, AND DORMANCY OF POTATO TUBERS[1]

LAWRENCE P. MILLER

When dormant tubers of the potato (*Solanum tuberosum* L.) are treated with chemicals that break the rest period, extensive changes in catalase and peroxidase activity, pH, reducing substances, and sucrose content take place within a few days of the application of the treatment and before visible sprouts appear (5, 6, 7, 8, 12). It has not been possible, however, to show any close correlation between the extent of any of these changes and the efficacy of the chemicals in breaking the rest period. Some chemicals differ little with respect to their effectiveness in breaking dormancy but show very marked dissimilarities in their effect on the chemical changes resulting.

The various chemicals seem to differ less in their activity in increasing the sucrose content of the tubers than in any of the other properties under consideration. Also treatments of non-dormant tubers have been found to produce smaller changes in pH and catalase and peroxidase activity than treatments of dormant tubers but treatments of non-dormant tubers resulted in increases in sucrose even when the tubers were already relatively high in sugar. Further experiments on the effect of various chemical treatments on the sucrose content of potato tubers were therefore made and are reported in this paper.

Previous work has shown that treatments of potato tubers with ethyl mercaptan, hydrogen sulphide (12), ethylene chlorhydrin (18), or hydrocyanic acid (9) greatly increase respiration. The present paper reports further investigations on the influence of chemical treatments on the respiratory rate as well as on the relation between the sugar changes and the respiratory effects.

Treatments of whole tubers with ethylene chlorhydrin, ethylene bromohydrin, acetaldehyde, hydrogen sulphide, and hydrocyanic acid increased the respiratory rate, as measured by the CO_2 output, several hundred per cent. These increases took place before the increases in sucrose content occurred, and were, therefore, not a result merely of a higher concentration of the carbohydrate substrate for respiration. Treatments of cut pieces of tubers produced smaller increases. A close correlation between the effect of a chemical in increasing respiration and in breaking dormancy was not observed.

In connection with a study of a number of chemicals in affecting the sucrose content no additional chemicals were found which uniformly in-

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creased the sucrose content to the same degree as do potassium thiocyanate, thiourea, and ammonium dithiocarbamate, which break dormancy very successfully. However, certain of the chemicals, notably acetone, had very definite effects on the sucrose content, increasing it as much as one hundred per cent, without inducing the growth of the dormant buds.

EFFECT OF CHEMICALS ON SUGAR CONTENT

Methods. For the experiments on the effect of various chemicals on the sugar content the tubers were cut into pieces weighing about 25 grams and including but one eye per piece. The pieces were soaked in a solution of the required concentration for one hour after which they were planted in soil without rinsing (4). Six days later twelve pieces were removed from the soil, washed, peeled, ground through a food grinder, and the juice squeezed out through several layers of cheesecloth. After centrifuging, aliquots of the juice were added to boiling water and made up to a definite volume upon cooling. The solutions were cleared with lead acetate and deleaded with sodium oxalate and the reducing sugars were determined before and after inversion with an invertase preparation (15) following the recommendations of Quisumbing and Thomas (14). The copper was determined by titration with potassium permanganate after solution of the cuprous oxide in a sulphuric acid solution of ferric ammonium sulphate. The sugar content is expressed in the tables as the number of milligrams in 5 cc. juice.

The treatments were usually made in series of six or eight lots of 24 pieces. Two lots were soaked in distilled water and served as controls while the others were soaked in solutions of the chemicals studied. Twelve pieces were taken for the analyses and the remaining twelve were used to obtain data as to the growth response, expressed as the number of days necessary for 50 per cent to appear above ground.

Potato tubers show considerable variation as to their sugar content and in order to interpret the results obtained it was necessary to have an estimate of the errors involved. The probable error of a single determination of the sugar content of a sample of 12 pieces was calculated from the data from 31 sets of duplicates of untreated tubers. Each set of duplicates represented separate lots of untreated tubers handled as separate samples throughout. The following formula was used:

$$\text{P. E. sing.} = \frac{.6745}{\sqrt{2}} \sqrt{\frac{\Sigma d^2}{n-1}}$$

where Σd^2 equals the sum of the squares of the differences between the two duplicates and n equals the number of sets of duplicates. The value obtained was ± 1.92 mg. Since we are concerned with differences between

the sugar content of the chemically treated sample and the mean of two untreated controls the probable error of this difference becomes ± 2.37.

Results. Some of the results obtained with completely non-dormant Green Mountain potatoes are shown in Table I. It is seen that potassium

TABLE I

EFFECT OF TREATMENTS WITH VARIOUS CHEMICALS ON THE SUGAR CONTENT OF NON-DORMANT GREEN MOUNTAIN POTATOES

Chemical	Concn., g. per liter	Change in sugar content, mg. in in 5 cc. juice	
		Reducing sugars	Sucrose
Potassium thiocyanate	20.0	+13.7	+16.5
Thiourea	20.0	+13.4	+19.2
Phenylthiourea	Satd. soln.	+36.2	+22.6
Thioacetamide	6.3	+24.1	+13.8
Acetamide	10.0	−6.9	−1.3
Urea	20.0	−6.2	+3.4
Potassium chloride	20.0	+1.9	+2.3
Sodium thiosulphate	20.0	−8.7	+2.3
Sodium sulphate	20.0	−5.2	+1.9
Sucrose	20.0	−0.6	+2.0
Dextrose	20.0	−3.3	+2.2
Levulose	20.0	+4.1	+1.1
Sugar content of untreated controls; average of 9		56.8	7.4

thiocyanate, thiourea, and phenylthiourea treatments increase the sucrose content of non-dormant tubers and thus produce effects similar to those previously reported for ammonium dithiocarbamate, thiosemicarbazide, thioacetamide, and hydrogen sulphide treatments of non-dormant potatoes (12).

The table also shows that the oxygen analogs of thiourea and thioacetamide, namely urea and acetamide, do not produce these sugar changes and that it is not possible to increase the sugar content of the tubers by soaking them in solutions of dextrose, levulose, or sucrose. These results have been verified in other experiments with other lots of tubers, both dormant and non-dormant.

Emphasis is placed on the changes in sucrose content because changes in sucrose are characteristic responses to these chemical treatments (5, 6). It happens that the tubers of Table I also show increases in reducing sugar but many tubers do not show any reducing sugar changes (see below, e.g.) as a result of chemical treatments while treatments with certain chemicals invariably result in sucrose increases.

For a more comprehensive study of the effect of various chemicals on the sugar content and sprouting response of dormant tubers, four different

lots of potatoes were employed, namely, tubers of the Irish Cobbler variety from South Carolina and Maryland and tubers of the Irish Cobbler and Bliss Triumph variety grown in the Institute gardens. These tubers varied considerably in their original sugar content so that the response of tubers of widely different nature, as far as the sugar content was concerned, was tested. The average total sugar content of the untreated samples of the tubers from South Carolina and Maryland during these experiments was 64 and 55 mg. per 5 cc. of juice respectively. In the potatoes from South Carolina this consisted of free reducing sugars to the extent of about 75 per cent of the total, while the Maryland potatoes were relatively lower in reducing sugars, 80 per cent of the total sugar content consisting of sucrose. The Bliss Triumph and Irish Cobbler potatoes from the Institute gardens had an average total sugar content of only 13 and 15 mg. per 5 cc. of juice respectively, and this consisted entirely of sucrose; no free reducing sugars were detected in these tubers, either treated or untreated, throughout the course of these tests.

The results are summarized in Table II which shows the correlation between the change in sucrose content and number of days required for 50 per cent of the pieces to show sprouts above ground in the case of 48 treatments. The numbers and letters in the table designate the kind of potatoes used and the chemical treatments applied as explained in the footnote attached. No tests in which 50 per cent of the untreated tubers sprouted in less than 58 days are included and in most cases these did not sprout until more than 70 days after treatment. It will be noted that the treatments which brought about very large changes in sucrose, also broke dormancy successfully, and consisted of treatments by chemicals previously known to be effective. There is a considerable range in which treatments causing similar increases in sucrose content may or may not break dormancy. Thus treatments with acetone (Nos. 2 to 8) were not effective in inducing growth (except in one out of 12 cases), although they produced sugar increases of over one hundred per cent in some of the tests, while some of the treatments with other chemicals broke dormancy without causing larger sugar increases. A considerable number of tests were made with sodium and potassium thiosulphate (Nos. 21, 22, and 29 to 32) but these chemicals were not effective in breaking dormancy although they are related to other sulphur compounds which are effective (12).

EFFECT OF CHEMICALS ON RESPIRATORY RATE

TESTS WITH WHOLE TUBERS

Methods. The carbon dioxide given off was used as a measure of the respiratory activity. The method employed was the same as that described previously (12). The tubers were placed in a desiccator with a tight-fitting lid (as an extra precaution the lids were always tied on securely with

TABLE II

RELATION BETWEEN EFFECT OF VARIOUS CHEMICAL TREATMENTS ON THE SUCROSE CONTENT AND DORMANCY OF POTATO TUBERS*

Change in sucrose content, mg. in 5 cc. juice	Number of days for 50 per cent to appear above ground														
	15–20	20–25	25–30	30–35	35–40	40–45	45–50	50–55	55–60	60–65	65–70	70–75	75–80	80–85	85–90
+35–40			19C		33D										
+30–35				20A											
+25–30			34D					32B							
+20–25				9D			13B						4B	13B	7C
+15–20				11D									6C, 3C 3C		
+10–15		35A	5B	18A 14B		16D 23A			27A	7D					8C
+5–10	10A			22B			31B		28A	27A, 23A 16A		17A 6D		3D, 2C 22C, 14B	30C, 29C 15B
+0–5								25A 12A		26A 24A		17A		21C 29D	
−0–5										1D					3C

* The different lots of potatoes used are differentiated as follows: A, South Carolina Irish Cobbler; B, Maryland Irish Cobbler; C, Institute Bliss Triumph; D, Institute Irish Cobbler. The numbers indicate the chemicals and the concentrations employed as follows: 1, acetamide, 10 g. per l.; 2, acetone, 100 cc. per l.; 3, 150 cc. per l.; 4, 166 cc. per l.; 5, 200 cc. per l.; 6, 150 cc. acetone saturated with benzyl disulphide per l.; 7, 150 cc. acetone plus 10 g. sodium thiosulphate per l.; 8, 100 cc. acetone saturated with sulphur per l.; 9, ammonium dithiocarbamate, 3.75 g. per l.; 10, 6.25 g. per l.; 11, 7.5 g. per l.; 12, diethyl ammonium diethyl dithiocarbamate, 5 g. per l.; 13, ethyl acetate, 20 cc. per l.; 14, 30 cc. per l.; 15, 40 cc. per l.; 16, phenylthiourea, saturated solution; 17, potassium sulphocarbonate, 10 g. per l.; 18, potassium thiocyanate, 10 g. per l.; 19, 15 g. per l.; 20, 20 g. per l.; 21, potassium thiosulphate, 10 g. per l.; 22, 10 g. per l. for 16 hours; 23, sodium bisulphite, 10 g. per l.; 24, sodium carbonate, 10 g. per l.; 25, sodium diethyl dithiocarbamate, 20 g. per l.; 26, sodium hydrosulphide, 10 g. per l.; 27, sodium sulphite, 10 g. per l.; 28, 15 g. per l.;29, sodium thiosulphate, 10 g. per l.; 30, 10 g. per l. for 16 hours; 31, 12.5 g. per l. for 16 hours; 32, 25.0 g. per l. for 16 hours; 33, thioacetamide, 6.25 g. per l.; 34, thiourea, 15 g. per l.; 35, 20 g. per l.

twine) and carbon dioxide-free air was drawn through at the rate of about 14 liters per hour. Temperature was kept constant at 26.7° C. by means of a thermostatically controlled water bath. The CO_2 given off was absorbed in $Ba(OH)_2$ in Van Slyke-Cullen tubes (24), each containing 50 cc. of a standard solution (approximately saturated). The tubes were arranged in series and a sufficient number used so that the last few tubes in the series showed no precipitate. At the rate at which the air was drawn through the apparatus in these experiments all the carbon dioxide coming off was absorbed by the first two to three tubes and no appreciable precipitation occurred in subsequent tubes until the $Ba(OH)_2$ in the first tube was exhausted. At suitable intervals the tubes showing any $BaCO_3$ were removed and fresh ones substituted. This change could be made in a few minutes and it was thus possible to get a continuous record of the CO_2 output of a given lot of tubers for as long a period as was desired.

For the determination of the CO_2, the $Ba(OH)_2$ with the precipitated $BaCO_3$ in the tubes was washed into a volumetric flask and made up to volume, and an aliquot withdrawn and titrated, after the precipitate had settled. The quantity of $Ba(OH)_2$ neutralized by the CO_2 could then be calculated since the strength of the $Ba(OH)_2$ before absorption of CO_2 was known.

The tubers were subjected to the vapors of the chemicals in the containers used for the respiration studies. Twenty-four hour treatment periods were usually employed. The treatment was considered ended when the stream of air was started through the desiccators. This rapidly swept out any remaining vapor. In cases where chemicals were used which react with $Ba(OH)_2$ suitable solutions were inserted between the desiccators and the $Ba(OH)_2$ tubes in order to absorb the chemicals. These solutions had to be so chosen that they would absorb the chemicals in question but no CO_2. Silver nitrate was used to absorb hydrocyanic acid, cadmium sulphate for hydrogen sulphide, and iodine for ethyl mercaptan.

The amount of CO_2 given off was determined usually at about 24 hour intervals and the average rate for this period calculated in terms of the number of milligrams of CO_2 given off by 100 grams fresh tissue per hour. In the case of each treatment the respiratory rate of an untreated control which was kept in a closed desiccator without any chemical during the treatment period was of course also determined. These determinations of respiratory activity were confined only to the treatment period and the first five or six days thereafter and were ended before any visible sprouts appeared.

An estimate of the accuracy of the method for the determination of the CO_2 given off was obtained from 22 duplicate determinations made on untreated tubers of the South Carolina Irish Cobbler potatoes of the 1931 crop. These duplicates represent determinations made on the same day

but on different samples of tubers. Using the formula previously given for the estimation of the probable error from a series of duplicates, the probable error of a single determination was found to be ±0.14 mg. CO_2 per 100 g. per hour. Four tubers were used in each test. In subsequent experiments the number of tubers used was larger and varied from 8 to 20 or more.

An examination of the tables will show that in practically every case the respiratory rate of the untreated samples is lower in the first determination shown than in subsequent ones. This is due to the fact that this deter-

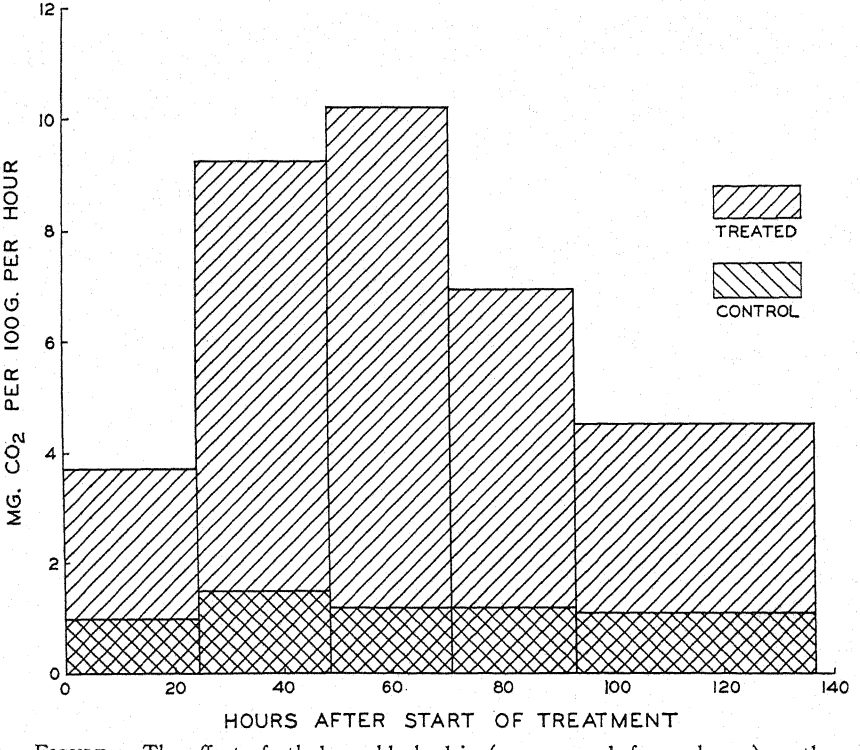


FIGURE 1. The effect of ethylene chlorhydrin (1.2 cc. per l. for 24 hours) on the respiratory rate of potato tubers.

mination included a 24 hour treatment period during which the tubers were kept in sealed containers without a stream of air passing over them.

Results. The effect of ethylene chlorhydrin treatments of various lots of Irish Cobbler and Bliss Triumph potatoes on the respiratory rate is shown in Figure 1 and Table III. In Figure 1 the area of each block represents the total amount of CO_2 given off per 100 grams tissue during the interval represented and the top line of each block shows the average respiratory rate during this period. The data from which this graph is constructed as

well as that of four other tests not given diagrammatically are shown in Table III. It is seen that in every case there resulted a prompt and pronounced rise in the respiratory rate which reached a maximum about 50 to 60 hours after treatment when values from 3.3 to 8.3 times that of the corresponding untreated samples were obtained. Subsequently the respiratory rates of the treated samples gradually fell but they were still considerably higher than those of the corresponding controls a week after the beginning of the treatments.

An examination of Table IV shows that increases over several hundred per cent were also obtained in treatments with ethylene bromohydrin, hydrogen sulphide, acetaldehyde, and hydrocyanic acid. The general shape of the curves for the respiratory rate of the tubers treated with these chemicals is the same as that for the chlorhydrin treatments, exhibiting a prompt rise with a maximum about 50 to 60 hours after the start of the treatments, and subsequently a gradual decrease. Treatments with ethyl sulphide, ethyl disulphide, and hydrochloric acid vapors resulted in smaller but nevertheless definite increases in the respiratory rate.

The reason for the tests with hydrochloric acid vapors was that a number of substances slightly acidic in nature, such as hydrogen sulphide, ethyl mercaptan, hydrocyanic acid, and carbon dioxide (23), had been found to increase respiration and it was thought that perhaps ethylene chlorhydrin produced its effect due to the gradual formation of small amounts of hydrochloric acid resulting from hydrolysis (3). The results obtained do not indicate, however, that hydrochloric acid vapor is effective in increasing respiration.

All the chemicals so far considered produced an increase in respiratory activities which was quite pronounced and was still evident about a week after the application of the treatments. The only chemicals studied which decreased the carbon dioxide output of the tubers were the primary alcohols. Concentrations of ethyl alcohol (Table V) which have some action in breaking dormancy (8) produce decided decreases in the respiratory rate which in one case reached a value as low as one-tenth that of the untreated controls. These effects were obtained with both dormant and non-dormant potatoes and if a series of concentrations of the alcohol was employed the extent to which the respiration was decreased was in line with the concentrations used. It is seen from Table V that after about one hundred hours, at least with the lower concentration of ethyl alcohol, the treated samples usually have recovered and respire at about the same rate as the controls.

Table VI shows the result of an experiment in which tubers are treated with methyl, ethyl, and isopropyl alcohols at a concentration of 2.5 cc. per liter. It is seen that all these alcohols produced large decreases in the carbon dioxide output. Judged from the time in which the tubers regained their normal rate the retarding effect of the alcohols was in the order methyl,

TABLE III

EFFECT OF ETHYLENE CHLORHYDRIN VAPOR TREATMENTS ON THE RESPIRATORY RATE OF POTATO TUBERS

South Carolina Irish Cobbler 1931			Institute Irish Cobbler 1932			Institute Bliss Triumph 1932			Immature Irish Cobbler 1932			New Jersey Irish Cobbler 1932		
Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.	
	Tr.*	Ck.		Tr.**	Ck.		Tr.†	Ck.		Tr.‡	Ck.		Tr.†	Ck.
0– 45	4.93	1.12	0– 25	3.70	0.99	0– 29	2.67	1.24	0– 26	6.26	2.72	0–26	3.72	2.33
45– 69	5.84	1.26	25– 48	9.24	1.51	29– 50	4.81	1.85	26– 44	16.47	3.52	26–45	10.36	3.15
69–139	3.57	1.09	48– 71	10.24	1.24	50– 68	6.24	1.77	44– 71	12.85	2.66	45–92	7.01	2.09
139–185	2.88	1.08	71– 93	6.97	1.25	68–119	4.11	1.34	71–168	7.87	2.03			
185–237	2.60	0.93	93–137	4.50	1.12	119–165	4.01	1.42						

* 1 cc. 40% ethylene chlorhydrin per liter for 24 hours; Ck. = untreated control.
** 1.2 cc. per liter for 23 hours.
† 1 cc. per liter for 27 hours.
‡ 1.2 cc. per liter for 24 hours.

TABLE IV

EFFECT OF THE VAPORS OF VARIOUS CHEMICALS ON THE RESPIRATORY RATE OF POTATO TUBERS

Ethylene bromohydrin			Hydrogen sulphide; Ethyl sulphide				Ethyl disulphide			
Hrs. from beginning of treatment	Mg. CO_2 per 100 g. g. per hr.		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.			Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		
	0.5 cc. per l., 24 hrs.	Control		0.3 g. $Na_2S.9H_2O$ per l., 24 hrs.	$(Et)_2S$ 0.3 cc. per l., 24 hrs.	Control		0.1 cc. per l., 24 hrs.	0.05 cc. per l., 24 hrs.	Control
0– 50	2.98	0.91	0– 42	4.23	2.21	1.40	0– 46	2.82	1.85	1.51
50– 73	8.91	1.43	42– 91	5.91	2.28	1.24	46– 67	3.79	2.50	1.91
73– 98	5.36	1.31	91–120	4.85	1.77	1.24	67–119	2.60	1.30	1.46
98–119	4.69	1.26	120–143	4.40	1.69	1.19	119–144	3.21	1.17	1.75
			143–168	3.44	1.42	1.09	144–168	2.97	1.72	1.44

TABLE IV (*Continued*)

Hydrochloric acid			Acetaldehyde			Hydrocyanic acid		
Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.	
	0.5 cc. per l., 21.5 hrs.	Control		0.6 cc. per l., 24 hrs.	Control		0.09 g. cyano-gas per l., 24 hrs.	Control
0– 45	1.93	1.08	0–26	4.16	0.65	0–29	3.02	1.71
45–115	1.83	1.11	26–45	5.14	0.90	29–52	4.44	1.98
115–162	1.48	1.16	46–97	2.75	1.11	52–71	4.27	2.00
162–213	1.41	1.01						
213–233	1.23	1.02						

TABLE V

EFFECT OF ETHYL ALCOHOL ON THE RESPIRATORY RATE OF POTATO TUBERS*

Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.			Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.	
	3.6 cc. per l., 24 hrs.	1.8 cc. per l., 24 hrs.	Control		3.0 cc. per l., 24 hrs.	Control		1.2 cc. per l., 24 hrs.	Control
0– 42	0.09	0.30	1.11	0–29	0.35	1.71	0–25	1.21	2.08
42– 65	0.71	1.10	1.24	29–52	0.67	1.98	25–46	1.26	3.12
66–116	0.76	1.00	1.17	52–71	1.22	2.00	46–89	2.82	2.96

* The first two tests here shown were made on dormant Irish Cobbler potatoes, the last one on non-dormant tubers of the Green Mountain variety.

ethyl, and isopropyl, which agrees with the order of effect of these alcohols on potato tissue determined in other ways (22).

TESTS WITH CUT PIECES

Methods. The method used for the determination of respiratory activity in the case of the chemical treatments of cut pieces was the same as that for whole tuber treatments except for the changes necessary due to differences in the method of treatment. The cut pieces were soaked for one hour in solutions of the chemical studied and were then planted in soil. When determinations of the respiratory rate were to be made samples of from 10 to 18 pieces were removed from the soil, washed in tap water, dried with cheesecloth, weighed, and placed in the desiccators used for the determinations. If the respiratory rate was to be determined immediately after the end of the treatments the pieces were taken without any previous planting in soil. A new lot of pieces was taken about every 24 hours or oftener for

TABLE VI

EFFECT OF METHYL, ETHYL, AND PROPYL ALCOHOLS ON THE RESPIRATORY RATE OF IRISH COBBLER POTATO TUBERS

Hrs. from beginning of treatment	Respiratory rate, mg. CO_2 per 100 g. per hr.			
	Methyl alcohol*	Ethyl alcohol*	Isopropyl alcohol*	Control
0– 42	0.16	0.14	0.17	0.87
42– 65	0.41	0.35	0.50	1.40
66–115	1.07	0.61	0.34	1.11
115–166	0.98	0.94	0.55	1.01

* 2.5 cc. per liter for 24 hrs.

it was not practical in these tests to determine the respiratory rate on the same material throughout the course of the experiment as was done in the case of the whole tubers, since there was some tendency for the pieces to rot after several days under the conditions under which the determinations were carried out. This difficulty was avoided when fresh samples of pieces were taken about every 24 hours.

Results. The effect of chemicals on the respiration of potato tubers when the treatments are made on cut pieces is considerably complicated by the marked action of cutting in influencing the respiratory rate. While intact tubers usually gave off from 1 to 2 mg. CO_2 per 100 g. per hour under the conditions of these experiments, cut pieces (weighing about 25 grams each) from the same tubers gave off 10 to 17 mg. per 100 g. per hour a number of hours after the wounding.

If tubers subjected to ethylene chlorhydrin vapors were cut into pieces with one eye each at the end of the treatment period and the respiration determined on treated and untreated pieces results as shown in the first

TABLE VII

EFFECT OF ETHYLENE CHLORHYDRIN TREATMENTS ON THE RESPIRATION OF CUT PIECES OF POTATO TUBERS

Vapor treatments of whole tubers, cut into pieces at end of treatment						Dip treatments, cut pieces					
Institute Irish Cobbler, 1932, two experiments						New Jersey Irish Cobbler, 1931			New Jersey Irish Cobbler, 1932		
Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.	
	Treated*	Control		Treated**	Control		Treated†	Control		Treated†	Control
24–42	12.21	12.76	25–47	11.21	9.82	25– 43	16.12	13.63	0– 26	7.21	6.41
42–49	17.63	15.92	48–70	12.53	12.77	45– 93	9.87	10.01	26– 47	14.62	8.42
49–72	12.19	13.31				93–118	6.95	5.84	48– 71	13.25	6.94
72–93	9.27	9.59				119–165	3.73	3.76	71–116	6.38	4.45
94–140	5.93	5.78				165–211	3.24	3.35			

* 1.2 cc. of 40% ethylene chlorhydrin for 23 hours.

** 1.2 cc. for 24 hours.

† Cut pieces dipped into solution containing 30 cc. chlorhydrin per liter, then stored in sealed container for 24 hours. Controls dipped in water and similarly stored.

part of Table VII were obtained. The treated samples respired at about the same rate as the controls but the respiratory rate of both samples was higher than that resulting from the greatest stimulation obtained by treatments of whole tubers. In experiments where treatments were made by the dip method (4), which is equivalent to a vapor treatment of cut pieces, there results some stimulation of the respiration of the treated samples (Table VII).

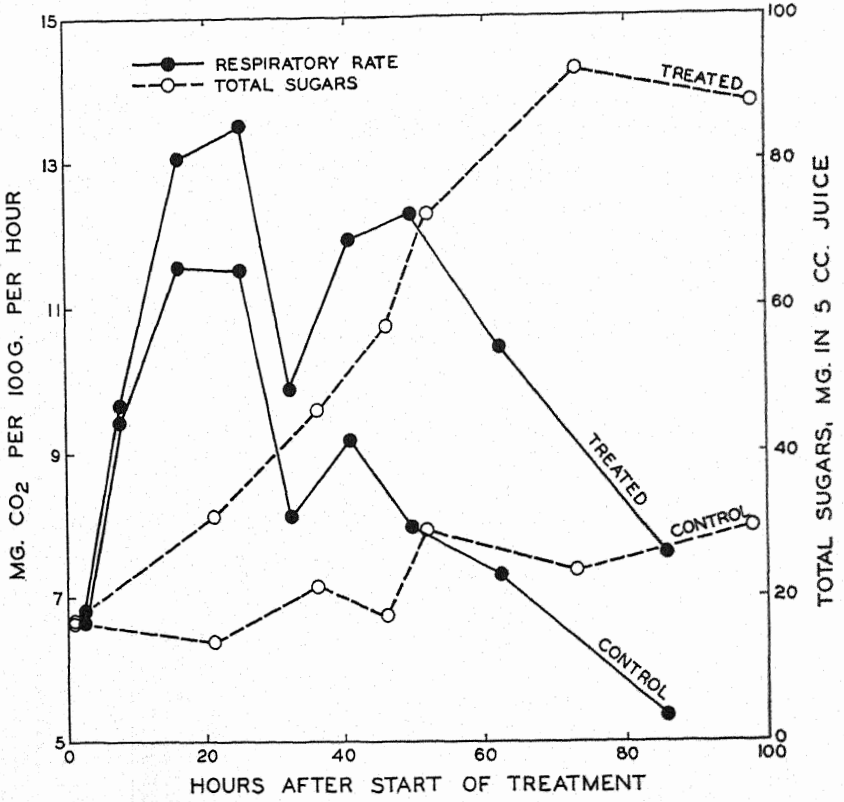


FIGURE 2. The effect of a treatment with potassium thiocyanate (15 g. per l.) on the respiratory rate and sugar content of cut pieces of potato tubers.

The effect of treatments with potassium thiocyanate, thiourea, and thioacetamide, in which cut pieces are soaked for one hour in solutions of the chemical under consideration, is shown in Figure 2 and Tables VIII and IX. The solid lines in Figure 2 represent the respiratory rate of pieces of potato tubers which had been treated with a solution containing 15 grams of potassium thiocyanate per liter and of corresponding control pieces which had been soaked in distilled water. The amount of carbon dioxide given off was determined at rather frequent intervals in order to

TABLE VIII

INFLUENCE OF TREATMENTS OF CUT PIECES OF POTATO TUBERS WITH POTASSIUM THIOCYANATE ON THEIR RESPIRATORY RATE AND SUGAR CONTENT

Potassium thiocyanate, 15 grams per liter

Maryland Irish Cobbler					Institute Bliss Triumph					Institute Bliss Triumph				
Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Total sugars at end of respiration run. Mg. in 5 cc. juice		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Total sugars at end of respiration run. Mg. in 5 cc. juice		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Total sugar at end of respiration run. Mg. in. 5 cc. juice	
	Tr.*	Ck.	Tr.	Ck.		Tr.	Ck.	Tr.	Ck.		Tr.	Ck.	Tr.	Ck.
2– 20	9.13	8.89	43.4	34.9	1– 19	11.36	10.09	52.6	44.8	1– 4	6.79	6.63	—	—
23– 44	12.25	8.93	91.0	51.7	19– 23	15.99	10.80	58.2	40.7	4–11	9.68	9.44	—	—
44– 68	14.19	7.99	122.9	40.7	23– 26	11.83	10.27	44.3	34.1	11–21	13.12	11.56	30.9	13.6
69– 95	9.57	4.94	128.5	46.5	26– 30	13.25	10.03	56.9	27.8	21–28	13.49	11.51	—	—
96–118	6.15	4.64	102.6	47.6	30– 33	14.61	10.85	46.1	30.2	28–36	9.87	8.11	45.6	21.6
					33– 42	12.32	9.35	62.5	35.6	36–46	11.90	9.18	57.2	17.1
					43– 58	12.95	9.81	68.7	37.8	47–52	12.25	7.96	72.7	28.7
					50– 65	12.44	9.94	69.7	42.4	52–73	10.41	7.30	92.7	23.6
					65– 89	9.01	—	63.4	20.1	74–98	7.60	5.32	88.1	29.8
					89–136	6.10	4.25	63.6	26.1					

* Tr. = Treated pieces soaked for one hour in solution of chemical; Ck. = Control pieces soaked for one hour in distilled water.

get a more accurate picture of the changes in rate which occur during the first few days after the cutting. The mg. of CO_2 per 100 g. tissue per hour are plotted against the number of hours since the start of the treatment, the hour plotted in each case being the time intermediate between the beginning and end of the period during which the CO_2 was being collected. Thus from the total amount of CO_2 given off from the sixth to the tenth hour after treatment, for example, the average rate during these four hours would be calculated and plotted at eight hours. It is seen from Figure 2 that the respiratory rate of both the treated and check samples is much higher than that of intact tubers at once after cutting, and that it reaches a maximum about 20 hours after the cutting and a second maximum about 50 hours after the start of the experiment. It follows, of course, that if the periods during which the amount of CO_2 is determined are longer, these

TABLE IX

INFLUENCE OF TREATMENTS OF CUT PIECES OF IRISH COBBLER POTATO TUBERS WITH THIOUREA AND THIOACETAMIDE ON THEIR RESPIRATORY RATE AND SUGAR CONTENT

Thiourea, 15 grams per liter					Thioacetamide, 5 grams per liter				
Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Total sugars at end of respiration run. Mg. in 5 cc. juice		Hrs. from beginning of treatment	Mg. CO_2 per 100 g. per hr.		Total sugars at end of respiration run. Mg. in 5 cc. juice	
	Tr.*	Ck.	Tr.	Ck.		Tr.	Ck.	Tr.	Ck.
2– 19	8.24	8.43	81.8	66.1	2– 20	8.42	7.97	69.8	70.4
21– 44	12.61	10.97	99.5	65.9	24– 46	13.35	11.24	80.3	58.8
48– 71	8.88	7.78	103.9	61.9	50– 71	10.73	7.46	70.2	65.9
75– 96	7.72	5.44	110.1	62.5	73– 93	8.07	5.98	73.9	68.6
98–118	6.96	4.83	95.2	59.3	96–140	—	3.94	—	57.1
121–165	4.83	3.42	99.7	57.0	144–166	5.29	4.12	65.5	55.7
168–190	4.60	3.29	94.8	58.9	168–188	5.17	3.55	72.5	52.6

* Tr. = Treated pieces soaked for one hour in solution of chemical; Ck. = Control pieces soaked for one hour in distilled water.

maxima will not become evident due to the averaging effect which results. For this reason some of the data given in Tables VIII and IX do not show double maxima.

It is seen that treatments with potassium thiocyanate, thiourea, and thioacetamide result in increases in the respiratory rate, which are, however, considerably smaller than those occurring from vapor treatments of whole tubers with various chemicals. These increases are in practically every case already evident in the determinations made immediately after treatment, but the differences become larger about 50 to 100 hours after treatment. At this time the respiratory rate of both treated and untreated pieces is dropping rapidly, due to the formation of suberized layers,

presumably, but the drop is more rapid in the controls so that there results at this stage a considerable percentage difference between the respiratory activity of the two samples.

A number of tests were made on the effect of treatments with urea and acetamide on the respiratory rate and these showed that treatments with these chemicals do not produce increases in respiration similar to those resulting from the corresponding sulphur analogs. Thus in one experiment in which the respiratory rate was calculated from the amount of CO_2 given off from the 120th to the 143rd hour after treatment, cut pieces treated with thioacetamide gave off 8.37 mg. CO_2 per 100 g. per hour during this period while pieces treated with acetamide and a control gave off 4.53 and 4.74 respectively. In another test in which the CO_2 given off from the 74th to the 95th hour after treatment was determined, pieces treated with thiourea respired at the rate of 11.25 mg. per 100 g. per hour while corresponding pieces treated with urea and a control respired at the rate of 7.67 and 7.89 mg. respectively.

RELATION BETWEEN EFFECT ON RESPIRATION AND EFFICACY IN BREAKING DORMANCY

Increases in respiration have been considered by some as an important factor in breaking dormancy (1, 17). The experiments reported in this paper do not show a very close relation between these two processes. While it is true that in the case of the vapor treatments studied, ethylene chlorhydrin was found to be the most effective in increasing respiration and it is also the most effective of these treatments in breaking dormancy, a close correlation between the increases in respiration and efficacy in breaking dormancy was not found to exist in the case of the other chemicals. Thus ethyl mercaptan (12) produces increases in respiratory activity almost as large as result from chlorhydrin treatments and yet its dormancy-breaking activity is considerably less than that of chlorhydrin. Ethyl alcohol depresses the respiration, and its efficacy in breaking dormancy is about the same as that of the mercaptan. Perhaps the most striking evidence indicating the absence of a close relation between respiration and the breaking of dormancy is that cutting the tubers into pieces with one eye each, such as were used for planting, will increase the respiration many-fold without breaking dormancy. Whole tuber treatments with ethylene chlorhydrin, while they result in large increases in respiration as compared with untreated tubers, never increase the respiration to as high a value as that reached when the tubers are cut into pieces, yet the ethylene chlorhydrin-treated whole tubers produce visible sprouts in a few weeks even if stored in bags in a relatively dry room while untreated cut pieces from the same lot of tubers planted in soil do not sprout for several months.

RELATION BETWEEN THE RESPIRATORY RESPONSES AND THE SUGAR CHANGES

With whole tubers. Since these treatments increase both the respiratory activity and the amount of sugar in the tubers, it is natural to inquire into a possible relation between these two responses. Table X summarizes data obtained in connection with vapor treatments of whole tubers in which the

TABLE X

EFFECT OF VARIOUS CHEMICAL TREATMENTS OF POTATO TUBERS ON THE RESPIRATORY RATE CONTRASTED WITH THE EFFECT ON THE SUGAR CONTENT

Chemical	Concn. cc. per l., 24 hrs.	Respiratory rate			Total sugars at end of respiration test. Mg. in 5 cc. juice	
		Hrs. from beginning of treatment	Treated	Control	Treated	Control
Ethylene chlorhydrin	1.2*	0–23	4.49	3.14	62.5	80.7
	1.2*	0–23	6.42	1.83	13.4	22.7
	1.2*	0–26	3.00	1.10	13.4	20.0
		26–50	5.24	1.34	2.2	20.0
	1.2	26–45	10.36	3.15	23.7	25.6
Ethyl alcohol	3.6	42–65	0.71	1.24	8.4	19.7
	1.8	42–65	1.10	1.24	13.8	19.7
	3.0	29–52	0.67	1.98	28.1	32.2
	3.6	26–45	0.41	0.90	13.9	17.1
	2.5	42–65	0.35	1.40	28.9	42.5
Methyl alcohol	2.5	42–65	0.41	1.40	34.9	42.5
Isopropyl alcohol	2.5	42–65	0.50	1.40	18.1	42.5
Acetaldehyde	0.6	25–45	5.14	0.90	6.4	17.1
Hydrocyanic acid	**	29–52	4.44	1.98	17.6	32.2
Hydrogen sulphide	†	26–45	—	3.15	7.2	25.6
Ethyl mercaptan	0.42	26–45	5.37	3.15	2.1	25.6

* 21 hr. treatment.
** HCN liberated from 0.09 g. cyanogas per l.
† Gas liberated from 0.42 g. $Na_2S.9H_2O$ per l.

sugar content of the tubers was determined at the time when the respiratory rate was at its highest. In each case a part or all of the sample, the respiratory rate of which had just been determined, was taken for the sugar analyses. The data show that at the time when the respiratory rate is at its maximum the sugar content of the treated sample is lower and not higher than that of the control. This result was obtained with all the chemicals investigated. The sugar increases which result from treatments with some of these chemicals (5, 6, 12) do not occur until later when the respiratory rate is decreasing rapidly.

The increases in respiration are therefore not a result of the sugar increases but take place quite independently of these changes. One might suppose that the opposite relation exists, that is, that the treated samples are lower in sugar because of the greatly enhanced respiratory rates. Such an explanation, however, does not hold for the alcohol treatments which depress both the respiratory rate and sugar content at the same time.

With cut pieces. The relation between the respiratory responses of treatments of cut pieces and the changes in sugar are complicated by the effect of cutting alone on these two processes. Tables VIII and IX and Figure 2 show data obtained in our experiments with treatments of cut pieces with potassium thiocyanate, thiourea, and thioacetamide. The respiratory rate and sugar content of both treated and control samples were determined at frequent intervals, the samples used for the sugar analyses always being the ones of which the respiratory rate had just been determined. In Figure 2 is shown a test in which the sugar changes in the treated sample were very large and there was also a small increase in the control. It is seen, however, that in both cases the broken lines, representing the sugar content, rise while the respiratory rate is falling. An examination of the tables shows that an increase in the sugar content of the untreated pieces does not always occur and it is therefore certainly not possible in these cases to ascribe the large respiratory increases resulting from cutting as due to changes in sugar content. In the treated samples sugar increases always took place but the largest increases are not usually evident until after the respiratory maxima have occurred and the respiratory activity is decreasing.

The increased activity resulting from cutting has been reported to take place to a large extent in the first few layers of cells at the cut surface (20) and in our samples these layers were diluted by the large amount of internal tissue present. Both the sugar analyses and respiratory rate were, of course, based on the whole piece.[2] A consideration of all the data does not indicate that it is possible to explain the respiratory behavior of either the treated or control pieces on the basis of changes in the concentration of sugar.

DISCUSSION

The effect of cutting on the respiratory rate of potato tubers has received a great deal of attention (2, 10, 11, 16, 20, 21) and the occurrence of a double maximum as a result of wounding has been previously observed by a number of workers (10, 11, 16, 20). Analyses of slices taken from the cut surface have been reported to show considerable increases in sugar (11). The relation between the respiratory changes and sugar changes

[2] In these studies on the relation between the respiratory rate and the sugar content the samples for sugar analyses were, of course, not peeled as was done in the sugar studies reported in an earlier section of the paper.

resulting from wounding has been studied but contradictory results have been reported, one worker (10, p. 87) concluding that the increased respiration "can be explained logically on the basis of the increase in the sugar content of the cells," while another (11, p. 454) considers that the two processes "do not seem to be dependent on each other." In our experiments analyses of the whole piece did not always show increases in sugar. In any event the percentage increases in sugar resulting from cutting are very small as compared to the percentage increases in respiration.

The Institute Bliss Triumph potatoes of the 1932 crop used in some of these tests did not contain any free reducing sugars and drastic treatments such as wounding or treatment with chemicals which increased the sucrose and respiratory rate greatly did not result in the accumulation of reducing sugars in amounts sufficient to be detected in our methods of analysis (about 0.3 mg. per 5 cc. juice). This result is of interest when viewed in relation to the data presented by Onslow (13, p. 266–273) in connection with the idea that γ fructose arising from sucrose is the substrate for respiration. Data from experiments by Evans, Emmett, and Bridel and Bourdouil, who worked with apples, pears, and bananas, respectively, are shown (13) indicating that in respiring organs, when sucrose is available, the amount of sugar respired is approximately equal to half the sucrose hydrolyzed, and the gain in hexose is about equal to half the loss in sucrose. The results with this lot of tubers are not in accord with the idea that the glucose from the sucrose molecule should remain behind unless one would be willing to assume that the glucose as soon as set free is at once changed back to sucrose or starch.

It will be noted that the nature of many of these chemicals which stimulate respiration is not such that one would expect them to further oxidation processes. In fact some of them show the properties of anti-oxidants. Hydrogen sulphide, ethyl mercaptan, thiourea, thioacetamide, and cyanides exhibit strong inhibitory effects on the oxidase (tyrosinase) in potato which catalyzes the darkening of tissue or juice when exposed to the air (12). It is of interest that hydrocyanic acid in proper doses also increases both the rate and depth of respiration (breathing) in man and other animals, being considered one of the strongest stimulants of the respiratory center; although in larger doses it produces death under asphyxial symptoms, by hindering the oxidative processes of the tissues (19, p. 796).

SUMMARY

1. When potato tubers were treated with the vapors of ethylene chlorhydrin, ethylene bromohydrin, hydrogen sulphide, acetaldehyde, hydrocyanic acid, or ethyl mercaptan increases of several hundred per cent in the respiratory rate, as measured by the CO_2 output, took place. The curves

representing the respiratory activity of tubers treated with these chemicals all have the same general shape, showing a prompt rise soon after the beginning of the treatment (treatment periods of about 24 hours were used in these tests), a maximum after about 50 to 60 hours, and then a gradual decrease until a value approaching that of corresponding untreated tubers is reached about a week after treatment. Lesser increases resulted from treatments with ethyl sulphide, ethyl disulphide, and hydrochloric acid vapor. Treatments with methyl, ethyl, and isopropyl alcohols decreased the respiratory rate. A close correlation between the effect of a chemical on the respiratory rate and its efficacy in breaking dormancy was not observed.

2. Treatments of cut pieces of potato tubers with potassium thiocyanate, thiourea, and thioacetamide also increased the respiratory rate. Urea and acetamide did not produce such increases.

3. Although treatments with many of these chemicals increase the sugar content of potato tubers, the respiratory responses to the treatments cannot be explained on the basis of sugar changes. This is especially pronounced in the case of vapor treatments of whole tubers; at the time of the highest respiratory activity the sugar content of the treated tubers is actually lower than that of the controls, and by the time the increase in sugar is evident the respiratory rate is rapidly falling. In the case of treatments of cut pieces of potato tubers the data do not indicate that the respiratory behavior of either treated or untreated pieces (cutting alone markedly affects respiration) can be explained on the basis of sugar content.

4. A study of the relation between the activity of a chemical in increasing the sucrose content of potato tubers and in breaking dormancy failed to show a very close correlation in that certain treatments, as with acetone, for example, produced considerable sucrose increases without breaking dormancy.

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STUDIES ON MOSAIC AND RELATED DISEASES OF DAHLIA[1,2]

PHILIP BRIERLEY[3]

For more than 20 years a disease known as stunt has caused concern to growers of dahlias (*Dahlia variabilis* Desf.) in the United States. Essentially any plant which remained short and bushy has been termed stunted, and the term has grown to be a catch-all rather than the name of a specific disease. Various authors have recognized this situation, and have sought to distinguish the supposed virus stunt or mosaic disease. The demonstration of inclusion bodies in the cells of mottled and dwarfed dahlias by Goldstein, and Brandenburg's successful transfer of dahlia mosaic by grafting, proved that at least one virus disease affects this plant. The writer began a study of dahlia mosaic in the summer of 1930 and has continued the work through the summers of 1931 and 1932. The purpose of this paper is to present the results of this study and to record certain observations on other virus diseases of dahlia encountered during the work.

REVIEW OF LITERATURE

Stone (35, p. 78) recorded the occurrence of stunting of dahlias in Massachusetts as early as 1911, and expressed the opinion that the trouble was not due to fungi or insects.

Crosby and Leonard (7, p. 471 and Figs. 61–63) described injury to dahlia by the tarnished plant bug, *Lygus pratensis* L. Their figure of a dahlia plant injured by this insect is in agreement with the usual conception of stunt.

Norton (25) stated that probably the worst disease of dahlia is a constitutional trouble in which plants are dwarfed, bushy, and subject to sunburn, with poor flowers or none. He regarded the cause as obscure but it seemed to be carried over in roots. Norton stated that he recognized and reported the disease in 1909. In 1923 he (26) found this disease could ap-

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[2] The writer wishes to acknowledge his indebtedness to Drs. L. M. Massey and C. E. F. Guterman of Cornell University, to Dr. L. O. Kunkel, formerly of Boyce Thompson Institute, to Dr. B. O. Dodge of the New York Botanical Garden, and to other members of the staffs of these institutions for advice and criticism from the beginning of the work to the reading of the manuscript.

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parently be eliminated by selection of healthy stock. The same author reported to the Plant Disease Survey in 1926 (24, No. 50, p. 449) that sucking insect attacks, red spiders, and bad weather conditions were responsible for some stunting in dahlias but plants so affected did not produce stunted plants the following year. He recognized also a distinct disease characterized by bushy plants with few late flowers and yellowish-green leaves reduced in size which did persist from year to year. This disease did not seem to spread rapidly, and affected plants often failed to live over winter. Since some insects cause stunt, and some probably carry stunt from plant to plant, he advised control of sucking insects as a check on both forms of stunt.

Dudley and Wilson (8, p. 10) in connection with a study of the potato leafhopper allowed 20 nymphs to feed on a caged dahlia plant. The plant was diseased in seven days and dead after 20 days; a control plant remained normal at the end of the test.

Howe (19) brought the experiment of Dudley and Wilson to the attention of dahlia growers. He suggested that stunt or dwarf in which affected plants remain short and bushy, and flowers are poorly formed and undersized, may not be different from what is known as the mosaic disease. He noticed that small brownish cracks often appear in the skin of the crown, the necks of the roots, or in the roots themselves. The cause was not certainly known but persisted from season to season in affected roots. Suspecting sucking insects, especially the potato leafhopper, as carriers, he advocated roguing and spraying. The same author (20) again called attention to stunt in a later publication.

Adams (24, No. 37, p. 391) reported symptoms in dahlias in Delaware similar to leaf roll and mosaic in potatoes. In the following year (24, No. 50, p. 449) he mentioned stunt, dwarf, and leaf curl as terms applied to this trouble by growers, and found the disease a limiting factor in production. In 1927 he (1) reported that the stunt or dwarf disease of dahlias failed to persist when roots from diseased plants were grown in the greenhouse in 1926. Cuttings from these plants made in 1927 also proved healthy. Adams considered the stunting in question "associated in part with soil or feeding factors."

Kunkel (21, p. 678) failed to transmit aster yellows to dahlia by means of the vector *Cicadula sexnotata* Fall., and did not consider stunt disease in any way related to the feeding of this insect.

Connors (3, p. 20) attributed 90 per cent of the "runting" of dahlias to thrips injury. He recognized a transmissible disease of the mosaic type known as stunt, but he did not consider it common. Various cultural conditions and injuries which may cause dwarfing were enumerated. Hamilton and Connors (13) briefly described the injuries to dahlias produced by thrips, leafhoppers, and tarnished plant bugs. Connors (4, 5) again listed

various causes of stunt, and mentioned two virus diseases, namely rugose mosaic and ring-spot. White, writing a section on stunt for a recent bulletin by Connors (6, p. 21), expressed the view that there are several virus diseases which will cause dwarfing, mottling, distortion of leaves, and asymmetrical blooms in dahlias.

Goldstein (11) found intracellular bodies similar to those recorded from mosaic-diseased tobacco, Hippeastrum, corn, sugar-cane, and wheat in the cells of dahlia plants affected with mosaic and dwarf.

Brandenburg (2) was the first to record transmission of a virus disease of dahlia, reporting positive results in transmitting dahlia mosaic by grafting. He found this virus persisted in diseased plants from year to year, but not in the soil. Attempts to transmit the mosaic virus by mechanical methods or by *Mycoides persicae*[4] failed. Chlorotic areas along the veins, and leaf deformations, were described and illustrated. He also observed decrease in number of blooms in diseased plants, and, in one variety, streaking of the flowers. Inclusion bodies found in the sieve tubes of diseased and apparently healthy plants were described in detail, but the author left open the question of their relation to the disease.

Weiss (39) considered that no one had proved that dahlia stunt fulfills the requirements of a virus disease, and, in view of the many records of recovery, suggested that it may be due to insect injury or defective culture.

Tilford (36, 37, 38) recognized two types of stunt in Ohio, one of which is the result of leafhopper injury, the other probably a virus disease transmissible by insects, which persists from year to year and has been found to spread. The latter disease is characterized by bushy growth, cracks in the stem and tubers, and repressed tuber development.

Smith (33, 34) reported a ring-spot disease of *Solanum capsicastrum* transmissible to dahlia. He has established the identity of this ring-spot with the spotted wilt of tomato described from Australia, and has shown that *Thrips tabaci* Lind. is a vector.

MATERIALS AND METHODS

SOURCE OF STOCK

Among the first requirements for an experimental study of virus diseases of dahlias was a supply of healthy stock of suitable varieties. In the early summer of 1930 twenty roots of each of three varieties were purchased from a commercial grower on Long Island. All the plants of the varieties Le Toreador and Faith Slocombe proved to be infected with mosaic when grown in the greenhouse. These varieties are of the tolerant

[4] In a letter to the writer dated Nov. 12, 1930, Brandenburg states that *Mycoides persicae* referred to in his paper is a synonym of *Myzus persicae* Pass. This aphid is known in the United States as *Myzus persicae* Sulz.

type (Fig. 4 C), do not stunt, and therefore were considered healthy by the grower. In the third variety, Mariposa, 18 plants proved healthy, two mosaic, when grown in the greenhouse.

During the late summer of 1930 the varieties Jersey's Beauty, Catherine Wilcox, Snowdrift, and Robert Scott were selected as representative of the symptom types then designated as veinal mosaic, rugose mosaic, yellow-top stunt, and dwarf. Plants of these four varieties and of the varieties Attraction and Florence Finger, rugose mosaic types, were tagged in the fields of commercial growers on Long Island and the selected hills purchased intact. Jersey's Beauty proved to be uniformly mosaic, tolerant, and difficult to diagnose because of masking. The plants tagged as healthy in 1930 were thus doubtless mosaic when marked but showed no symptoms at the time. Of 48 plants of the other five varieties 25 proved healthy and 23 mosaic. The appearance of mosaic is attributed to late infection in 1930, not evident at the time of selection. From this stock of 25 healthy plants a sufficient number of cuttings were made to provide for the experiments of 1931. Some of these plants grown in the field in 1931 provided an abundance of material for the work carried on in 1932. Healthy plants of a few other varieties supplied by the New York Botanical Garden were used in a limited number of tests.

Catherine Wilcox and Robert Scott proved most useful as test varieties. The former develops well defined mosaic symptoms (Fig. 1) and the latter is strikingly dwarfed (Fig. 7) when infected with the mosaic virus. The varieties Attraction and Snowdrift proved more difficult to diagnose, especially in field experiments where hopperburn was a complicating factor.

In addition to mosaic plants of the varieties mentioned above which were purchased at the same time as the healthy selections, a quantity of diseased material was supplied by the New York Botanical Garden. Most of the plants which gave unsatisfactory results in the display bed at that institution during the three-year period of this study were turned over to the writer.

METHOD OF PROPAGATION

Cuttings of known varieties were used almost exclusively in experiments on transfer of the virus and in the majority of the field tests. Vegetatively propagated plants of suitable varieties offer the advantages of uniform symptoms which can be diagnosed early and accurately, but are open to the theoretical danger of carrying masked viruses. Seedlings, on the other hand, are less likely to carry masked viruses, but, because of the hybridity of commercial dahlia stocks, show variations in reaction to the same virus.

Cuttings were made according to a method devised by Dr. P. W. Zimmerman of the Boyce Thompson Institute. Each clump of roots was num-

bered, then divided as for ordinary planting. The divisions were buried in a layer of peat on a greenhouse bench. Shoots developed promptly. When these shoots had reached a suitable size they were cut off, leaving one or two nodes next to the crown to develop further shoots. The cuttings were then set directly into potting soil in four-inch pots and the pots plunged in moist peat. They were shaded with a single layer of cheesecloth which was kept moist by frequent light sprinklings for about a week. Rooted plants were thus available in a week or ten days from the cutting date with a minimum expenditure of space and labor.

SYSTEM OF CONTROL PLANTS

Since each cutting was marked with the number of the stock plant and the date it was possible to check the performance of each plant against that of other members of the line. When mosaic appeared in any line, all the members of that line were excluded from further use in experiments. In addition to this control on performance of individual plant progenies, blocks of plants were set aside as controls against chance infections by insects. With proper attention to greenhouse fumigation no natural infection of greenhouse-grown dahlias from natural spread of the diseases here discussed, with the possible exception of oakleaf, was detected.

GREENHOUSE FUMIGATION

To avoid severe injury to dahlias in the greenhouse by mites and thrips, it was found necessary to fumigate at intervals with naphthalene vapor according to the method described by Hartzell and Wilcoxon (14). This fumigation, carried out at irregular intervals when necessary, was satisfactory in preventing injury by the pests mentioned. The potato leafhopper and other leafhoppers were never numerous in the greenhouse which was chiefly used. All greenhouses in which dahlias were grown for the purpose of this study were fumigated by vaporizing free nicotine at intervals of about one week as a precaution against aphid infestation.

DAHLIA MOSAIC

NAMES

Norton (25) and Stone (35), who first called attention to this disease, do not give it a definite name. Howe (19, p. 26) mentions "yellows" and "mosaic disease" as names applied by growers, and in 1923 (20, p. 181) refers to "stunt or dwarf." Connors (3, p. 10) uses the term "runting" to include mosaic and other troubles. Martin, in summarizing reports to the Plant Disease Survey, adds the names "leafcurl" and "rosette" (24, No. 65, p. 419). The last three terms have not gained general use, and "yellows" is seldom applied. "Mosaic," "dwarf," and "stunt" have been frequently used, the last most frequently, by American writers. All of these

names seem to have been applied at least in part to the disease under discussion, but some of them, notably "stunt," have been used loosely to apply to hopperburn and other injuries as well. Not only has the term "stunt" in American usage often included diseases and injuries of other causes, but it is singularly inapplicable to mosaic in the more tolerant varieties. Such varieties have evidently been overlooked in treatments concerned with stunt. In Germany, a disease, which from Brandenburg's description (2, p. 48) appears to be the same as the one considered here, is known as "Mosaikkrankheit," "Zwergkrankheit," and "Stockkrankheit" (Pape 27, p. 197, and Foerster and Schneider 9, p. 89), the English equivalents of which are mosaic-, dwarf-, and stunt-disease.

HISTORY AND RANGE

The country of origin of dahlia mosaic is unknown. The first records for the United States are from Maryland in 1909 (Norton 25, p. 387) and from Massachusetts in 1911 (Stone 35, p. 78). Diseases probably dahlia mosaic, at least in part, are reported also from California (31, p. 562), Connecticut (24, No. 29, p. 435), Delaware (24, No. 37, p. 391), Georgia (24, No. 73, p. 382), Indiana (24, No. 65, p. 418), Kansas (24, No. 65, p. 419), New Jersey (3, p. 10), New York (19, p. 26), Ohio (24, No. 55, p. 366), Pennsylvania (15, p. 73), Tennessee (10), Utah (24, No. 73, p. 382), and Wisconsin (24, No. 55, p. 366). Later reports from many of these states are also on record. The writer has found dahlia mosaic common in Connecticut, New Jersey, and New York, or throughout the region surveyed. The disease is undoubtedly generally distributed in the United States. It is present in Holland (28, p. 17), in Germany (2), and probably in England (34, p. 320). Inasmuch as the disease persists in the roots which are commonly sold and exchanged, it is very likely to have a distribution coextensive with dahlia culture.

IMPORTANCE

Dahlia "stunt," in its broad sense, including insect injuries as well as mosaic, is generally conceded to be the most serious disease of dahlia in the United States. Opinions differ on the relative importance of leafhoppers, thrips, tarnished plant bugs, mosaic, and cultural defects as causes of "stunt." The writer considers mosaic the most serious disease of dahlias in the region surveyed.

The character of the injury produced by mosaic varies strikingly with the tolerance or intolerance of the variety. Varieties of the tolerant group are not conspicuously damaged, but even these are doubtless below normal in performance when affected. In the semi-tolerant group the most evident loss is involved in shortening of the flower stem which accompanies partial dwarfing and unsightly appearance of the plants. In varieties of the in-

tolerant group, mosaic plants often fail to flower, or bloom later than healthy plants (Fig. 6 A). Such plants have an extremely unsightly dwarf habit and are worthless for cut flowers or for garden display. A further source of loss is the disappearance from the trade of intolerant sorts which are highly desirable aside from their reaction to mosaic.

In the display of dahlias at the New York Botanical Garden, which included stock from a large number of commercial dahlia growers, 28 per cent of the plants were found mosaic in 1930, 56 per cent in 1931, and 30 per cent in 1932. Of 102 plants purchased from growers by the writer 65 were

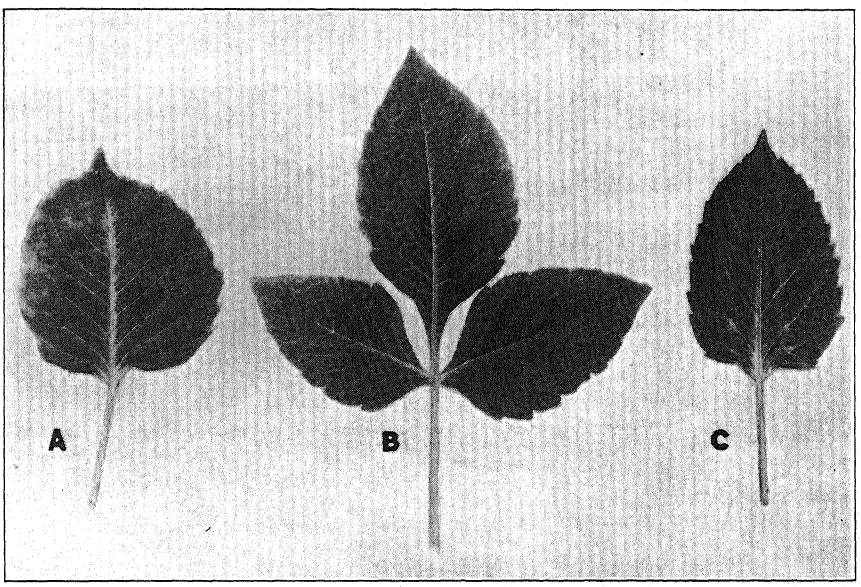


FIGURE 1. Dahlia mosaic in the variety Catherine Wilcox. Variations in the vein-banding pattern. A. Typical pattern. From a greenhouse plant inoculated by *Myzus persicae*. B. Initial symptoms in a greenhouse plant following grafting. Rare. C. Symptoms in a field-grown plant. Natural infection. Not uncommon.

mosaic when received. In commercial fields tolerant varieties, especially Jersey's Beauty, which is often difficult to diagnose because of masking, may be found practically 100 per cent affected. Intolerant varieties have been seen from 18 to 89 per cent mosaic in commercial fields.

SYMPTOMS

The most characteristic symptom of dahlia mosaic is vein banding (Figs. 1, 2, 5 A, 12). The normal green color develops irregularly in the mosaic leaf, bands adjacent to the midrib or branch veins remaining yellowish-green or pale green when the remainder of the leaf has reached nor-

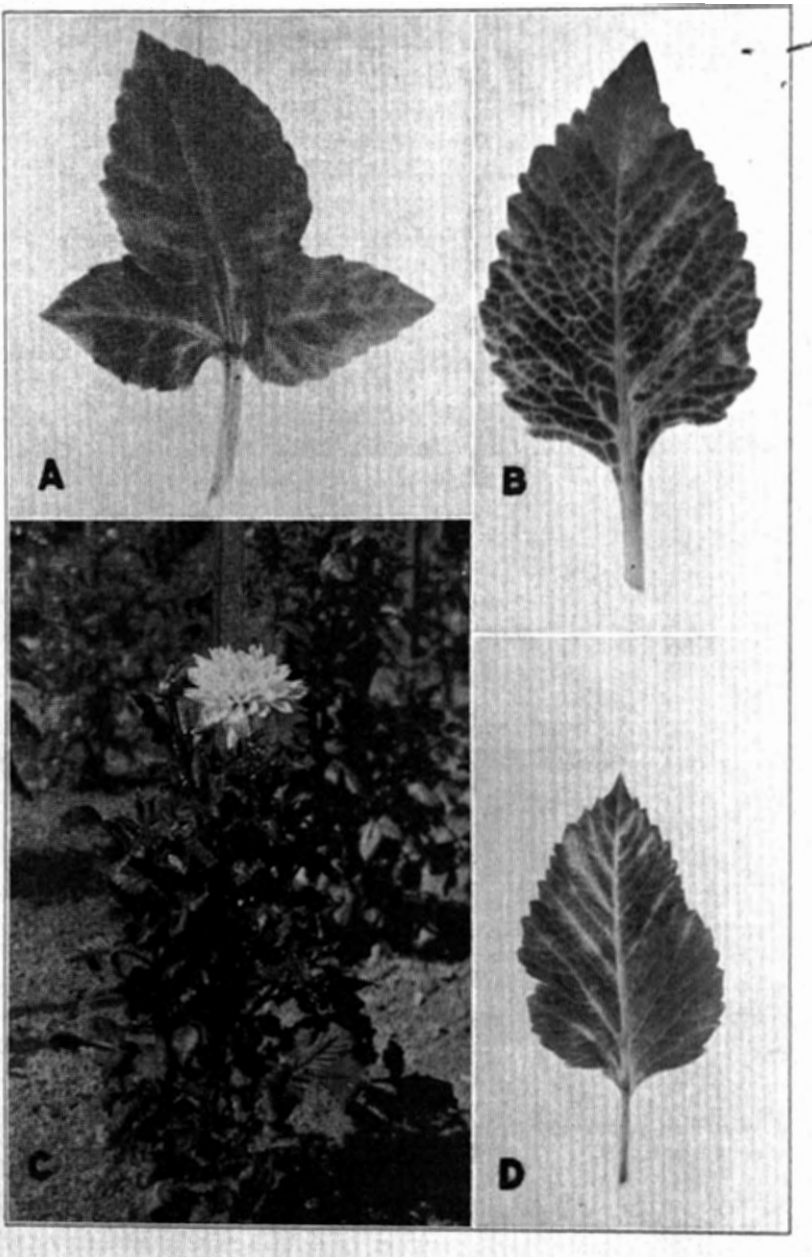


FIGURE 2. Symptoms of dahlia mosaic. A. Veinlets prominent over broad diffuse chlorotic bands in the variety Eureka. B. Chlorotic pattern in the variety White Empress. C. Field symptoms of mosaic in the variety Mrs. Margaret W. Wilson. Note nearly normal flower on extremely short stem. Plant is 27 inches tall. Photographed in August, 1931. D. Typical vein-banding pattern in the variety Jersey's Beauty.

mal color. The width and form of this banding varies in different varieties, and to a less extent in individuals of a given variety. The typical coloring of the bands is uniform within a variety but varies among different sorts from a green only slightly paler than normal to a bright yellow. As the affected leaf grows older the discolored areas tend to approach the normal green of the remainder of the leaf and the color pattern may become masked. Vein banding is the most reliable diagnostic symptom of dahlia mosaic known, appearing in varieties which show no distinctive distortion, necrosis, or dwarfing. Although the presence of this symptom has proven positive evidence of mosaic infection, its absence does not prove the absence of the virus, since under conditions of poor growth or severe insect infestation the symptom may fail to develop or be obscured by other abnormalities. In the variety Robert Scott this symptom rarely appears. This variety, however, shows other characteristic symptoms of mosaic.

In some varieties (Fig. 2 A) the yellowish bands occupy a considerable area of the affected leaf and the veinlets as viewed from above are seen to stand out sharply as brownish or purplish lines. Accompanying vein banding in many cases there is an unequal growth of the two sides of the affected leaf, or a slight twisting of the apex of a pinna.

In certain varieties such as Snowdrift (Fig. 3 C) leaves of mosaic plants may become generally yellowed, the margins roll upward on the midribs, and the lateral pinnae are more or less twisted. Vein banding may be poorly expressed (Fig. 3 D) and the whole symptom complex is distinguished with difficulty from that of hopperburn in the field.

Some varieties such as Miss Bridgeport (Fig. 3 A, B) and Doris Wilmore (Fig. 4 A) show marked distortion, rugosity, and blistering of leaves when mosaic. Others, of which Robert Scott is the only well established representative, develop areas of necrosis on the midveins. These areas commonly appear about midway in the length of individual pinnae and bring about a sharp downward turning of the pinna at that point (Fig. 7). This vein necrosis, together with the characteristic dwarfing that accompanies it, is as valuable for diagnosis as vein banding.

Shortening of the internodes is prominent in mosaic plants of all the intolerant group of dahlias, and somewhat less prominent in the semi-tolerant group. In the tolerant group the variety Mrs. I. de Ver Warner appears to be only slightly shortened by mosaic infection. Accompanying the shortening of the main stem in intolerant sorts is a tendency to force the lateral shoots, which are in turn shortened, producing the familiar short, bushy, "crippled" habit designated as "stunt." The flower stems are likewise short in such varieties so that it is often impossible to cut a flower with suitable stem length without cutting away the whole plant (Fig. 2 C).

Accompanying the shortening of the stem is a tendency to shorten the

roots (Fig. 6 B, C). This symptom has been noted in a number of varieties of the intolerant group which may be readily diagnosed as mosaic on the basis of top symptoms when affected. From a limited number of observations it appears that shortening of the roots of mosaic plants is not recog-

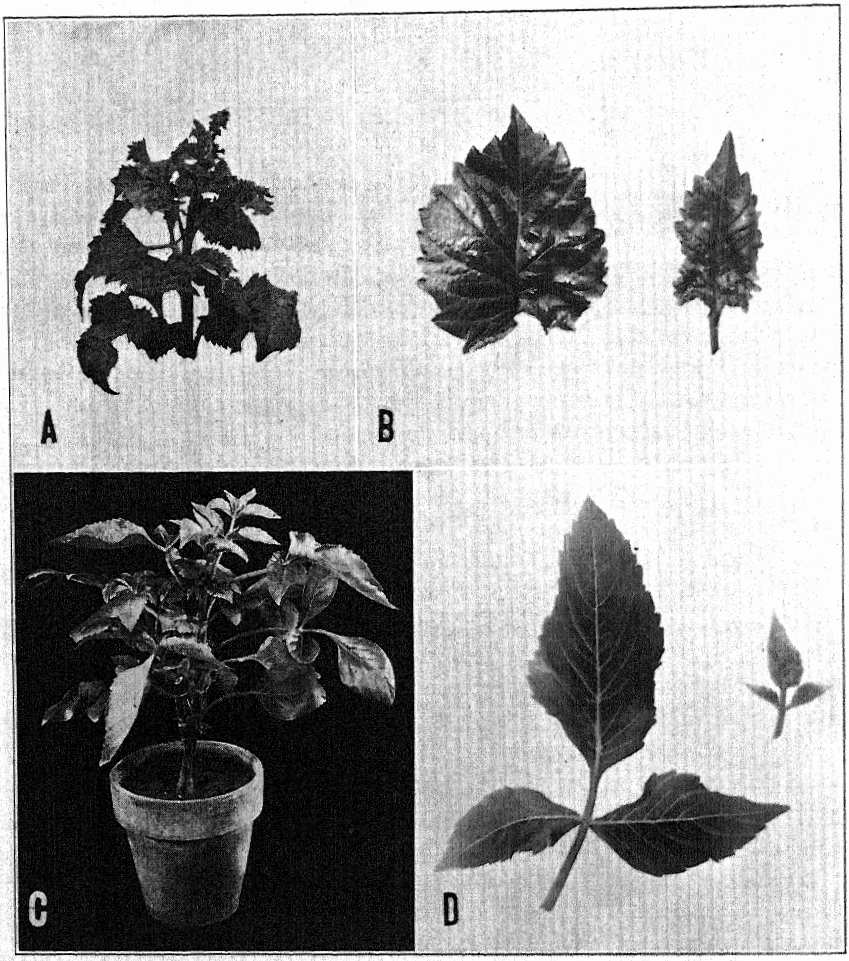


FIGURE 3. Symptoms of dahlia mosaic. A. A 6-inch shoot of the variety Miss Bridgeport, grown in the greenhouse. Note short internodes and leaf distortion. B. Two leaves from A showing wrinkling and distortion of leaves. C. A plant of the variety Snowdrift infected by grafting to mosaic Jersey's Beauty. Note yellowing and distortion of leaves, and short internodes. Six-inch pot. D. Two leaves from C showing obscure vein banding.

nizable in tolerant varieties, and that plants of intolerant varieties infected during the current season produce roots indistinguishable from healthy roots (Fig. 6 C). In view of the fact that shortening of the roots may well

be brought about by causes other than mosaic, this symptom is not regarded as of general diagnostic value.

The flowers of mosaic dahlias are commonly normal. In the most tolerant varieties such as Mrs. I. de Ver Warner there is no evident difference between the flowers of healthy and diseased plants (Fig. 5 B). In the semi-tolerant group shortening of the flower stalk is the only effect which tends to make the flowers unsalable. In many of the intolerant sorts flowers are developed later on mosaic plants (Fig. 6 A) and may be smaller than normal or even lacking. Some plants badly stunted by mosaic develop ap-



FIGURE 4. Variation in field symptoms of dahlia mosaic. A. Variety Doris Wilmore, intolerant. Fifteen inches tall. B. Variety Calizona, semi-tolerant. C. Variety Faith Slocombe, tolerant. Meter stick indicates height. All photographed in August, 1931.

parently normal flowers on extremely short stems (Fig. 2 C), and others develop defective blooms.

No change in the coloring of the flower due to mosaic infection has been established by the writer. Brandenburg (2, p. 47) describes and figures a change in color distribution in the variety Paradiesvogel due to mosaic. The writer has seen individual cases of streaking in the flowers of mosaic plants of three varieties but the streaking has not uniformly accompanied mosaic infection in these varieties. In view of the fact that Lawrence (22, p. 128, and 23, p. 270) has shown that similar color patterns are heritable the writer prefers not to claim that these few cases of pigment changes associated with mosaic were caused by mosaic infection.

The "mosaic" and "dwarf" diseases, with which Miss Goldstein (11) found cellular inclusions associated, are undoubtedly both dahlia mosaic. The writer has not studied these inclusions.

ETIOLOGY

Dahlia mosaic is shown to be a virus disease by the type of symptoms developed, by the persistence of the infectious principle in vegetative parts

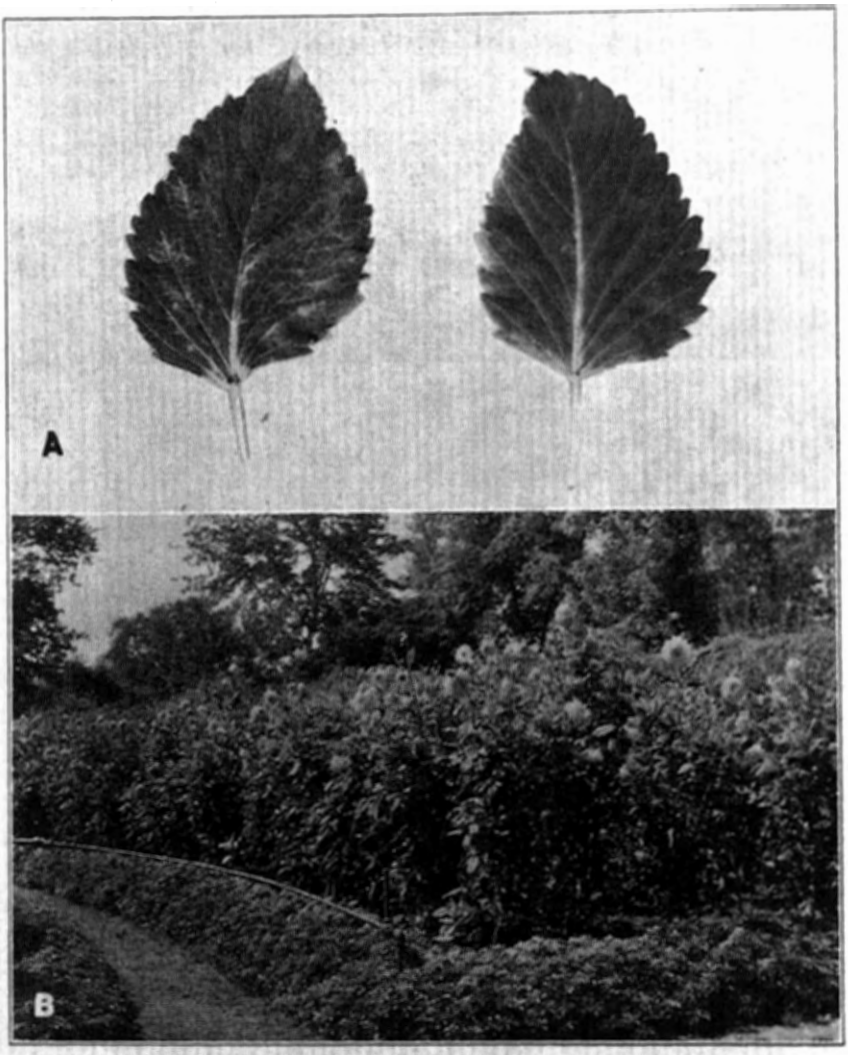


FIGURE 5. Symptoms of dahlia mosaic in the variety Mrs. I. de Ver Warner, tolerant. A. Mosaic and healthy leaves. Note that chlorotic symptoms are clearly expressed. B. A block of Mrs. Warner including 66 mosaic and 2 healthy plants, the latter indistinguishable from the mosaic in general habit. Note good flowers and long stems.

of mosaic plants, and by the transmissibility of this infectious principle by grafting and by *Myzus persicae* Sulz. Brandenburg (2, p. 52) found no evi-

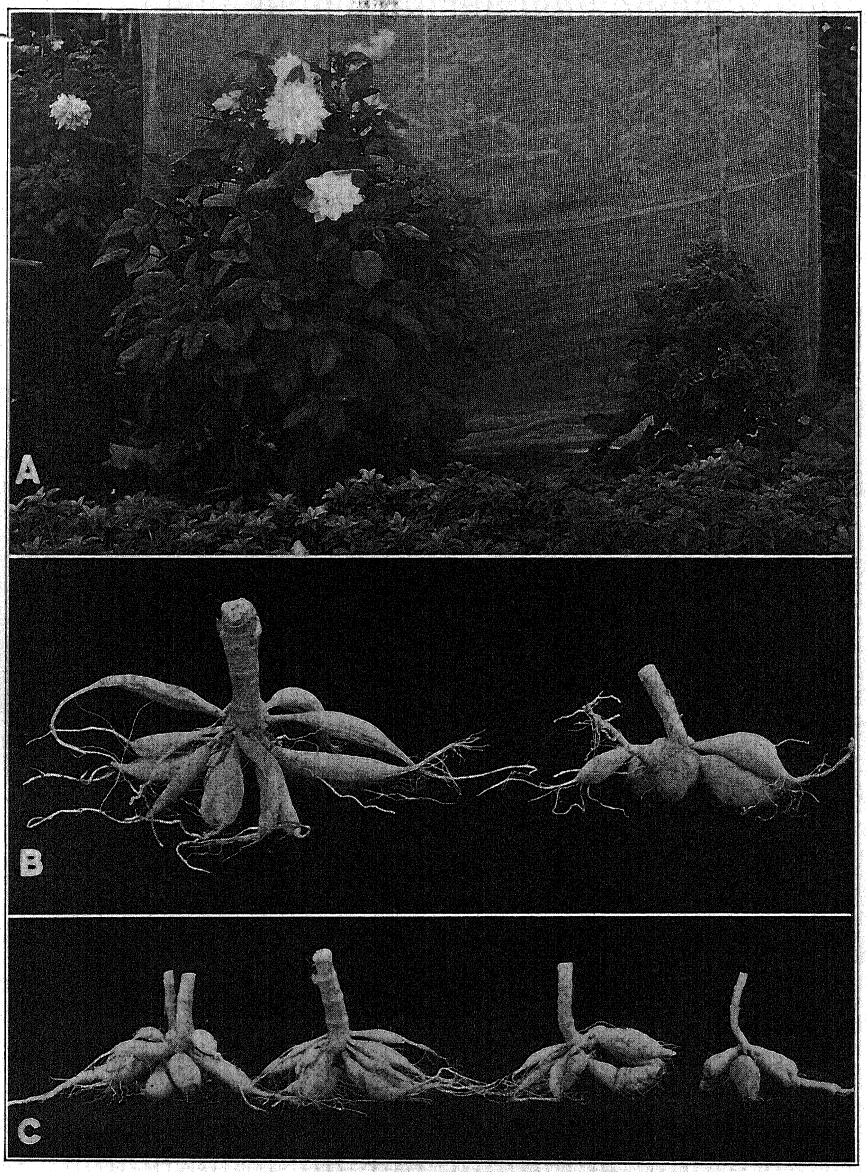


FIGURE 6. A. Healthy and mosaic plants of the variety Snowdrift, photographed in September, 1932. B. Roots of the plants shown in A. C. Roots of the variety Esther R. Holmes. Left to right: two healthy plants, a plant infected during the current season, and a plant mosaic for at least one year.

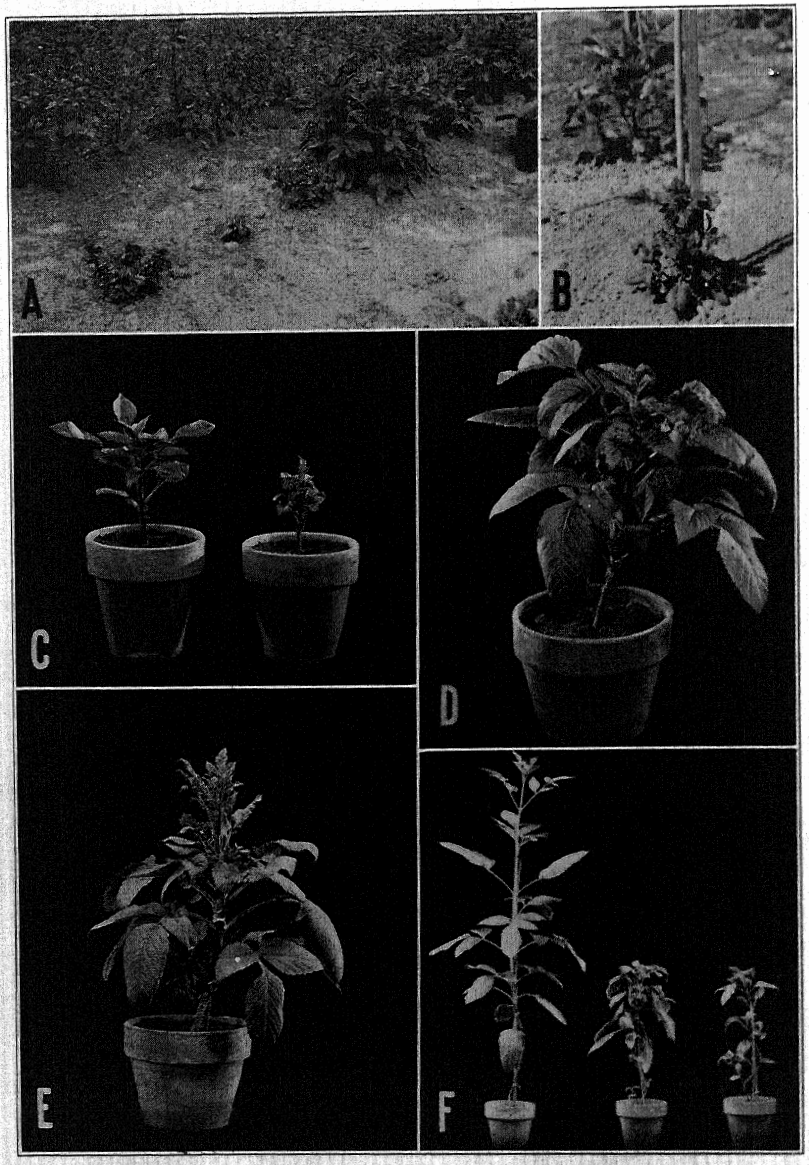


FIGURE 7. Symptoms of mosaic in the variety Robert Scott. A. Three mosaic plants and one healthy plant in a commercial field in August, 1930. B. Plant in the left foreground in A as it appeared in August, 1931. C. Healthy and mosaic cuttings at the stage commonly used in grafting. Four-inch pots. D. A plant infected following grafting. Eight-inch pot. E. The same plant after further growth. F. Healthy control plant and two plants infected by *Myzus persicae*. Note shortening of internodes and leaf distortion is common to all mosaic plants shown.

dence that the dahlia mosaic virus is soil-borne. The writer has found no evidence suggestive of persistence in soil, and no evidence of seed transmission. No mosaic appeared in 44 seedlings grown from seed of mosaic Catherine Wilcox. Three types of seedling dahlias grown from commercial seed proved free of mosaic. The symptom designated as "stipple chlorosis" (Fig. 16 D) appeared in a number of these seedlings. This condition has not been found to be transmissible by grafting. The significance of "stipple chlorosis" is unknown, but it is not a symptom of dahlia mosaic. There is some indication that mosaic dahlias do not set seed as freely as healthy plants.

Persistence in Vegetative Parts

The mosaic virus persists from year to year in diseased roots. Thirty-eight roots representing 30 varieties marked diseased by B. O. Dodge in 1929 were found to be mosaic in 1930. Two hundred fourteen plants mosaic in 1930 again developed characteristic symptoms in 1931; 399 mosaic in 1931 proved mosaic again in 1932. The virus has been shown to persist over winter in 129 varieties. Diseased stock of 55 of these sorts has been grown for three or more successive seasons, and 19 varieties have been grown in the mosaic state for four years. Typical symptoms of mosaic appeared in 265 diseased plants grown in the greenhouse, and in 61 which were grown in cloth cages out-of-doors. These plants were largely root divisions of plants with previous mosaic records. The vein-banding symptom has been relied on almost exclusively in diagnosis of mosaic in these tests, although it is supported by other symptoms in all but the most tolerant sorts. In only four cases has mosaic, diagnosed as positive on this basis, failed to appear in the following year.

Cuttings taken from stocks known to be diseased have proved to be infected in all cases. In 1931 one hundred five cuttings representing five varieties proved to be mosaic in agreement with the 27 plants from which they were taken. On the other hand both healthy and diseased cuttings were taken in 1931 from four plants diagnosed as healthy in the field in September, 1930. Two of these plants were of the variety Robert Scott, and two of the variety Snowdrift. Diseased cuttings of each variety when grafted to healthy Catherine Wilcox produced mosaic in the latter, and healthy cuttings from the same lines produced no effect on the Catherine Wilcox stock. It is believed that these partially infected hills were the result of infection which took place late in 1930, the virus penetrating only a part of the hill before it was divided.

Failure of Mechanical Transmission

Brandenburg (2, p. 57) was unable to produce infection in dahlias by injection of expressed sap of mosaic leaves into stems, petioles, and mid-

ribs. He also reported that contact of the cut surface of a healthy cutting with the sap of a diseased plant which adhered to the cutting knife was without effect. The writer has tried five methods of mechanical inoculation which have proven effective in transmitting other plant viruses, but he has found no evidence that the dahlia mosaic virus is transmissible in this manner.

The methods tested were as follows: (a) Expressed sap of mosaic leaves was rubbed over the surface of several leaves of each healthy plant with cheesecloth (18). (b) Expressed sap was applied to several leaves of each plant by means of a glass rod of the type devised by Samuel (30). (c) Expressed sap was pricked into leaf axils by means of No. 0 black insect pins. This method is a modification of one found effective in transmission of the virus of lily mosaic by Guterman (12). (d) Diseased leaves were superimposed on healthy leaves and No. 0 black insect pins were pushed through the chlorotic areas of the mosaic leaf into the healthy leaf. About 100 of such punctures were made in each of several leaves of each inoculated plant. This method is a modification of one used by Sein (32) in transferring the virus of sugar-cane mosaic. (e) A piece of diseased tissue bearing a bud was inserted in a slit in the stem of a healthy plant. None of the inserted buds survived, and no transfer of the virus occurred. Kunkel (21, p. 681) was able to transmit the virus of aster yellows by budding but only in those cases in which the inserted bud lived.

Because of space limitations in the greenhouse all plants were grown in the open field after inoculation. Natural infection appeared in the inoculated plants, but not more than in uninoculated controls. In 1931, thirteen of 82 inoculated plants and 11 of 63 controls became infected. In 1932, three of 60 inoculated and two of 58 controls were mosaic at the end of the growing season. The time of appearance of symptoms showed no correlation with the dates of inoculation. There is, therefore, no evidence that infection resulted from any of the writer's mechanical inoculations. The absence of natural spread in the greenhouse, where the same knife was used throughout in making cuttings and grafts without any attempt to remove or destroy the virus between cuts, lends further support to the conclusion that the virus of dahlia mosaic is not readily transmitted. Additional support is furnished by the outcome of a series of grafts in which the diseased scions were removed after short intervals. Infection resulted in the four stocks with which the diseased scion was left in contact for six days or more, but no infection appeared in the four stocks from which the diseased scion was removed after four days or less.

Transmission by Grafting

Brandenburg (2, p. 57) first reported transmission of dahlia mosaic by grafting. In one experiment healthy scions on diseased stocks developed

weak symptoms after some time. When he grafted diseased scions on healthy stocks, symptoms did not appear in the stocks until the following year. The writer found symptoms developed in 42 days following an approach graft made in July, 1930. At the close of 1930 it appeared that approach grafting could be expected to yield results within a reasonable time and that mechanical methods of inoculation would not prove effective. Accordingly the method of approach grafting was applied to the problems presented by diverse symptom types in different varieties. The data from 273 grafts made during the three seasons are summarized in Table I.

TABLE I

TRANSMISSION OF DAHLIA MOSAIC BY GRAFTING DISEASED SCIONS ON HEALTHY STOCKS

Stock variety	No. of scion varieties		No. of plants grafted	
	Tested	Positive	Positive	Negative
Attraction	18	18	32	3
Calvin Coolidge, Jr.	2	2	2	1
Catherine Wilcox	68	56	110	37
Collarette Seedling	2	2	4	1
Coltness Gem Seedling	2	2	4	1
Eva Williams	1	1	1	0
Florence Finger	6	6	6	0
Galli Curci	1	1	1	0
Jane Cowl	1	1	1	0
Mariposa	2	2	2	0
Papillon	2	2	2	0
Robert Scott	13	11	16	2
Rose Fallon	1	1	1	0
Snowdrift	17	15	39	7
Totals	71*	64*	221	52

* Eliminating duplicates.

Controls. All grafted plants with one exception were grown in the greenhouse. The cuttings of all varieties listed as stocks in Table I were free of mosaic when grafted as shown by subsequent performance of the root clumps from which the cuttings were taken and further, in nearly all the cases, by the performance of sister cuttings grown parallel. Additional plants grown in field cages and in the open field furnished further evidence on the reliability of individual plant lines.

The healthy cuttings as well as the root clumps from which they were taken were kept in the same greenhouse. The number of cuttings was large in the early summer and gradually diminished as plants were used. In 1931 the supply of plants was small and the stock of controls was allowed to diminish to 20 at the close of the season. In 1932 the supply of plants was limited only by space and labor requirements. On August 12 two hundred forty-one uninoculated cuttings representing ten varieties and twelve uninoculated seedlings of two sorts served as controls in the greenhouse. By

October 11 the supply had been narrowed to specific control blocks consisting of 55 plants of Catherine Wilcox, 2 Galli Curci, and 21 Robert Scott in this greenhouse. Seventy uninoculated plants were continued until October 29, and 35 until November 28 or later. In a separate greenhouse 36 plants of Catherine Wilcox served as controls on a series of 59 grafts to that variety. This block of controls was maintained parallel to the grafted plants from July 30 to October 29, and controls were brought to the main greenhouse with the grafted plants retained on that date. No mosaic infection appeared in any control plant grown under glass in either year. Many plants which remained healthy in the greenhouse following ineffective inoculations or insect transfers furnished additional evidence in support of this conclusion. Twelve grafts in which healthy scions were set on healthy stocks of different varieties showed no change in stock or scion reaction.

Diverse symptom types are varietal reactions to one virus. The chief object of grafting diseased scions of a number of varieties on healthy plants of certain test varieties was to determine the interrelation of apparently distinct types of disease of the virus type found in dahlias. Five types tentatively regarded as distinct at the beginning of this study may be illustrated as follows: 1. (Mild mosaic) Plants of Mrs. I. de Ver Warner (Fig. 5), Jersey's Beauty (Fig. 2 D), Faith Slocombe (Fig. 4 C), and Le Toreador showed vein banding but no evident dwarfing or distortion. 2. (Rugose mosaic) Plants of Calizona (Fig. 4 B) and other varieties showed vein banding together with rugose to blistered leaves when mosaic. 3. (Rugose rosette) Affected plants of Casper G. Ware, Mrs. M. W. Wilson (Fig. 2 C), Doris Wilmore (Fig. 4 A), and others combined the symptoms of Types 1 and 2 with marked rosetting. 4. (Yellow top rosette) Certain plants of Snowdrift (Figs. 3 C, D, and 6 A), White Empress, and a few other varieties were classed separately as showing yellowing of the upper leaves and marked dwarfing without evident mottling. 5. (Dwarf) Robert Scott plants (Fig. 7) suffered the most severe dwarfing but showed no mottling.

On further observation of selected representatives of these types in the greenhouse and field through three seasons it was found that the type of reaction is relatively uniform within a given variety. The vein-banding symptom characteristic of Type 1 varieties may be found to appear in varieties of Types 2, 3, and 4, if the plants are examined carefully throughout a growing season. Vein banding also occurs in Type 5 but so rarely that it is of no value in diagnosis. Degree of dwarfing was found unworkable as a distinction between Types 2 and 3. Plants graded as Type 3 in the field may be graded 2 in the greenhouse or in field cages, and the degree of rosetting used as a criterion of Type 3 in grading a new variety must be entirely arbitrary. Current season mosaic in a Type 3 variety commonly appears as Type 2. The varieties Snowdrift and White Empress, which

originally formed the nucleus of Type 4, may show mottling in the field, and cannot be logically excluded from Type 3. Varieties of uncertain classing but showing definite vein banding are treated as of Type 1. Since all of these five symptom types appear to be merely varietal responses to mosaic, the terms tolerant (Type 1), semi-tolerant (Type 2), and intolerant (Types 3, 4, 5) are more useful for purposes of general discussion.

Mosaic from 18 varieties transferred to healthy plants of Attraction produced the same type of symptoms characteristic of mosaic plants of this variety naturally infected in the field. Attraction is an intolerant variety (Type 3 or 4) showing typical vein banding, yellowing, distortion of the leaves, and dwarfing. The number of transfers from the five symptom types was 13, 6, 5, 1, and 7 respectively. Eighteen of the stock plants carrying infection derived from each of the five symptom types were grown in the field together with naturally infected Attraction plants in the following season. No differences in the symptoms developed were detected.

Healthy plants of the variety Catherine Wilcox were infected with mosaic from 56 varieties. The number of transfers from the five symptom types was 44, 30, 22, 5, and 9 respectively. Thirty-eight of the stock plants infected from representatives of each of the five types showed the usual Type 2 or 3 reaction characteristic of naturally infected plants of this variety when grown in the field in the following season. Catherine Wilcox plants have shown some variation in the vein-banding pattern expressed (Fig. 1 A, B, C) but these variations have not proved characteristic of plants inoculated from particular sources.

The mosaic virus was transferred to Florence Finger from six varieties, representing the symptom Types 1, 2, and 3. The reaction of the stock plants of this variety was of Type 3 regardless of source of the virus.

The Robert Scott variety which shows the distinctive Type 5 reaction characterized by marked dwarfing, stiff foliage, and vein necrosis reacts in the same manner regardless of the source of the mosaic virus. Mosaic virus from nine plants of Type 1, three of Type 2, two of Type 3, and two of Type 5, brought about this characteristic reaction in healthy plants of Robert Scott. The response of this variety to the mosaic virus from relatively tolerant varieties such as Chautauqua Salute, Le Toreador, Mariposa, and Mrs. I. de Ver Warner, offers striking evidence that the differences in symptom types are varietal reactions to one mosaic disease rather than distinct diseases.

Snowdrift plants naturally infected in the field show a Type 3 or 4 reaction. Plants grafted with diseased scions of 15 varieties, including 25 plants of Type 1 reaction, three of Type 2, two of Type 3, one of Type 4, and eight of Type 5 reacted in the same manner. Twenty-seven of the grafted plants grown in the field the following season showed no differences in symptom type from Snowdrift plants naturally infected.

Calvin Coolidge, Jr. reacted in the same manner to mosaic scions of Types 1 and 2. Mariposa showed the same response to the virus from Types 2 and 5. Individual seedlings of Coltness Gem and Collarette varied in reaction to mosaic, but the reaction was not correlated with the source of the virus. A uniform reaction is not to be expected from seedlings from a commercial packet.

Mosaic plants of several varieties of which no healthy stocks were available were grafted with mosaic scions of other varieties. In case different viruses were involved, it was thought that the resulting combination of diseases might show symptoms distinct from the original reaction of the mosaic stocks. No evidence of change in the symptom type of the stock variety was detected, however, when mosaic plants of Faith Slocombe, Francis La Rocco, Jersey's Beacon, Jersey's Beauty, Jersey's Olympus, and Watchung Sunset were grafted with diseased scions of other varieties.

The evidence detailed, together with less extensive evidence from insect transmission experiments, indicates that the symptom types thus far discussed are varietal reactions to one mosaic virus.

Failures. In 52 cases no infection was observed following grafting of a mosaic scion on a healthy stock. Twenty-one of these failures are attributed to death of the scion before union was effected. Included in this number are four grafts in which the scion was removed after contact of four days or less. Twenty failures may be attributed to the late date at which the graft was made inasmuch as the proportion of takes following August grafts was low and no takes resulted from September grafts. All August grafts were made to the stock variety Catherine Wilcox, consequently a high proportion of failure appears in this variety. Eleven failures are not to be explained on the basis of early death of the scion or to grafting late in the season. In some of these cases the failure of symptoms to develop appears to have been due to slow growth of the stock plant. Six grafts which resulted in no evident transfer of the mosaic virus in 1931 showed definite symptoms in 1932. It is probable, therefore, that a number of the grafts recorded as failures would prove positive if the stocks could be grown again.

Transmission by Myzus persicae

Methods. Two types of insect cages were used in field tests and two other types in the greenhouse. Twelve movable wooden cages covered with cheesecloth of 40 meshes to the inch were used in the experiments at Yonkers. At the New York Botanical Garden a permanent wooden frame so divided as to form 24 units in a double row of 12 was provided. It was necessary to cover all field cages with new cloth each season. Greenhouse cages were available at the Boyce Thompson Institute. Lantern globe

cages consisted of the largest size of globe available, the tops covered with 40-mesh cheesecloth.

Leafhoppers and plant bugs were handled with a suction apparatus of the type illustrated by Kunkel (21, Fig. 4). Aphids and thrips were transferred by means of a camel's hair brush.

Identification of insects. Twenty-two slides of aphid species were identified for the writer by Dr. Edith M. Patch. Eighty collections of aphids in alcohol and seven slides of aphids were identified by Dr. P. W. Mason. The species of *Empoasca* used in 1930 and 1931 was diagnosed as *E. fabae* Harris by Dr. Albert Hartzell. One composite sample collected from field cages in 1930 was identified as this species by Dr. F. W. Poos. One sample of *Lygus pratensis* L. was named by Mr. H. G. Barber.

Experiments of 1930. In the 1930 field cage experiments 16 healthy plants of the variety Mariposa were grown in various combinations, with diseased plants, with insects, and with both insects and diseased plants. A colony of *Myzus persicae* which appeared as a natural infestation on a mosaic dahlia in the greenhouse on July 9 was cultured on this plant in a greenhouse cage until July 28 when individuals were transferred to four healthy plants in field cages. One of these plants developed mosaic symptoms on September 13, and two more on September 24. The fourth plant showed mosaic symptoms in the following season. No infection appeared in four plants caged without insects, and none in eight plants exposed to feeding of insects other than *M. persicae.* The other insects tested were *Aphis rumicis* L. from nasturtium (*Tropaeolum majus* L.) and *Empoasca fabae* from dahlias in the field. Two healthy plants were infested with each of these insects in the absence of mosaic dahlias and two healthy dahlias were caged throughout the growing season with each insect in the presence of mosaic dahlias.

Experiments of 1931. An experiment with field cages at Yonkers in 1931 was designed to determine the nature of the disease called "dwarf" in the variety Robert Scott. All the plants grown in this series of cages were of this variety. The fact that shoots from a number of roots with a previous history of "dwarf" appeared healthy in the first stages of growth in the greenhouse, together with the general absence of chlorotic symptoms in this variety, suggested that the "dwarf" disease was not of the virus type. According to the original plan of the experiment, therefore, one healthy and one "dwarfed" plant were set in each of the first five cages, and two plants "recovered" from "dwarf" were set in each of the remaining five cages on June 25. In the last five cages it was planned to attempt to reproduce "dwarf" by the direct feeding effects of some insect. By July 30 it was evident that the apparent recovery of "dwarfed" plants was a temporary effect, and that the causal agent not only persisted in affected plants but was transmissible by grafting. On July 30, therefore, apparently

healthy cuttings were set between the two diseased roots in these five cages. Subsequent experiments have shown that "dwarf" is the mosaic reaction of the variety Robert Scott.

Myzus persicae was transferred to two cages from a natural infestation which appeared on a mosaic dahlia in a field cage at the New York Botanical Garden. One plant infested with this species showed mosaic ("dwarf") symptoms on August 21. The other plant to which *M. persicae* was transferred on August 17 showed no symptoms in 1931 but was mosaic in part in 1932. No mosaic appeared in 1931 in three cages to which no insects were added, and none in five cages into which insects other than *M. persicae* were introduced. The other insects tested were *Empoasca* sp. and *Illinoia solanifolii* Ashmead from potato (*Solanum tuberosum* L.), and *Aphis gossypii* Glover from lily (*Lilium* sp.). *Empoasca* sp. introduced into two cages on August 4 and *A. gossypii* placed in one cage July 16 multiplied on the diseased plants and migrated to the healthy plants but no virus symptoms resulted in 1931 or 1932. *Illinoia solanifolii* placed in two cages July 16 and August 5 failed to multiply. No infection appeared in 1931 but both plants exposed to this insect were mosaic in part in 1932. The infection of these two plants may have resulted from contamination by *M. persicae* in the last weeks of growth after rain and wind had rendered the cages no longer insect-tight.

An experiment in the 24 units of the permanent cage at the New York Botanical Garden in 1931 was conducted along similar lines. Healthy cuttings of the variety Catherine Wilcox from individual plant lines which proved uniformly healthy were grown in each cage. Mosaic plants were set in each cage except four control cages in which only healthy plants were grown. Insects were placed on the diseased plants on the dates shown in Table II and allowed to migrate to the healthy plants throughout the season. Transfers from the diseased to the healthy plant were made when natural spread was slow. *Aphis gossypii* was obtained from lilies, *Empoasca* sp. and *Illinoia solanifolii* from potato, and *A. rumicis* from nasturtium. *Myzus persicae*, *M. pseudosolani* Theobald, *M. circumflexus* Buckt., and *Aphis* sp. No. 21 appeared as natural contaminations in some of the cages, apparently gaining entrance during the three-day interval between planting and completion of the cage. *Aphis* sp. No. 21 is probably an undescribed form near *A. abbreviata* Patch, according to Dr. P. W. Mason. Dr. Patch called it *Aphis* sp., not *A. gossypii*. No attempt was made to destroy the aphids which thus appeared, but the experiment was adjusted to include them in the tests. All the insects mentioned multiplied and fed on both diseased and healthy plants. The results are summarized in Table II.

As shown in Table II the mosaic virus was transmitted to two of three healthy plants exposed to feeding of *Myzus persicae*. The third plant re-

mained healthy in 1932 also. No infection appeared in eight plants not exposed to insects, and only one plant of 17 colonized by insects other than *M. persicae* became infected during 1931. This plant was infested with *Aphis rumicis* in a cage adjacent to a colony of viruliferous *M. persicae* which may have penetrated from one compartment to the other through some defect in the cloth wall. On August 19 a severe hailstorm damaged the tops of many of the cages and the aphids intermingled freely before the damage could be repaired. Mosaic infections resulting in the following season for this reason cannot safely be attributed to any one species. Seven plants healthy at the close of 1931 showed mosaic symptoms in the greenhouse in 1932. These seven plants were from a group of cages at the south end of the block so situated that viruliferous *M. persicae* may have been distributed to them by wind accompanying the hail.

TABLE II

EXPERIMENTS ON INSECT TRANSMISSION OF DAHLIA MOSAIC IN FIELD CAGES AT THE NEW YORK BOTANICAL GARDEN, 1931

Insects tested	No. healthy plants exposed	Date infested	Result
Myzus persicae	3	July 13–20	1 mosaic Aug. 10 1 mosaic Sept. 8 1 healthy
*M. pseudosolani**	1	July 20	No mosaic but killed by aphids
*M. circumflexus**	1	July 27	Healthy
Aphis rumicis	4	July 13	3 healthy, 1 mosaic Sept. 8
A. gossypii	3	July 13–27	Healthy
Illinoia solanifolii	4	July 13–27	Healthy
Empoasca sp.	4	July 16–Aug. 4	Healthy
Aphis No. 21	2	July 13	Healthy
Control cages no insects	8	—	Healthy

* Appeared on one of the three parts infested with *Aphis gossypii.*

On August 14 two healthy plants of Catherine Wilcox in pots were set in one of the cages and infested with *Myzus persicae* from a recently infected plant of this variety. This compartment suffered relatively little damage from hail and no admixture of other species was detected. The two pot plants became well infested but developed no mosaic symptoms. On September 22 they were removed to the greenhouse and fumigated. No mosaic symptoms appeared up to October 29. The one plant which survived storage was mosaic in the following season. Controls of the same individual plant line remained healthy.

Myzus persicae from the mosaic plant of Catherine Wilcox infected in this same field cage was placed on three healthy plants of Catherine Wilcox and two healthy Robert Scott plants in a greenhouse cage on August 14. Aphids became abundant on all the pot plants by August 31. One of the Robert Scott plants became mosaic on August 27. The other four plants

remained healthy until harvest on October 29. Only one survived storage. This Robert Scott plant was mosaic in the greenhouse in June of the following season and was used successfully as a source of infection in a field cage at the New York Botanical Garden in 1932. Two controls of the same individual plant line remained healthy throughout 1931 in the greenhouse, and through August 1932 in the open field.

Striking injury to both healthy Catherine Wilcox and mosaic Le Toreador accompanied the feeding of enormous numbers of *Myzus pseudosolani* in one field cage. Since the effect somewhat resembled mosaic, a test was conducted to determine whether the injury might be due to a virus carried by *M. pseudosolani*. Two healthy plants of Catherine Wilcox in a greenhouse cage were each infested with about 50 aphids of this species on August 22. The characteristic angular yellow spots simulating mosaic (Fig. 16 G) appeared on the younger leaves in five days, and the injury was comparable to that observed in the field cage by September 1. On this date the aphids were destroyed by fumigation. As the plants developed further in the greenhouse, the symptoms persisted on the injured leaves but new leaves which developed subsequent to the aphid infestation were normal. Both plants survived storage and both were healthy throughout the following season in the open field. The injury associated with *M. pseudosolani* is, therefore, evidently a direct feeding effect, and not a virus symptom. Miss Hoggan (17, p. 208) reports similar injury to tobacco and other plants by this species.

Experiments of 1932. Insect transmission tests were conducted at Yonkers and at the New York Botanical Garden along the same lines as in previous years. Both a diseased and a healthy plant were set in each test cage and the insects placed on the mosaic plant. All diseased plants showed typical symptoms except one plant of Jean Chazot with a previous record of both ring-spot and mosaic which appeared to be healthy. Mosaic but not ring-spot was transferred from this plant by *Myzus persicae*. Two other plants showed ring-spot symptoms in addition to mosaic. All healthy plants were cuttings from individual plant lines which proved uniformly healthy in the greenhouse as well as in the controls provided within the experiment. In preparing the compound cages at the New York Botanical Garden for planting, all weeds were carefully removed and the soil and walls sprayed with a strong pyrethrum preparation. The cage was then completed and made tight. Plants were set in all cages later, through the sleeve entrances provided. These precautions reduced the number of natural infestations, but one colony of *M. pseudosolani* appeared. This colony was allowed to multiply throughout the season. The injury characteristic of this species appeared again, but no virus transfer occurred.

Empoasca spp. collected from dahlia were introduced into two cages. They multiplied freely in both cages and produced typical marginal burn.

Both Catherine Wilcox and Robert Scott were somewhat shorter in these cages than when not infested by leafhoppers. *Lygus pratensis* L., collected from dahlia and calendula (*Calendula officinalis* L.), were placed in one cage. The mosaic plant of Le Toreador in this cage was injured but not dwarfed, and mosaic symptoms were clearly expressed. The healthy Catherine Wilcox plant was dwarfed by injury to the terminal shoot but no mosaic symptoms appeared. *Myzus persicae* was taken from two sources.

TABLE III

EXPERIMENTS ON INSECT TRANSMISSION OF DAHLIA MOSAIC IN FIELD CAGES IN 1932

Insects tested	No. healthy plants infested	Date infested	Condition of healthy plants to Sept. 29
Myzus persicae	14	June 1–Aug. 22	7 mosaic, 7 healthy
Empoasca spp.	2	July 26	Healthy
Myzus pseudosolani	1	July 15	Healthy
Lygus pratensis	1	Aug. 1	Injury, but no mosaic
Controls, no insects	25	—	Healthy
Controls, non-viruliferous *Myzus persicae*	6	June 6–July 25	Healthy

A colony from peach (*Prunus persica* (L.) Stokes) was used to infest six cages, and a colony from calendula served to infest 11 cages. Both colonies appear to have been non-viruliferous when collected, and both proved capable of acting as vectors. Considerable difficulty was experienced in establishing this species on dahlias early in the summer, and repeated additions were made to several cages. Only the last or successful introduction is shown in the summary charts in Figures 9 and 10. When once established this species multiplied readily.

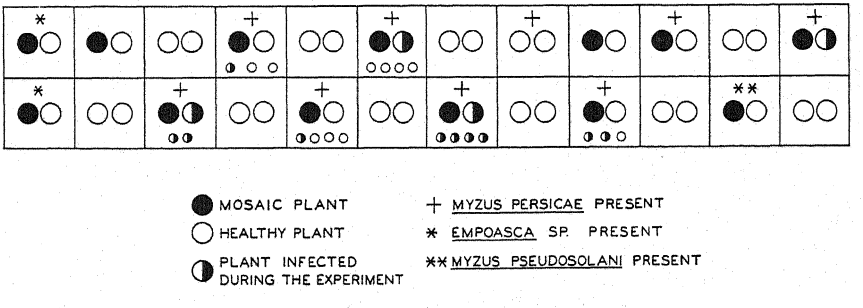


FIGURE 8. Ground plan of field cages at New York Botanical Garden in 1932. Smaller circles indicate pot plants introduced for short periods.

The results of these experiments are summarized in Table III. The virus was transmitted by *Myzus persicae* in seven of 14 cages in which it was colonized on diseased plants. The various types of controls supplied are illustrated in Figure 8, which shows the arrangement of tests in the

compound cage at the New York Botanical Garden. Tests were arranged similarly in the cages at Yonkers. No infection appeared in plants caged without insects, with insects other than *M. persicae*, or with non-viruliferous *M. persicae*.

Supplementary tests were conducted by placing healthy potted plants in field cages in which viruliferous *Myzus persicae* was present. The varieties used were Robert Scott and Catherine Wilcox. All plant lines involved were uniformly healthy in the greenhouse throughout the season, and all were represented in the 68 controls grown in the greenhouse parallel to the test plants. The plants were set in the cages for the periods shown in Table IV. Aphids from mosaic plants in the cages were shaken over the

TABLE IV

TRANSMISSION OF DAHLIA MOSAIC TO POTTED PLANTS PLACED IN FIELD CAGES IN THE PRESENCE OF MYZUS PERSICAE

Date of exposure to viruliferous aphids	No. of plants exposed	No. infected		
		Sept. 26	Oct. 31	Dec. 6
Aug. 15–Sept. 6	9	1	3	—
Aug. 24–Sept. 6	11	3	7	—
Aug. 13–Sept. 12	15	7	12	—
Sept. 12–26	30	0	0	3
Exposed to non-viruliferous *Myzus persicae* Sept. 12–26	10	0	0	0
Greenhouse controls	68	0	0	*

* Of 44 controls retained to November 28, and eight retained to December 6, all remained healthy.

pot plants on the date these were placed in the cages, and also subsequently, if necessary, until infestation of each resulted. At the end of the period of exposure the plants were removed from the cages, dusted with nicotine to avoid mixture of aphids from different cages during transit, then returned to the greenhouse and fumigated. Plants of the first series were grown in the greenhouse until October 31, and those of the second series until November 28, unless mosaic symptoms appeared earlier. As shown in Table IV, 22 of 35 pot plants exposed to feeding of viruliferous *M. persicae* in the earlier series developed mosaic symptoms before October 31. In the later series, however, 30 plants similarly exposed developed no symptoms by October 31, and only three of these showed symptoms by December 6. Two of these had received supplementary light to induce further vegetative growth.

Figures 9 and 10 show the interrelation of the insect transmission tests previously summarized. The experiments with pot plants confirmed the presence of viruliferous insects indicated by the results of the field cage experiments proper in five cages. They failed to confirm positive results from the field cages proper in two cases, but furnished positive results from

six cages in which no previous transfer of the virus was recorded. If the data from the field cage experiments proper and from the pot plant tests are considered together, it appears that transmission took place in 13 out of 14 cages in which *Myzus persicae* was present and supposedly viruliferous. At the same time no infection appeared in 19 cages from which this aphid was absent, and none in three cages in which *M. persicae* was present but non-viruliferous. In the one field cage from which positive results were expected but failed to appear, the aphids introduced apparently failed to feed on the mosaic plant.

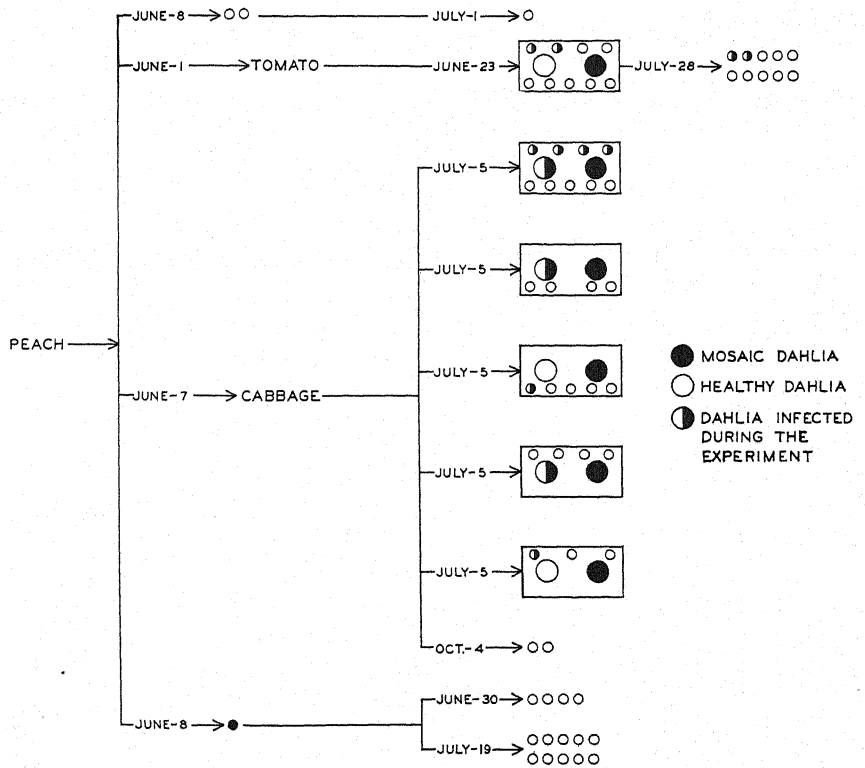


FIGURE 9. Diagrammatic summary of experiments with a colony of *Myzus persicae* from peach in 1932. Rectangles indicate field cages, circles indicate pot plants in globe cages when not shown within a field cage.

The field cage experiments just detailed show that *Myzus persicae* is a vector of the dahlia mosaic virus. Inasmuch as this aphid has been shown to exert a selective action in transmitting one virus only from a mixture of two in tobacco (Hogan, 16) it was thought that experiments with this vector might reveal as complexes certain of the disease types found in

dahlias. The one result detected which appears to be of significance in this connection is the failure of ring-spot symptoms to appear in any of the plants inoculated by the insect. *M. persicae* transferred mosaic only from plants showing both mosaic and ring-spot in three cases. No evidence has been found to suggest that the mosaic symptoms designated in a previous section as Types 1 to 5 inclusive have any significance other than varietal reactions to one mosaic virus. Catherine Wilcox inoculated with the virus

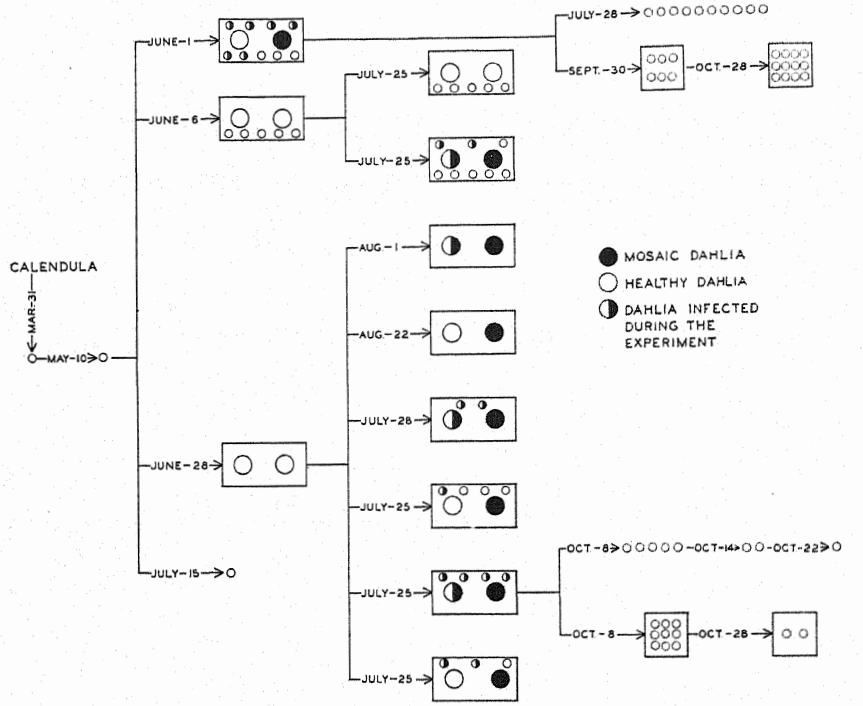


FIGURE 10. Diagrammatic summary of experiments with a colony of *Myzus persicae* from calendula in 1932. Rectangles indicate field cages, squares, greenhouse cages. Smaller circles indicate pot plants in globe cages unless shown within a field or greenhouse cage.

from Doris Wilmore, Jean Chazot, Jersey's Beauty, Le Toreador, Mrs. I. de Ver Warner, and Robert Scott developed the usual symptoms characteristic of this variety. Similarly, Robert Scott infected following aphid transfer from Catherine Wilcox, Faith Slocombe, Jersey's Beacon, Jersey's Beauty, and Le Toreador showed vein necrosis and dwarfing as usual. Since most of the aphid transfers took place late in 1932, these reactions to virus derived from different varieties have been observed through only a part of one season. As far as observed, however, they support without exception the conclusion that only one mosaic virus is involved in the varietal reactions designated as Types 1 to 5 inclusive.

The large proportion of takes recorded in certain cages (Figs. 9 and 10) indicates that *Myzus persicae* is an efficient vector when certain undetermined conditions are supplied. The relatively low proportion of exposed plants which became infected in this group of tests as a whole, namely 32 out of 79, emphasizes the fact that requirements for infection and expression of symptoms are exacting. A large number of apparent failures may be attributed to the poor conditions for expression of symptoms in the late summer and fall. With due allowance for this fact, the total time required for a non-viruliferous colony to acquire the virus and transmit it, and for the infected plant to develop mosaic symptoms, is surprisingly variable. In one cage all of these steps were completed in the 24-day interval between July 15 and August 8. In another cage many aphids placed on the mosaic plant on the same day and from the same source were found on the healthy plant three days later as in the previous case, but the healthy plant developed no symptoms up to the last record on September 29. Attempts to analyze the factors involved in data such as these by culturing *M. persicae* on pot plants in greenhouse cages and under lantern globe cages have largely failed as will appear from the following paragraph.

Myzus persicae taken from peach June 1, and grown on healthy tomato (*Lycopersicon esculentum* Mill.) plants in globe cages in the insectary to June 23, were placed on mosaic Le Toreador in a field cage on June 23 and 28. Forty or more individuals were transferred from the mosaic plant to each of ten healthy dahlias on July 28. The plants were grown in globe cages in the insectary to August 6, then fumigated and grown in the greenhouse to September 26. Two plants of Robert Scott showed mosaic symptoms on August 19. The remaining eight plants were healthy through September 26. Thirty-two control plants were healthy in the greenhouse September 26. Ten healthy cuttings exposed to supposedly viruliferous *M. persicae* under similar conditions on July 19, ten on July 28, and 15 cuttings and 26 seedlings infested on September 30 or later developed no mosaic symptoms. These tests are included in the diagrammatic summaries in Figures 9 and 10.

Field observations suggest that expression of the chlorotic symptoms of dahlia mosaic is dependent on active vegetative growth. In the fall months dahlias make very little new growth and the character of growth is modified by the tendency to blossom. It has been shown by Zimmerman and Hitchcock (41) that this tendency to bloom is a response to short day length, and that vegetative growth may be continued into the winter months when a long day length is provided by supplementing sunlight with artificial light. It seemed probable that many of the failures recorded for both grafts and aphid transfers in August and September might be attributed to lack of expression of symptoms in plants actually infected with mosaic. To test this hypothesis a block of 35 dahlias were provided

with four hours extra light daily from two 1500 watt lamps. Twenty of the plants included in this test had been exposed to supposedly viruliferous *Myzus persicae*, two to non-viruliferous *M. persicae*, and eight were greenhouse-grown controls. The extra light was continued nightly from October 28 to November 28. Two plants which had been exposed in a field cage showed typical mosaic symptoms on December 6. No further infection appeared in the other 18 plants exposed to infection or in the ten control plants.

All of the experiments conducted with the peach strain of *Myzus persicae* are shown in diagrammatic form in Figure 9 and all tests with the calendula strain of this vector in Figure 10. Both strains are shown to have been non-viruliferous when collected, and both have proven effective as vectors in some cases. Accepting all tests in which plants were exposed to feeding of *M. persicae* from mosaic dahlia, we find 13 transfers out of 61 plants exposed to the peach strain, and 21 out of 85 for the calendula strain. There is, therefore, little difference in the effectiveness of the two strains. The low number of takes may be explained partly by the failure of symptoms to develop in the fall. It seems evident, however, that other peculiarities in the behavior of the vector are involved.

Seasonal History of Dahlia Mosaic

As far as known the only source of inoculum of dahlia mosaic in nature is found in mosaic dahlias. The mosaic virus persists over winter in stored roots of affected plants. The only natural agent of inoculation known is the green peach aphid, *Myzus persicae*. The few data available indicate that this vector does not carry dahlia mosaic when it first infests dahlias.

When a mosaic scion is grafted on a healthy dahlia the inoculation is not effective at once. The conditions which must be fulfilled before inoculation becomes effective have not been determined, but they are probably related to the union of stock and scion. If the scion dies or is removed from the stock before these conditions are fulfilled, no infection results. The results of one preliminary test indicate that removal of the mosaic scion after four days or less prevents infection, but removal after six days or more does not.

The interval between inoculation and appearance of symptoms is commonly termed the incubation period, although much of this interval is taken up by the process of development of visible symptoms which may more properly be designated the latent period of infection.

In Table V all the data on the incubation period from 221 grafts and 45 transfers by *Myzus persicae* are summarized. The data from grafting experiments are shown graphically in Figure 11. A well defined mode appears in the fifth-week class, and in 55 per cent of the grafts symptoms appeared within six weeks. One block of 34 grafts made in August show

TABLE V

INCUBATION PERIODS OF DAHLIA MOSAIC. DISTRIBUTION OF INFECTIONS FOLLOWING GRAFTING AND FOLLOWING TRANSFER OF MYZUS PERSICAE ACCORDING TO WEEKS ELAPSED BETWEEN INOCULATION AND APPEARANCE OF SYMPTOMS

Weeks to symptoms	No. of grafts, all data from three seasons. Stock variety						Number of transmissions by *Myzus persicae*, three seasons			
	Attraction	C. Wilcox	R. Scott	Snowdrift	Others	Totals	Aphids moved to healthy plants in greenhouse	Pot plants infested in field cages	Plants grown in field cages in presence of vector	Totals: all three methods of infestation
3	0	2	2	0	0	4	1	0	0	1
4	2	8	5	5	1	21	2	0	2	4
5	6	24	3	13	7	53	0	8	0	8
6	12	14	3	8	7	44	0	1	2	3
7	7	10	1	4	1	23	0	6	3	9
8	1	32(4)*	0	2	2	37(9)*	0	0	0	0
9	1	3	0	2	2	8	0	4	4	8
10	1	12(6)*	2	3	0	18(12)*	0	0	0	0
Over 10	2	5	0	2	4	13	1	7	4	12
Totals	32	110(76)*	16	39	24	221(187)*	4	26	15	45

* Omitting 34 grafts made in August 1932 (see text).

an abnormal distribution with respect to the remainder. The data for 136 grafts made during 1931 show close agreement in distribution with the total data for the three seasons, with the August grafts omitted. It is evident that the data from the August grafts are not homogeneous with the rest. If the data for grafts to individual stock varieties are considered separately, the same type of curve is indicated. The mode appears in the sixth-week class in Attraction, in the fifth-week class in Catherine Wilcox and Snowdrift, in the fourth-week class in Robert Scott, and in the fifth or sixth week in the miscellaneous varieties. The lower mode for Robert Scott reflects the ease with which early symptoms are recognizable, and the higher mode for Attraction reflects the difficulty experienced in diagnosing mosaic in early stages in this variety. If the data from four varieties used as scions in ten or more grafts are ranged in a similar man-

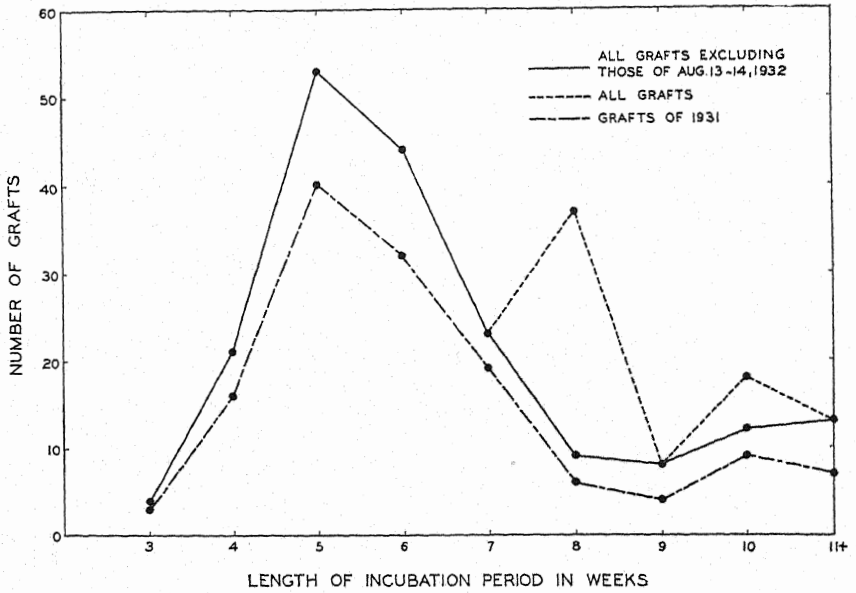


FIGURE 11. Frequency curves for length of incubation period of dahlia mosaic following grafting.

ner, modes are indicated in the fifth-week class for each of these scion varieties, and the scattering of data on either side of the mode is very similar to the distribution in Figure 11. It seems that the shape of the curve is relatively uniform and not influenced by the scion variety or by the variety of the stock plant except in so far as the latter affects the accuracy of diagnosing early stage symptoms.

The data from transmission of *Myzus persicae* fail to show the same regularity of distribution. This is not surprising in view of the small number of observations. Moreover, the data from insect transfers are subject

to a further error in that the date of inoculation is not known accurately in most cases, because of the procedure in exposing plants to aphid feeding. This error is eliminated where transfers of aphids have been made to pot plants in the greenhouse, is relatively small where pot plants have been exposed for a short time to viruliferous aphids in field cages, and is very large in the case of plants grown in field cages throughout the season in the presence of the vector. There is a tendency to group in the fourth- and fifth-week classes in the data less subject to the error mentioned. If the data on incubation period following aphid transfer are grouped according to variety exposed to infection, a mode appears in the fifth-week class for Catherine Wilcox but not for the varieties Robert Scott and Mariposa.

In Figure 11 two characteristics are outstanding, namely, the well marked early maximum and the tendency to tail out to the right. The 34 grafts made in August, which do not fit the curve from the other data, are the only August grafts in the entire series. Unfortunately observations on these grafts were not made at frequent intervals, and they appear bunched in the eighth- and tenth-week classes. If observations had been made regularly, some spread in the incubation period would have occurred and the tailing out to the right in the curve would have been accentuated more smoothly. The fact that August grafts are not homogeneous with grafts made in the three preceding months with respect to the ensuing incubation period recalls the fact brought out earlier that many August grafts and all September grafts have failed to show symptoms in the current growing season. The suggestion that date of inoculation has an influence on the length of the incubation period is at once apparent.

If all the 221 grafts which have yielded positive results are grouped according to date of grafting and weeks to symptoms, a positive correlation is suggested. The correlation is in fact positive and significant ($r = +0.326 \pm 0.041$). However, if the data for the August grafts are omitted, the correlation is not significant. These data, gathered for other purposes, show indications that the date of inoculation influences the length of the incubation period, and that the influence is strongest where late season inoculations are concerned. The low coefficient of correlation indicates that the date of the graft is not the only factor operative, and that the tailing out to the right cannot be accounted for solely on this basis. The interval under consideration is largely occupied by the process of expression of visible symptoms. Field observations suggest that this process is influenced primarily by the rate of vegetative growth, and that the date of inoculation works indirectly through the slower growth rate which obtains in dahlias in late summer.

The minimum incubation periods observed were 14 days for one graft and 13 days for one aphid transfer. Analysis of the available data on the duration of the incubation period, therefore, brings out the following

points: 1. Symptoms may appear in two weeks under very favorable conditions. 2. About half the infections are evident within six weeks. 3. The other half become evident after longer intervals dependent upon various factors, among which is the date of inoculation. 4. The date at which infection takes place cannot be deduced accurately from the date of first appearance of symptoms.

When symptoms begin to appear in plants grown in the greenhouse the order in which they become evident varies with the variety and to a less extent in individual plants within a variety. In Catherine Wilcox, for example, a slight rugosity in the young leaves is usually evident shortly before vein banding becomes recognizable in the same leaves. In some cases, on the contrary (Fig. 1 B), the chlorotic symptom is first evident. In Snowdrift (Fig. 3 C, D) yellowing and rolling of the younger leaves and abnormal orientation of the leaflets on the petiole are commonly first detected. The vein-banding symptom appears later and is commonly poorly expressed. In Robert Scott the downward turning of the leaflets at the point where vein necrosis is to appear, precedes the necrotic symptom in appearance. Shortening of internodes becomes evident after chlorotic symptoms in varieties showing this symptom. In field-grown plants the dwarfing effect may precede the chlorotic symptoms under conditions unfavorable for expression of the latter.

The chlorotic, necrotic, and dwarfing effects are evident only in the organs developed or matured after inoculation (Fig. 7 D, E). Plants of intolerant sorts infected after making considerable normal growth consequently may appear to be semi-tolerant. Secondary infections may sometimes be distinguished from primary infections in this manner. On the other hand, slow development of symptoms in spring in plants carrying primary infection often renders such a distinction difficult. The symptoms expressed by mosaic plants in the second, third, and following years are apparently indistinguishable. In other words, there appears to be no "running out" or progressive severity of symptoms from year to year (Fig. 7 A, B). Mosaic plants of intolerant varieties may be lost in storage but so far as observed are not killed by the mosaic infection.

EPIPHYTOLOGY

Delayed Appearance of Symptoms in Early Stages of Growth

It has been observed repeatedly that dahlias with a mosaic record in the previous season may appear to be healthy during the first month after planting. It has been previously mentioned that this peculiarity led to misinterpretation of the nature of "dwarf," the mosaic reaction of Robert Scott, when this variety was first grown in the greenhouse by the writer. Figure 7 B shows one of several plants of this variety from known mosaic lines of 1930 which showed no mosaic symptoms when grown in the green-

house from June 6 to July 1, 1931. These plants were transplanted to the field on July 1. They made little further growth after setting in the field, were suspected of mosaic on July 9, and called typical mosaic on July 24. The photographs show the appearance of one plant on August 18. Twelve plants of a similar stock of this variety set in cloth cages in the field on June 25 were considered healthy until July 9, and first diagnosed as definitely mosaic July 15.

A similar performance was observed in Robert Scott in 1932. The stock was from a field-grown plant healthy at last record in 1931. The source plant was bedded in peat and several cuttings taken from it in April, 1932, before it was suspected of mosaic. Two cuttings were set in a field cage for confirmation of symptoms on May 19. The first definite mosaic symptom, downward curling of the pinnae, was recognized on June 27. Some vein necrosis was recognized on July 5, and vein banding, uncommon in this variety, on July 11. Other cuttings of this line developed mosaic symptoms in the greenhouse at the same time.

A further instance of delayed appearance of symptoms was found in the variety Doris Wilmore. The plant in question (Fig. 4 A) was a striking "stunt" in 1931, and showed both mosaic and ring-spot symptoms. In 1932 the root clump was divided, and one division set in the greenhouse, the other in a field cage. The division in the greenhouse showed mosaic symptoms when one foot high after about one month from planting. The division set in the field cage on May 20 first showed ring-spot symptoms on one leaflet on June 23, and first showed mosaic symptoms on July 25. The plant had by this time reached a height of five and one-half feet and bore three normal flowers. All subsequent growth showed distortion characteristic of mosaic in this variety, and the general aspect of the cage-grown plant was similar to that of the field-grown plant of the previous year except for the fact that the stunting effect became evident at five and one-half feet in the cage as contrasted with one and one-half feet in the field.

Many further instances of this delay in appearance of symptoms could be cited. The phenomenon is common, but not universal, in plants grown in cages, greenhouse, or open field, and in known mosaic stock as well as in plants infected late in the previous year and showing symptoms for the first time. It occurs in tolerant, semi-tolerant, and intolerant sorts. That the virus is present in the symptomless early shoots is shown by the fact that cuttings removed at this stage develop symptoms later. The delay in appearance of symptoms presents serious difficulties in the practical problems of roguing in the field and of selection of healthy cuttings in the greenhouse.

Masking During Later Stages of Growth

The chlorotic symptoms of dahlia mosaic frequently become masked during the growing season. As explained by Brandenburg (2, p. 46) these

symptoms result from unequal development of the chloroplasts in the green and the paler areas of the leaf, the retarded areas gradually approaching the normal green intensity. It is an extremely common experience to find well developed vein-banding symptoms on some leaves when other leaves of the same plant, and even of the same shoot, appear normal (Fig. 12). In some cases no symptoms occur in a pair of leaves when the pairs immediately above and below show characteristic vein banding. In the field, symptoms are more commonly recognizable in the leaves of shaded lateral shoots than in exposed mature leaves.

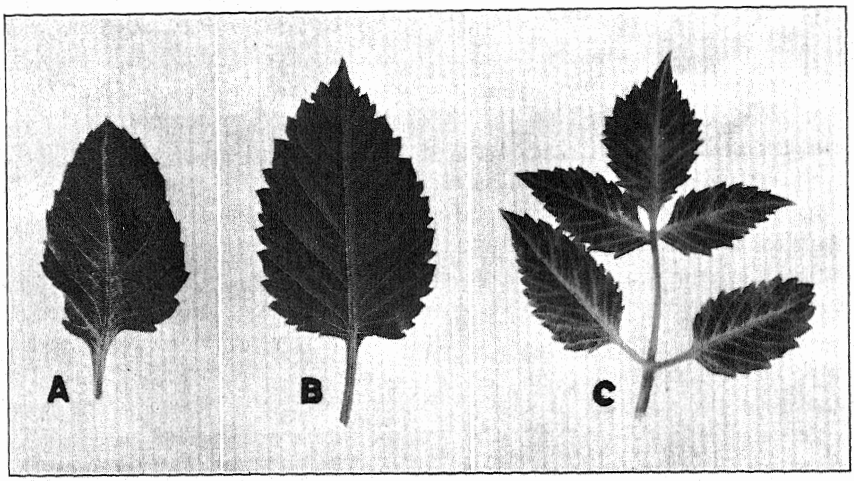


FIGURE 12. Dahlia mosaic symptoms in the variety Treasure Island. A. Symptoms evident in one leaflet of a compound leaf. B. Symptoms absent in leaf (one leaflet shown) two nodes above on same shoot. C. Symptoms evident in younger shoot from base of same plant.

The conditions which determine masking of the chlorotic pattern of dahlia mosaic are not known. These chlorotic symptoms are usually more clearly expressed in cloth cages than in the open field. In the field they are often expressed in one plant when masked in an adjacent mosaic plant of the same variety. Hence masking does not seem likely to be a simple effect of an external factor such as temperature or light. The writer's observations point to a relation to growth.

Prevalence of Myzus persicae on Dahlia

Myzus persicae frequently passes the winter in greenhouses, and is commonly present in the spring when dahlias are propagated by cuttings. This vector also appears about the first week in May in the vicinity of Yonkers, New York, from eggs overwintered on peach. At this season of the year, however, the dahlia is not a preferred food plant. Considerable

difficulty has been experienced in inducing this species to feed on dahlias under experimental conditions in May and June although it develops rapidly on other plants at this time. The colonies which have thus far been established on dahlia with individuals from peach and from greenhouse plants alike have apparently been non-viruliferous. Similarly the colonies which have appeared from unknown sources on dahlias in field cages in July and August appear to have been non-viruliferous

Collections of this species were taken on dahlias in the field at Yonkers on July 16, 1931 and October 4, 1932. It was taken on dahlias at the New York Botanical Garden on September 9, 1930 and from two plots of dahlias at the same institution on October 4 and 21, 1932. The identifications were made by Dr. P. W. Mason. In addition to these positive records the writer has seen what he considers to be this species on dahlias in the field at Yonkers once on July 12, 1932 and on dahlias at both Yonkers and New York frequently in September and October. The vector has thus been found prevalent on other plants but not on dahlias in May and June. It appears sparingly on dahlias in July and in increasing numbers during September and October.

Rate of Spread of Mosaic Under Field Conditions

Estimates of the rate of spread during the growing season are shown for seven plots in Table VI. In collecting these data records were kept on the expression of symptoms in each individual plant on several dates

TABLE VI

RATE OF SPREAD OF DAHLIA MOSAIC UNDER FIELD CONDITIONS

Place and date	Mosaic when planted		Healthy when planted	Infected during season	
	No.	Per cent		No.	Per cent of healthy
N. Y. Botanical Garden 1930.	214	27.9	554	66	11.9
N. Y. Botanical Garden 1931.	419	56.5	322	50	15.5
N.Y. Botanical Garden 1932..	221	29.8	521	96	18.4
Nepera Park, Yonkers 1931..	236	52.8	211	33	15.6
Nepera Park, Yonkers 1932..	370	61.2	235	21	8.9
Institute Yonkers 1932.....	226	57.3	168	25	14.9
Isolation plot, N. Y. Botanical Garden 1932.....	22*	11.2	197	5**	2.5

* Replaced as soon as recognized.
** Diagnosis not certain in three of these.

through the season. Because of delayed appearance of symptoms in mosaic plants in the first stages of growth, early season infections cannot be distinguished accurately from infections of the previous year. For this reason all plants showing definite mosaic symptoms before August 1 were considered as mosaic when planted, and all plants with no mosaic record

previous to August 1 but showing definite symptoms later were considered current season infections.

The accuracy of estimates attained in this manner is dependent largely on the observer's familiarity with symptoms in the varieties involved, and on the number of observations made during the season. The estimates of rate of spread at the New York Botanical Garden are least accurate because the number of varieties grown was large and relatively few observations were made. These estimates, however, are in good agreement with those for the Yonkers plots. The plants in the Nepera Park plot were observed weekly through the season in 1931 and the two Yonkers plots were examined monthly in 1932. Estimates for the Yonkers plots were checked against data for the individual plant lines from field, greenhouse, and cage experiments of the previous and current seasons, and are, therefore, relatively accurate.

The estimates presented in Table VI and thus far discussed are based on infections which became evident between August 1 and the end of the growing season. It has been pointed out that many infections which take place late in the season do not become evident until the following year. If the rate of spread is to be computed on a yearly basis, all these estimates are too low. The writer has data on spread for a full year for only one plot. Of 178 plants apparently healthy at the close of the season in the Nepera Park plot of 1931, one hundred fifteen were grown again in 1932. Ten of these, or 8.7 per cent, showed mosaic before August 1. Adding this percentage to the 15.6 per cent recorded for the previous year, we find 24.3 per cent spread during a twelve-month period. The rate of spread of mosaic in the vicinity of Yonkers and New York City may, therefore, be estimated at 10 to 25 per cent. Tilford (36, p. 65) presents similar data on "dahlia stunt disease" in Ohio. Of 240 plants called healthy in 1928, 34.2 per cent were stunted in 1929. Tilford states that the plants were studied throughout the season.

The experiment designated as "Isolation plot 1932" in Table VI was intended to contain healthy plants only and was situated at a distance of some 300 yards from other dahlias. The original purpose was in large part defeated by the appearance of mosaic in 22 plants, representing lines healthy at last record in the field in 1931. These diseased cuttings were replaced by healthy plants as soon as symptoms were recognized, four on June 9, six on June 29, ten on July 7, and two on July 16. The performance of cuttings of the same lines in the greenhouse showed that these plants became infected late in 1931. Twelve diseased plants present in the plot in early July, however, furnished a source of inoculum within the plot to which the two definite and three possible cases of current season infection may be attributed. Symptoms were first detected in one Robert Scott plant August 15 and in one Snowdrift on September 12. Three other Robert

Scott plants which showed dwarfing on October 20 but none of the vein necrosis characteristic of mosaic in this variety, make up the total of five cases of current season infection shown in the table. The assumption, that healthy dahlias isolated from the nearest mosaic dahlias by some 300 yards would not become infected, seems to be supported by the outcome of the test.

PLANTS AFFECTED

All members of the genus *Dahlia* which have been adequately studied have proven susceptible to dahlia mosaic. This disease has been found in nature in members of the Single, Mignon, Collarette, Duplex, Peony, Incurved Cactus, Recurved and Straight Cactus, Semi-cactus, Formal Decorative, Informal Decorative, Ball, Miniature, Pompon, and Star or Orchid Flowering classes of dahlias. No records have been made on members of the Anemone class. *Dahlia maxonii* Safford and *D. imperialis* Roezl. have been infected by grafting, showing typical vein-banding symptoms. These species have not been seen affected in nature.

No plant outside the genus *Dahlia* has been experimentally infected with dahlia mosaic. Individuals of nine species representing eight genera of the tribe Heliantheae were grafted to mosaic dahlias without effect. None of the plants of genera other than *Dahlia* formed good unions with dahlia plants. These tests by the method of approach grafting are for this reason inconclusive. Individuals of nine species representing eight genera of the tribe Heliantheae were exposed to feeding of *Myzus persicae* from mosaic dahlias without effect. One plant of *Helianthus debilis* Nutt. (*H. cucumerifolius*) developed mosaic symptoms (Fig. 16 F) but subsequent tests indicate that this was not dahlia mosaic.

Of the plants tested only the dahlia and *Ambrosia trifida* L. have been seen naturally affected with mosaic or mosaic-like symptoms by the writer. An irregular chlorosis and puckering of the leaves of *A. trifida* is common on this weed about Yonkers, New York. The symptoms might be interpreted as the effects of aphid injury as they are commonly accompanied by aphids and resemble the effects of *Myzus pseudosolani* on dahlia (Fig. 16 G). However, sharply defined chlorotic rings which occasionally appear with or without the irregular chlorosis and puckering of leaves suggest that a virus disease occurs in *A. trifida* in this vicinity. No evidence has been obtained that this mosaic is transmissible to dahlia.

VARIETAL SUSCEPTIBILITY

No variety of dahlia is known to be immune to mosaic. It is reasonable to assume that some varieties are less subject to infection than others under field conditions because of differences in preference of the insect vector or vectors for different varieties as food plants, but this has not been shown to be true. Striking differences do appear in the tolerance of varieties to

mosaic infection. For example, when infected with mosaic the variety Robert Scott is so badly dwarfed and distorted as to be worthless, but Mrs. I. de Ver Warner suffers such slight injury that it is not recognized as diseased by growers. We term Robert Scott intolerant and Mrs. I. de Ver Warner tolerant. There is no sharp dividing line between tolerant and intolerant varieties when the thousands of sorts in use are considered. They fall into a continuous series with extremes such as the two varieties cited, and all intervening gradations are represented.

During the course of this study dahlia mosaic has been observed in 461 varieties. The records have been made largely at the New York Botanical Garden where approximately 1000 plants representing 200 to 300 varieties are grown each year. The number of a given sort is necessarily small and changes in the varieties represented are made from year to year. These records have been supplemented by the data collected from the writer's experimental material, and by a small number of observations from commercial fields.

These data do not justify the final classification of the varieties observed with respect to tolerance to mosaic. Of the 461 sorts recorded as mosaic only 178 can be classed even provisionally. In such a provisional classification 23 varieties may be called tolerant, 63 semi-tolerant, and 103 intolerant. The tolerant group includes Jersey's Beauty, Le Toreador, Mrs. I. de Ver Warner (Fig. 5), Trentonian, and others which show vein banding but no marked dwarfing or distortion when mosaic. The varieties Anna Marie, Asbury Park, Bonnie, Buckeye Bride, Caroline Meussdorfer, Congressman Wolverton, Eckford Century, Faith Slocombe, Jane Cowl, Jersey's Triumph, Martha Eppele, Mrs. Charles R. Sherman, Papillon, Queen of the Garden Beautiful, Roxy, Sagamore, Snowclad, and the Queensbury are regarded as tolerant on the basis of relatively few observations. The semi-tolerant group includes such varieties as Calizona (Fig. 4 B), Chautauqua Salute, and Jersey's Beacon, which, when mosaic, show some shortening of internodes and more or less distortion of leaves as well as vein banding. The intolerant group includes such varieties as Caspar G. Ware, Doris Wilmore (Fig. 4 A), Esther R. Holmes, Miss Bridgeport (Fig. 3 A, B), Mrs. Margaret W. Wilson (Fig. 2 C), Robert Scott (Fig. 7), Ruth Hottel, Snowdrift (Fig. 6 A), and White Empress. In this group the mosaic symptoms are severe enough to place the affected plant in the "stunt" class without question. Such plants are not commonly saved for propagation.

CONTROL

The soundest method of control of dahlia mosaic consists in growing selected healthy stock at a reasonable distance from any dahlias which may be mosaic. Several factors tend to make this solution relatively easy,

but several others tend to make it difficult of accomplishment. As favorable factors we may cite the apparent absence of seed transmission, the apparent absence of any reservoirs of inoculum in plants other than dahlias, the fact that the only known vector is not a strong flier as compared to leafhoppers, and the ease with which dahlias may be propagated. As unfavorable factors we may mention the difficulty with which the disease is diagnosed, the tendency for symptoms to become masked or obscured by insect injuries, the fact that a considerable number of infections take place in late season and do not become evident until the following year, the delay in expression of symptoms in early season, and the fact that dahlias are commonly propagated vegetatively rather than from seed. In general the problem of control of dahlia mosaic appears to be less formidable than the problems confronted in the control of similar diseases of other plants by this method. The writer's experience in building up a stock of over 1000 healthy dahlias for use in the 1932 experiments almost entirely by propagation from 25 plants healthy when purchased in the fall of 1930 indicates that this solution is feasible.

Roguing of affected plants from plantings largely healthy is a sound practice in general. In the control of dahlia mosaic, however, this practice should be subordinated to selection. When roguing is the first step taken in control, the large number of plants which cannot be diagnosed as definitely mosaic or definitely healthy are left in place. Roguing as a method accessory to selection, however, shows considerable promise as indicated in Table VI. It cannot be too strongly emphasized that removal of "stunts" alone will not suffice to eliminate mosaic. As far as the writer is aware this roguing of stunt is as far as any commercial grower goes at present, the object being to remove plants unfit for propagation rather than to eliminate sources of inoculum. There is some indication that mosaic plants of tolerant varieties are more dangerous sources of inoculum than stunted plants since the latter are less attractive to *Myzus persicae* under experimental conditions.

Control of the insect vector by greenhouse fumigation during the period when cuttings or seedlings are grown under glass is feasible and important. The few colonies of *Myzus persicae* tested at this season of the year have proven non-viruliferous. Spraying or dusting dahlias in the field for the purpose of controlling the aphid vector does not appear to be promising.

The question remains as to whether tolerant varieties offer a satisfactory solution of the problem of mosaic control. Of 178 varieties provisionally classed in the section on varietal susceptibility, only 23 were regarded as likely to be tolerant enough to give satisfactory performance when mosaic. In general a variety has been considered tolerant if (a) mosaic symptoms have been sufficiently well developed to justify positive

diagnosis, but (b) no serious deformation of the plant is apparent, and (c) confusion of mild current season symptoms with tolerance can be excluded. The degree of injury permitted in a sort classed as mosaic-tolerant must be determined arbitrarily. Plants of the most tolerant varieties such as Mrs. I. de Ver Warner appear to be somewhat shorter when mosaic.

The present tendency to retain tolerant varieties in company with more recent introductions is deplorable. Sound practice requires segregation. It is justifiable for a cut-flower grower with large stocks of tolerant varieties to continue to grow these when affected, inasmuch as the flowers are commercially acceptable and the performance remains relatively satisfactory. The objections to tolerance as a final solution of the problem lie in the restricted choice of varieties, and in the fact that even these are not as fine when mosaic. Moreover, some varieties tolerant of mosaic are susceptible to hopperburn and thrips injury, and some, such as Robert Scott, one of the most intolerant of mosaic, suffer comparatively little damage from leafhoppers and thrips. If all the problems of "stunt" are to be met by simple selection of resistant and tolerant sorts, therefore, the choice will be still further restricted. This is at best not a courageous line of attack, and in view of the fact that isolation and roguing seem practicable, it is hardly logical. Tolerance, therefore, is suggested merely as a temporary expedient.

DAHLIA RING-SPOT

A ring-spot disease of dahlia was first described by Conners (4, 5) who reported that about one in 1000 of the dahlias at the New Jersey Experiment Station were affected. No other reference to the disease has been found. The writer has observed ring-spot in New York, New Jersey, and Connecticut in 44 varieties in all. It has appeared in approximately two per cent of the plants grown each year at the New York Botanical Garden in 1930, 1931, and 1932. In the writer's stocks at Yonkers ring-spot has appeared only in plants brought from outside sources as mosaic or ring-spot. At the New York Botanical Garden there has been no compelling evidence of spread although ring-spot plants have shown a tendency to appear in groups in one or two instances. In commercial plantings, as far as observed by the writer, ring-spot is more localized in distribution than dahlia mosaic. Ring-spot was found in 9 to 44 per cent of the plants of three varieties, and present in a number of other varieties and in unnamed seedlings at one commercial planting on Long Island in 1931. In 1932 ring-spot was again common in this field. The relatively high percentages found in this locality and the occurrence in seedlings suggest that ring-spot spreads there. In other commercial fields on Long Island and in Connecticut and New Jersey, ring-spot occurs but indications of spread are less marked.

The symptoms of ring-spot appear as irregular concentric rings, irregular zigzag markings, intricate hieroglyphic patterns, and green islands (Fig.

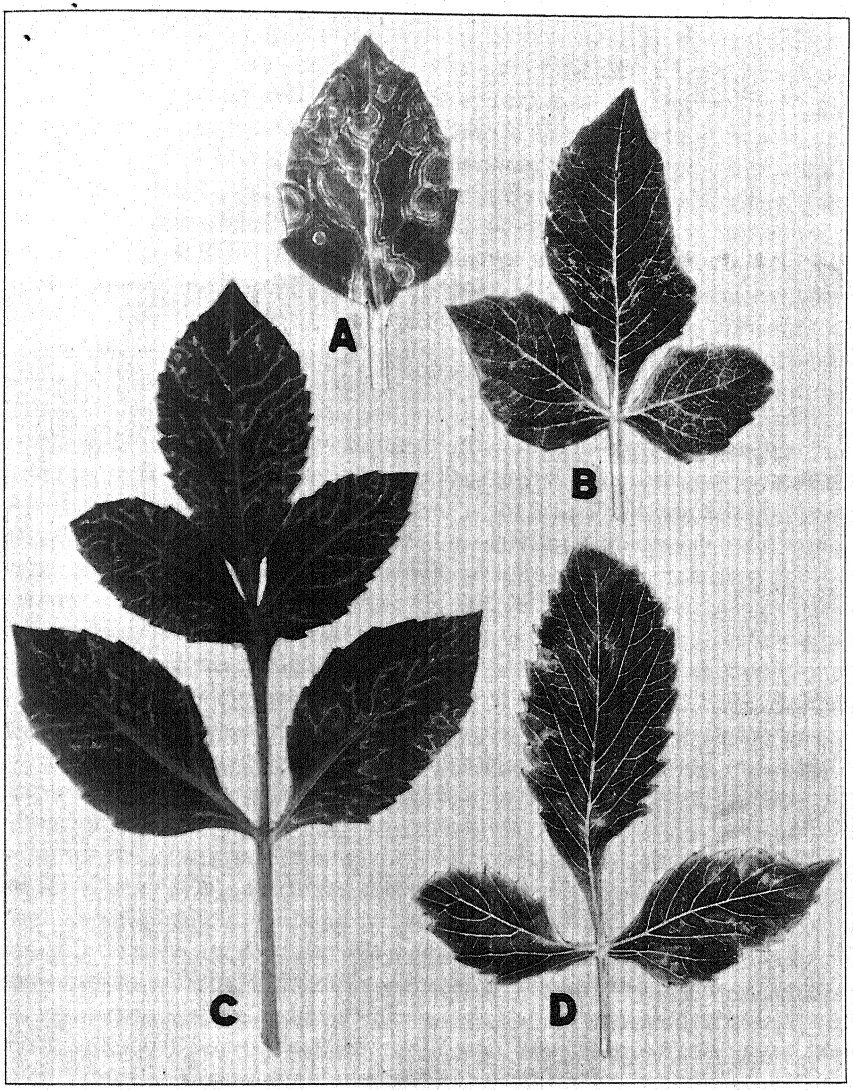


FIGURE 13. Symptoms of dahlia ring-spot (in Catherine Wilcox appearing after grafting; in others as natural infections). A. Chlorotic rings in the variety Chautauqua Salute. B. Chlorotic hieroglyphic pattern in the variety Watchung Wonder. C. Necrotic pattern in the variety Catherine Wilcox. D. Green Island enclosed in a chlorotic ring in the variety Mrs. Shirley Shaw.

13). The width and number of zones expressed vary within a given variety. The color of the chlorotic areas varies from pale green to yellowish-green in different varieties but is relatively uniform within a variety. In several plants observed in the field ring-spot symptoms have been first expressed as a chlorotic pattern, later becoming necrotic. This fact, together with the fact that both chlorotic and necrotic patterns have appeared in Catherine Wilcox following grafts to ring-spot stocks, forms a basis for the assumption that the chlorotic and necrotic patterns are symptoms of one virus disease. In some varieties only chlorotic symptoms have been recognized. As far as observed ring-spot plants are not stunted or distorted, but may be rendered unsightly if necrotic symptoms appear in a large number of the leaves.

RELATION OF RING-SPOT TO DAHLIA MOSAIC

The study of dahlia ring-spot has been hampered by the occurrence of this disease in combination with mosaic in most of the plants available. The writer assumed until recently that ring-spot was merely another symptom of dahlia mosaic. The evidence is still insufficient to show that the two diseases are due to distinct viruses but seems to point to that conclusion.

The persistence of ring-spot in affected plant lines as determined by expression of symptoms is erratic. No ring-spot appeared in subsequent years in two lines of Jersey's Beauty and one line of Fordhook Marvel diagnosed as ring-spot in 1930, nor was ring-spot detected in these lines by grafting tests. A third line of Jersey's Beauty selected as ring-spot in 1930 showed mosaic only in the field in 1931, and mosaic only in the greenhouse in 1932. A root division grown in a field cage in 1932, however, developed typical ring-spot symptoms as well as mosaic, but only mosaic was transferred from this by *Myzus persicae*. Both ring-spot and mosaic passed from a scion of this line to a healthy Catherine Wilcox stock in 1931. When the stock thus infected with both diseases was grown in the field in 1932, only mosaic symptoms appeared.

Of three plants of Jean Chazot which showed both mosaic and ring-spot in 1931, one failed to show either in 1932, possibly because of red spider injury. A second showed both diseases in the greenhouse, but neither in a field cage. Both mosaic and ring-spot were transferred from the greenhouse-grown plant by grafting, and mosaic only from the symptomless plant in the field cage by means of *Myzus persicae*. The third plant of this variety developed both diseases in both greenhouse and field and both diseases passed to a healthy plant grafted to the one grown in the greenhouse.

One plant of the variety Roxy developed ring-spot symptoms in the greenhouse in 1931 and a plant of The Choctaw showed ring-spot in the field this year but no further ring-spot symptoms appeared in either in the

following year, and no evidence of ring-spot was detected in either by grafting. Eight ring-spot cuttings of the variety Galli Curci at the New York Botanical Garden in 1932, probably derived from one of two plants of this variety which showed this disease in the previous year, showed no symptoms of mosaic in combination. Four slips of one of these ring-spot plants were grafted to three healthy Catherine Wilcox plants and one healthy Galli Curci in September, but no symptoms developed in the healthy stocks. This failure to express symptoms in late fall is probably due to slow growth of the dahlia as explained in the discussion of mosaic.

One plant of Doris Wilmore which showed mosaic symptoms in 1930 showed ring-spot also in some of the lower leaves in 1931. One root division grown in the greenhouse in 1932 showed mosaic only, and only mosaic was transferred from it by grafting. Another root division in a field cage showed ring-spot first, and after some delay, mosaic also, but only mosaic was transmitted to a healthy plant by *Myzus persicae*.

Persistence of ring-spot in vegetative parts has thus been shown in only six cases out of 16 expected. When scions of known ring-spot history were grafted on healthy stocks, ring-spot appeared alone in two cases, ring-spot and mosaic in three cases, mosaic only in 14 cases, and neither disease developed in ten cases. When scions of known ring-spot history were grafted on two stocks already showing mosaic symptoms no change in the symptoms expressed was detected. Scions of certain lines of the varieties Mrs. Anna Roche and Robert Scott, which showed mosaic symptoms only, produced mosaic only in healthy stocks in ten grafts, both mosaic and ring-spot in seven grafts, ring-spot only in two grafts, and no effect in one. Scions of these lines grafted to stocks already showing mosaic, produced no change in two cases, but in one case ring-spot symptoms in addition to mosaic appeared following such a graft.

Thirteen of the transfers of ring-spot by grafting were made to the stock variety Catherine Wilcox and one to the variety Attraction. Ring-spot was superimposed on mosaic in one plant of Jersey's Olympus. None of these varieties have been seen naturally affected with ring-spot. No transfer to Snowdrift was accomplished in six attempts and none to Robert Scott in two grafts. Neither of these varieties has been seen affected, but ring-spot has occurred in masked form in the latter. Varietal reaction may account for some of the peculiarities in the observed behavior of this disease. Ring-spot clearly shows a tendency to mask, but the conditions under which it masks are not evident. Symptoms have been expressed in plants grown in the field, in the greenhouse, and in cages in some cases, but have not appeared consistently under any of these conditions. The first symptoms expressed following grafting in early summer have usually been necrotic and those following grafts in late summer usually chlorotic. Seven

plants infected by grafting and grown again in the following year showed mosaic only in the second season.

The observations which point to the conclusion that ring-spot is distinct from mosaic are as follows: 1. The two diseases differ in prevalence. Mosaic has been found generally present in high percentages throughout the region surveyed. Ring-spot, although found throughout the same region, has been seen in low percentages with the exception of one or two localities. 2. Mosaic has been found to spread to 10 to 25 per cent of the healthy plants in field plots in the vicinity of Yonkers, but no convincing evidence of spread of ring-spot in this vicnity has been found. 3. The symptoms of ring-spot are distinct from mosaic in the varieties Catherine Wilcox and Galli Curci, although they appear to intergrade in Jean Chazot. 4 Following grafting, ring-spot symptoms tend to show before mosaic symptoms when the two diseases are in combination in the diseased scion, and the incubation periods recorded for ring-spot indicate a mode in the third- or fourth-week class as compared with the fifth-week class for mosaic. The difference here may be due to the relatively large number of instances in which the first ring-spot symptoms were necrotic. 5. No transmission of ring-spot by *Myzus persicae* has been detected, although this aphid transmitted mosaic to one or more healthy plants from each of three plants affected with both diseases.

YELLOW RING-SPOT

This disease was brought to the writer's attention in July, 1931, by M. B. Linford who showed photographs of the disease as it appeared in Utah and expressed the opinion that it differed from the ring-spot disease just described. On returning to Utah, Linford marked plants showing the disease and sent the writer roots of such plants in the fall. The symptoms developed in the variety Long Island in the greenhouse in 1932 are shown in Figure 14 A. This disease has not been previously described, as far as the writer is aware.

The symptoms in the variety Long Island are bright yellow concentric rings and zigzag patterns as shown in Figure 14 A. Symptoms appeared on the first pair of leaves developed and were expressed and persisted in each leaf developed through the season. Healthy plants of Catherine Wilcox and Robert Scott became infected following grafting to this plant, and a second Robert Scott was later infected from the Catherine Wilcox plant. Symptoms appeared in 24, 17, and 32 days, in these three grafts. The symptoms developed in one of the lower leaves near the graft union in the Catherine Wilcox plant are shown in Figure 14 B. Symptoms appeared throughout this plant during the summer but in much less brilliant form, often as a few rings or segments of rings near the tips of the leaflets. The conspicuous symptoms illustrated are, therefore, probably representative

of the initial reaction to the virus, and the less conspicuous symptoms may be more typical of the variety Catherine Wilcox. In both Robert Scott plants which became infected the symptoms appeared as yellow rings and segments of rings. These patterns appeared throughout both plants during the summer, and were often confined to the tips of leaflets as in Catherine Wilcox and never as strongly expressed as in the variety Long Island. The brilliant pattern in Long Island, therefore, is probably a varietal characteristic not to be expected in dahlias in general.

Two further attempts to transmit yellow ring-spot by grafting in September, 1932, failed. The failure is in agreement with experience with dahlia mosaic and ring-spot grafts in late summer. Attempts to transmit yellow ring-spot to ten plants of Catherine Wilcox by mechanical methods,

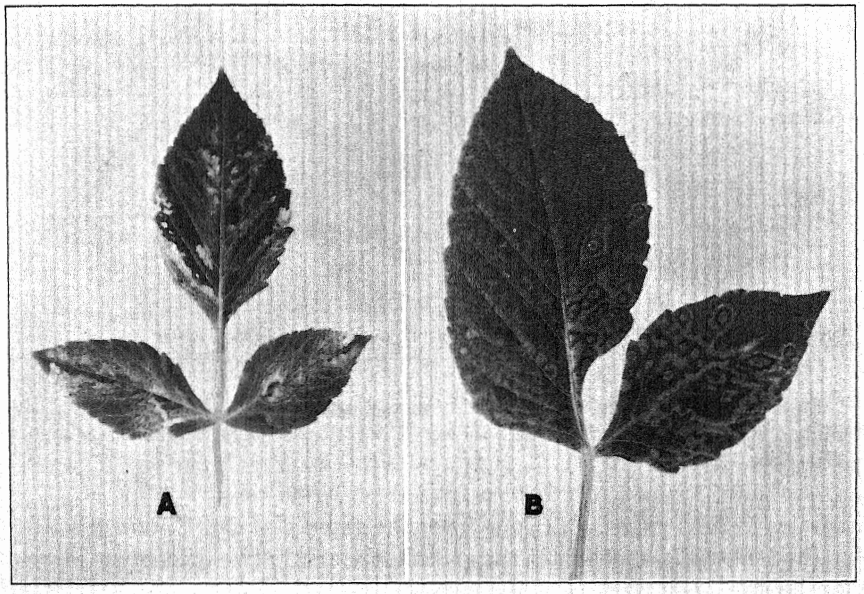


FIGURE 14. Symptoms of yellow ring-spot. A. Natural infection in the variety Long Island. B. Symptoms which appeared in the variety Catherine Wilcox grafted to Long Island.

five by the cheesecloth method, and five by the Sein method, failed. No spread of this disease was detected although yellow ring-spot plants were grown in the greenhouse with other dahlias from April to November. Affected plants were not grown out-of-doors because the disease seemed new to the region. So far as could be judged from greenhouse performance, no necrosis, distortion, or dwarfing appears in the varieties mentioned.

Yellow ring-spot is distinct from mosaic in the varieties Catherine Wilcox and Robert Scott, and from ring-spot in the former. It is distinguished

from ring-spot in general by prominent yellow patterns as contrasted with greenish and yellowish-green patterns. (Compare Figs. 13 and 14). As far as known yellow ring-spot differs from the other diseases in its distribution also.

OAKLEAF

The name oakleaf is applied to a disease first seen in the variety Calvin Coolidge, Jr. in the greenhouse in 1932 (Fig. 15 A). It has since been recognized in Catherine Wilcox in the greenhouse (Fig. 15 C) and in both these

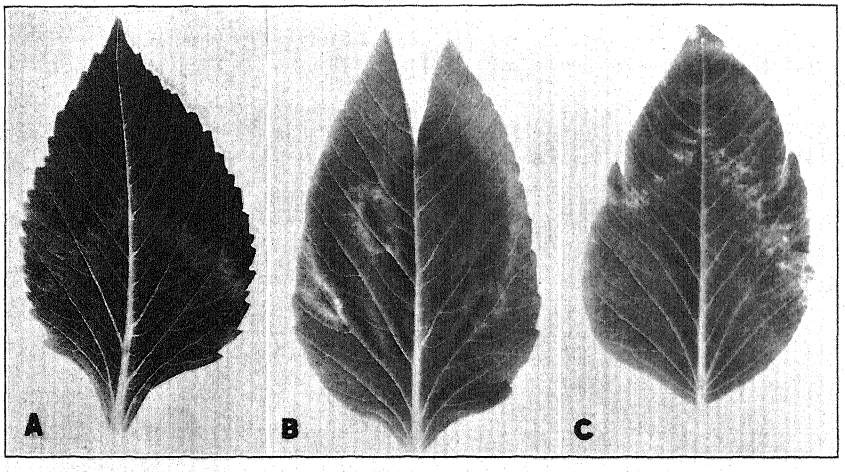


FIGURE 15. Symptoms of oakleaf. All natural infections. A. In the variety Calvin Coolidge, Jr. B. Chlorotic rings which appeared in the same plant with C. C. Symptoms in Catherine Wilcox.

varieties in field cages. Cuttings from the same plant of Calvin Coolidge, Jr., that showed symptoms in the greenhouse and in two field cages, showed none in the open field, and the two plants in field cages masked in late summer. What was probably the same disease appeared in one plant of Snowdrift in the greenhouse.

The symptoms of which the name oakleaf is descriptive are often well marked in Calvin Coolidge, Jr., and are of the same character but less distinct in Catherine Wilcox (Fig. 15 C). The rather poorly defined large chlorotic rings shown in Figure 15 B appeared on the same plant in the pair of leaves immediately below the pair showing the more typical pattern, and are, therefore, believed to be expressions of the same disease. Oakleaf is not known to involve dwarfing, distortion, or necrosis in the varieties mentioned. It has not been recognized in other varieties, nor in any variety in the open field.

Since the disease was confused with ring-spot and mosaic when first recognized, cross inoculations between Catherine Wilcox and Calvin Coolidge, Jr. were made by means of grafts to compare reactions. One of three healthy Catherine Wilcox plants grafted to oakleaf Calvin Coolidge, Jr. developed oakleaf in 52 days, but the symptoms soon masked in this plant and were never detected in the two parallel grafts. Calvin Coolidge, Jr. plants already showing oakleaf were grafted to mosaic Catherine Wilcox and mosaic Le Toreador. Two of three grafted plants developed typical mosaic symptoms. The occasional appearance of oakleaf symptoms in uninoculated Catherine Wilcox plants in the same greenhouse vitiates the evidence from the single graft in which oakleaf was apparently transmitted. Inasmuch as this disease was first recognized in 1932, no opportunity has been offered to test its persistence in affected plants. Oakleaf is tentatively described as a virus disease, therefore, solely on the basis of the symptoms expressed.

RELATION OF DAHLIA VIRUS DISEASES TO OTHER VIRUS DISEASES

In the early stages of this study it was thought possible that some of the chlorotic symptoms expressed in dahlias might be due to one or more of the common and readily inoculable viruses to which tobacoo (*Nicotiana tabacum* L.) is susceptible. Attempts to transfer a virus to Turkish tobacco by the cheesecloth method from dahlias showing mosaic, ring-spot, yellow ring-spot, and "stipple chlorosis" were without effect. An apparent transfer of oakleaf to tobacco in one test was not confirmed in a subsequent test. Dahlia ring-spot material of the type shown in Figure 13 B was tried on tobacco, and the tobacco ring-spot virus, studied by Wingard (40), Price (29), and others, was tried on healthy dahlias by W. C. Price in 1931, but also without effect. The symptoms developed in Turkish tobacco following the inoculation with oakleaf did not appear to be those of the tobacco ring-spot virus on this plant.

There is no evident relation of any of the diseases described in this paper to the tomato spotted wilt disease described on dahlia by K. M. Smith (33, 34). Smith finds the spotted wilt virus is transmitted by *Thrips tabaci* Lind. and is also readily inoculated into tobacco by mechanical methods. Our experiments indicate that dahlia mosaic, ring-spot, and yellow ring-spot are not transmissible to tobacco by mechanical methods and in our experience these diseases have failed to spread in the presence of thrips. Oakleaf appears to differ from spotted wilt in the symptoms expressed on dahlia.

OTHER SYMPTOMS IN DAHLIA WHICH MAY BE CONFUSED WITH MOSAIC

A chlorotic pattern which has been termed "stipple chlorosis" (Fig. 16 B, C) was first regarded as a symptom type of dahlia mosaic. This

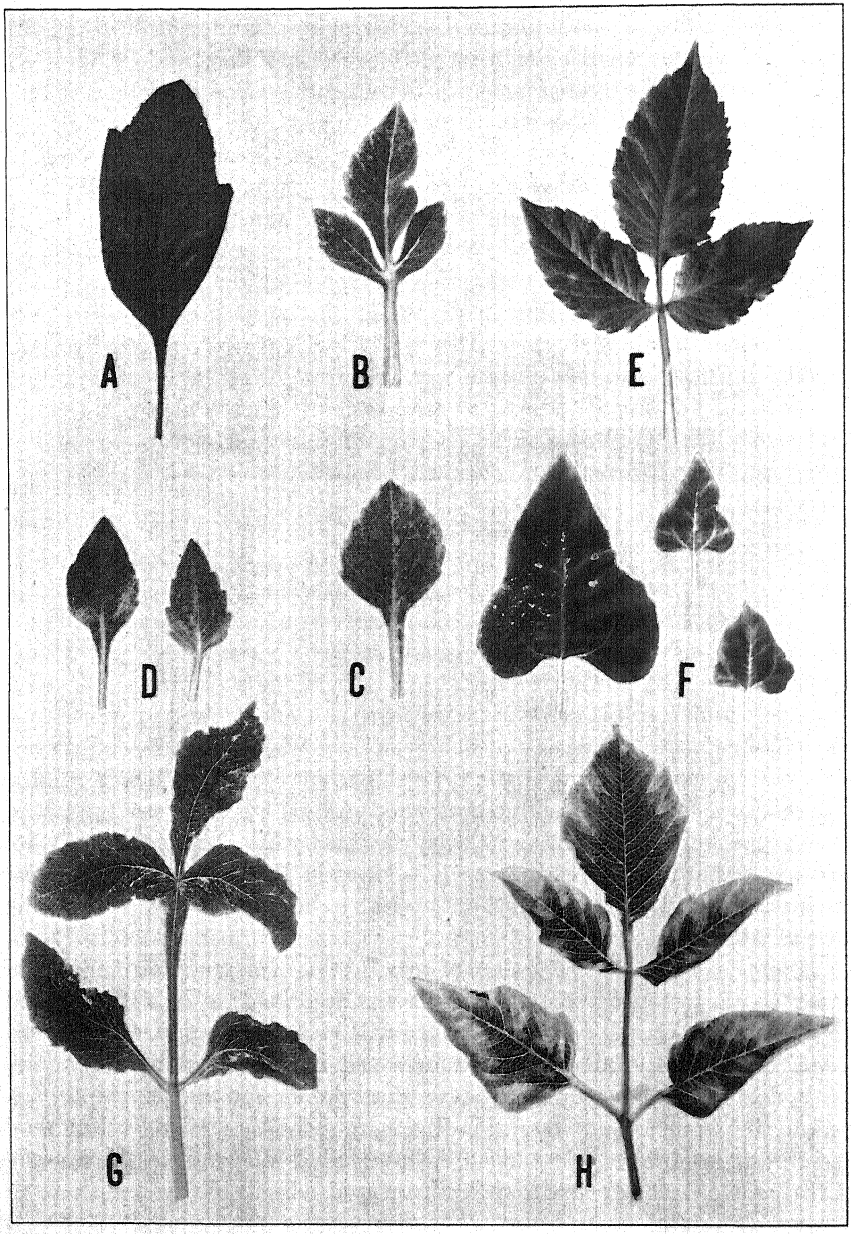


FIGURE 16. Symptoms which may be confused with dahlia mosaic. A. Necrotic streaks in leaf of Attraction. B, C. Stipple chlorosis in leaves of Snowdrift. D. Stipple chlorosis in leaves of a seedling dahlia. E. Yellow spots in leaf of Catherine Wilcox. F. A mosaic disease of *Helianthus debilis*. G. Injury to Catherine Wilcox by *Myzus pseudosolani*. H. Typical leaf symptoms of hopper-burn.

chlorotic stippling has appeared frequently in the varieties Snowdrift, Robert Scott, Papillon, and others in early stages of growth, and is sometimes seen in dahlias in the field. A similar spotting appeared in dahlias grown from seed in the greenhouse (Fig. 16 D). Larger scattered chlorotic spots of the type shown in Figure 16 E have appeared in the variety Catherine Wilcox in the greenhouse. All attempts to transmit a virus from symptom types such as these have failed, and the affected plants have developed normally in all other respects.

Streak-like symptoms shown in the variety Attraction in Figure 16 A have been observed four times, once each in this variety and in Robert Scott, and twice in Catherine Wilcox. The Robert Scott and Attraction plants showed typical mosaic symptoms also; the Catherine Wilcox plants had been exposed to mosaic infection in the fall but showed no other symptoms. This may be a mosaic symptom which is rarely expressed. The symptoms illustrated in Attraction appeared on September 25, 1931, and the remaining three cases appeared October 11, 1932.

The typical leaf symptoms of hopperburn are shown in Figure 16 H for comparison with the symptoms of the other diseases discussed.

SUMMARY

Dahlia mosaic has been found common throughout the region surveyed, namely Connecticut, New York, and New Jersey, and is probably widely distributed. All members of the genus *Dahlia* tested have proved susceptible but no suscepts have been found outside this genus.

Mosaic is not synonymous with stunt. The term "stunt" as applied in America includes mosaic in the less tolerant varieties of dahlias and also certain insect injuries. The more tolerant varieties which are not stunted by mosaic have evidently been overlooked in previous treatments of stunt by American authors.

The symptoms of mosaic are chlorotic bands correlated with the veins, leaf distortion, shortening of internodes and flower stems, and vein necrosis. Great variation appears in the reactions of different varieties, the more tolerant varieties showing only chlorotic symptoms.

Mosaic persists in vegetative parts of affected plants but has not been found to pass through seed. The virus has not been transmitted by mechanical methods.

Grafting experiments have established the fact that mosaic is a transmissible disease, and the further fact that the diverse symptoms shown by tolerant and intolerant varieties are merely varietal reactions to one mosaic.

Myzus persicae is shown to be a vector of dahlia mosaic. No clear evidence has been found to indicate that other insects tested can act as vectors of this disease.

The interval between inoculation and appearance of symptoms is commonly four to six weeks, but in some cases much longer. Late season infections in particular tend to show symptoms after a longer interval, often not until the following season.

The expression of symptoms in mosaic plants is often delayed in early season growth. The chlorotic symptoms of mosaic are frequently masked during the growing season. It is suggested that masking is determined by growth relations rather than by any single environmental factor.

Dahlia is not a preferred food plant of *Myzus persicae* in early summer. Limited evidence suggests that some infections take place in July, and that more occur in September and October.

The rate of spread of mosaic in the field has been found to be of the order of 10 to 25 per cent per year at Yonkers and New York.

Control of mosaic by selection and isolation of disease-free plants, supplemented by control of aphids during the period of greenhouse propagation, and roguing, is recommended. Tolerant varieties affected with mosaic should be segregated from the healthy stocks, if grown at all.

Dahlia ring-spot is generally distributed in Connecticut, New Jersey, and southern New York, but has been found in high percentages in a few localities only. This disease has been transferred by grafting but not by mechanical methods. The relation of ring-spot to mosaic is discussed.

Yellow ring-spot, seen only in dahlias received from Utah, has been transmitted by grafting but not by mechanical methods.

Oakleaf is tentatively described as a fourth virus disease of dahlia solely on the basis of symptoms expressed.

None of the four virus diseases of dahlia described in this paper have been connected with other known virus diseases.

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EFFECT OF THALLIUM SULPHATE ON THE GROWTH OF SEVERAL PLANTS AND ON NITRIFICATION IN SOILS

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Thallium sulphate, since its introduction for rodent control, has aroused much interest not only on account of its pharmacological effects on animals but also because of its toxicity to plants (4). Dilling (2) reported in 1926 that N/600 thallium sulphate completely prevented the germination of *Lepidium sativum*. Brooks (1) called attention to the possibility of soil sterilization over a long period, if not permanently, from the use of thallium sulphate for rodent control or extermination. He cited a case in Hawaii where the growth of grass was prohibited over a two-year period on a strip of soil, from 1 to 3 feet long and about one-half as wide, under bait which contained about 0.5 gram of this salt. It was assumed that this amount would sterilize about one cubic foot of soil. It is not clear from his statements whether the grass grew after the two-year period. McMurtrey (3) studied the effect of heavy metals on tobacco in an endeavor to account for the Frenching disease. He included thallium nitrate in the study, because nitrate deficiency had been reported to be responsible for the presence of this disease. The nitrate was applied at the rate of 35 and 75 parts per million of each of three sandy loam soils, after the plants had become established in pots. He reported that the stems of the plants were killed at the surface of the soil in many instances. The injury varied somewhat in the different soils. One part per million of the element thallium in solution cultures was injurious and in the later stages the effects were similar to those of plants having the Frenching disease.

Prof. S. C. Brooks of the University of California, Department of Zoology, suggested to Director William Crocker that an investigation of the toxic effects of thallium on soils be conducted at Boyce Thompson Institute for Plant Research. As a result the effect of thallium sulphate on the growth of several plants and on nitrification in soils has been studied. The thallium sulphate was employed in these investigations because it is utilized in the field as a poison for rodents.

MATERIALS AND METHODS

The soils employed in the culture studies were sandy loam, volatile matter content 3.4 per cent, pH 4.63; silt loam, volatile matter content 5.4 per cent, pH 5.40; and fibrous sedimentary peat, volatile matter content 83.5 per cent, pH 6.70. The pH values were determined by means of the quinhydrone electrode, the ratio of soil to water being 1 to 3. The thallium sulphate solution was incorporated with about 100 grams of soil which in turn was mixed with the remainder of the soil. One-gallon glazed jars were

employed as the containers. The nitrification studies consisted of adding 30 milligrams of ammonium sulphate to 100-gram portions of a garden loam soil. These were treated with different amounts of thallium sulphate, placed in Erlenmeyer flasks, and incubated 30 days at room temperature. The phenol disulphonic acid method was used in making the nitrate determinations, after dispersing ten minutes with the Bouyoucos dispersing machine.

In attempting to reduce the toxicity of the thallium by leaching, the thallium-treated soils were placed in one-gallon jars and on filter paper in large Büchner funnels. Different amounts of water were passed slowly through the soils. Thallium was determined as the iodide (5, p. 318).

EXPERIMENTAL RESULTS

Effect of thallium sulphate on the growth of different plants. Soybean (*Glycine max* Merr. var. Biloxi), wax bean (*Phaseolus vulgaris* L. var. Golden Cluster), wheat (*Triticum aestivum* L. var. Marquis), buckwheat (*Fagopyrum esculentum* Moench), alfalfa (*Medicago sativa* L.), and rye grass (*Lolium perenne* L.) seed were planted in sandy loam soil to which had been added thallium sulphate at the rates of 1.7, 8.5, 17.3, 34.6, 69.2, and 103.8 parts per million. Observations were made at frequent intervals and final notes taken 30 days after the seeds were planted. The smallest dose was slightly injurious and the next larger application was much more so to all except the wax beans. The presence of 17.3 parts per million of thallium sulphate proved to be very injurious to all except the wax beans. The above-ground portions of the soybean plants developed to about one-half the extent of those grown in the untreated soil. Portions of the leaves were yellow in color and few lateral roots were present. The wheat plants were about one-third as tall as the control plants and were lighter in color. The root development was greatly retarded. The rye grass, buckwheat, and alfalfa plants were injured to about the same extent as the wheat plants. The growth of the wax beans showed only slight retardation and the leaves were normal in color.

The next higher concentration, or 34.6 parts per million, prevented lateral root development of all plants except that of the wax beans. The soybean, wheat, and alfalfa plants were dead. The growth of the rye grass and buckwheat plants was retarded greatly. The wax bean plants were about one-half as large as those grown in the control soil and there was considerable lateral root development.

The presence of 69.2 parts per million of thallium sulphate solution in the soil resulted in the death of all except the wax bean and buckwheat plants.

The largest dose, or 103.8 parts per million of soil, did not prevent germination of the seeds but the plants were killed soon after they emerged from the soil.

Buckwheat was also planted in one-gallon jars of silt loam soil to which had been added in duplicate thallium sulphate at the rates of 1, 2, 3, 5, and 10 parts per million (Fig. 1). The average fresh weight of plants grown in the control cultures at the early blooming stage was 44 grams. The average yields of the plants grown in the thallium treated soil, in the order given, were 34, 30, 29.2, 22.5, 14, and 2.1 grams.

Surface applications of thallium solution were made to rye grass which had been established several months in one-gallon jars of loam soil. The treatments consisted of 2.65, 5.30, 7.95, and 12.25 pounds per acre. The smallest application did not hinder the development of the grass, the next larger one resulted in slight injury to it, the 7.95 pounds per acre application caused severe injury and the largest or 12.25 pounds per acre killed the plants.



FIGURE 1. Effect of thallium sulphate on the growth of buckwheat. Left to right: Control, 1, 2, 3, 5, and 10 parts per million of silt loam soil.

Another series of cultures was run in which corn (*Zea mays* L. var. Golden Bantam) was planted in sandy loam soil to which 1.05, 2.1, and 8.5 parts per million of thallium sulphate had been added. The containers were one-gallon glazed jars. The smallest dose retarded the growth of the tops and roots of the plants slightly, the next larger one strikingly so, and the largest application resulted in the death of the plants about six weeks after the seeds were planted. The leaves of all the plants, except those grown in the soil to which was added the smallest amount of the thallium, became yellow soon after they came through the soil.

The injury to the leaves of the corn plants was of the interveinal type; that of the soybean, wax bean, and buckwheat veinal; and that of the rye and wheat was of the general type. The alfalfa leaves were yellow at their base.

Young tobacco (*Nicotiana tabacum* L.) and tomato (*Lycopersicon esculentum* Mill.) plants also were very sensitive to thallium sulphate. They were planted in sandy loam soil which had been treated with this salt at the rates of 2.1, 8.5, 17.3, and 51.9 parts per million, respectively. The results are presented in Table I and illustrated by Figures 2 and 3. It should be noted that Frenching occurred in the late stages of development of the tobacco plants grown in all the cultures to which thallium sulphate was added except the ones which received 2.1 parts per million.



FIGURE 2. The effect of thallium sulphate on the growth of tobacco. Left to right: Control, 8.5, 17.3, and 51.9 parts of thallium sulphate per million of sandy loam soil.

TABLE I

EFFECT OF THALLIUM SULPHATE ON TOMATO AND TOBACCO PLANTS GROWN IN SANDY LOAM SOIL; DURATION OF EXPERIMENT 30 DAYS

Parts of Tl_2SO_4 per million of soil	Tomato		Tobacco	
	Av. height of 6 plants in inches	Root development	Av. height of 3 plants in inches	Root development
0	7.2	Normal	4.2	Normal
2.1	6.0	Normal	3.3	Normal
8.5	4.3	Marked retardation	2.5	No additional growth
17.3	3.0	No additional growth	1.0	No additional growth
51.9	2.5	No additional growth	0.0	No additional growth

Tobacco plants also were planted in one-gallon jars filled with silt loam soil to which thallium sulphate had been added at the rates of 1, 3, and 5 parts per million. The average fresh weight of three plants grown 35 days in the control cultures was 134 grams, and the average weight of those grown in the thallium treated soil, in the order given, were 138, 101, and 51 grams, respectively.

Effect of thallium sulphate on plants grown in different soils. The extent of the injury of thallium sulphate toward plants in different soils varies slightly. Corn was planted in sandy loam, silt loam, and fibrous sedimentary peat soil to each of which had been added 8.5, 17.3, and 51.9 parts



FIGURE 3. The effect of thallium sulphate on the growth of tomato. Left to right: Control, 8.5, 17.3, and 51.9 parts of thallium sulphate per million of sandy loam soil.

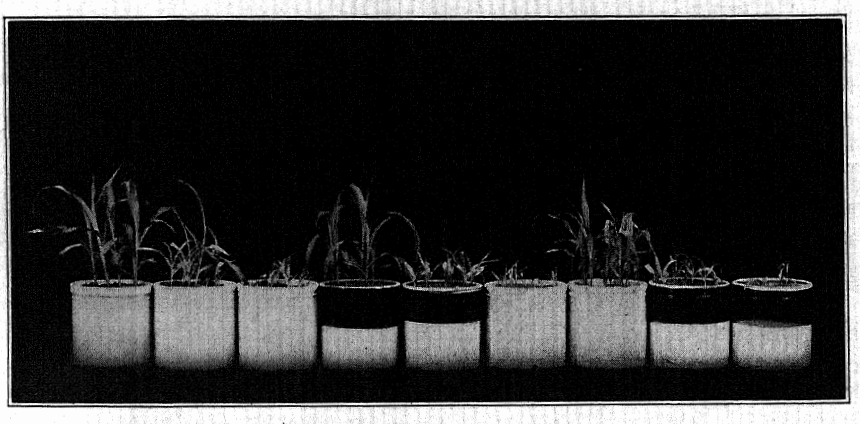


FIGURE 4. The effect of thallium sulphate on the growth of corn. A series, peat; B series, silt loam; and C series, sandy loam. Left to right in each series: Control, 8.5, and 51.9 parts of thallium sulphate per million of soil.

per million of thallium sulphate. The injury was visible in all cultures but was greatest in the sandy loam, slightly less in the silt loam, and still less in the peat. Fifteen days subsequent to the date the photograph (Fig. 4) was taken, the plants were dead in all cultures except the controls and the peat culture which contained the smallest dose of thallium sulphate.

Effect of commercial fertilizer on the inhibitory effects of thallium sulphate. In order to determine the effect of commercial fertilizer on the effect of thallium, tomato and tobacco plants were set in a sandy loam soil to which had been added 2.1, 8.5, 17.3, and 51.9 parts per million thallium sulphate. One set of cultures received in addition 6 grams of a 5–8–7 commercial fertilizer per 7 pounds of soil. The influence of the fertilizer on the toxicity of the thallium sulphate was negligible except in those cultures which contained the smallest application of the thallium. The leaves of the tomato plants grown in the fertilized soil to which 17.3 and 51.9 parts per million of thallium sulphate had been added were purple in color, especially on the lower sides and in the veins.

Effect of lime on the injurious action of thallium sulphate. The reaction of sandy loam was raised from pH 4.63 to 5.70 and 6.70 by the addition of calcium hydroxide, and thallium sulphate was added at the rate of 34.6 parts per million. The jars of soil were seeded to wax beans, wheat, and corn. Reduction in the injury by the thallium was not evident.

Effect of thallium sulphate on nitrate formation in the soil. The effect of thallium sulphate on the formation of nitrates in a loam soil taken from a garden was determined. Different 100-gram portions received doses of thallium sulphate solution ranging from 100 to 2400 parts per million. The nitrate content of the different cultures with the exception of the largest addition did not vary more than was to be expected from culture variations. The largest application retarded nitrate formation 5.8 per cent. The nitrate content of the soil in certain of the containers of the tobacco series also was determined. There were 47 and 61 parts per million of nitrates in the unfertilized and fertilized soils which had received 17.3 parts per million of thallium sulphate and 48.5 parts per million in the fertilized soil which had been treated with 51.9 parts per million of this salt. It would appear that the injurious action of the thallium sulphate was not due to the absence of nitrates in the soil.

Removal of thallium from solution by soils. The greater toxicity of thallium sulphate in some soils than in others indicates that they vary in their capacity to remove thallium from solution. This may be accompanied by base exchange and by the precipitation of thallium chloride. In order to determine to what extent soils remove thallium from solution, 25-gram samples of sandy loam and silt loam containing 100 cc. of a 5.5 per cent solution of thallium sulphate were shaken, let stand 24 hours, again shaken several minutes, and filtered. Aliquots of the clear filtrate were analyzed for thallium. Twenty-five grams of the soils in the order given removed 0.362 and 0.440 gram of thallium from solution.

The aluminum content of the soil extracts was increased markedly by the thallium treatment, that of the sandy loam extract was increased from less than one to 20 parts, and that of the silt loam from less than one to

15 parts per million by the addition of the thallium sulphate. The calcium displaced from 25 grams of these soils, in the order given, amounted to 0.047 and 0.064 gram. It is evident that base exchange took place when thallium sulphate was added to these soils.

Effect of leaching of thallium sulphate-treated soils on plant growth. Sandy loam and silt loam soils were treated with 2 per cent thallium sulphate solution at the rate of 1 cc. per gram, let stand 24 hours, placed in one-gallon glazed jars, and leached with different amounts of water. The soil in each jar was thoroughly mixed, fertilized, and corn planted in it. According to the data in Table II leaching did not reduce the injurious

TABLE II

Effect of Leaching Thallium-Treated Soils on the Growth of Corn

Inches of water leached through soil	Soil Class	
	Av. height of 6 plants in inches	
	Silt loam	Sandy loam
3	0.0	0.0
12	0.0	0.0
24	0.5	0.0
36	0.7	0.5
(Control) 36	8.5	6.3

action of the thallium significantly. Additional tests were run in which the soils containing 17.3 and 51.9 parts per million of thallium sulphate were leached slowly in Büchner funnels. Rye grass and wheat were planted in them. The results obtained did not show any evidence of the reduction of the thallium injury by leaching with 36 inches of water. It appears that soils, to which large applications of thallium sulphate are made will remain unproductive over long periods of time. Precautions should be taken to prevent this salt from coming into contact with the soil if productivity of it is desirable.

SUMMARY

1. The presence of 2.1 parts of thallium sulphate in one million parts of sandy loam slightly retarded the root and top development of soybean, wheat, buckwheat, alfalfa, and rye grass, and 8.5 parts of this salt in one million parts of soil were very injurious to these plants. Similar relationships occurred with corn, tobacco, and tomato plants. Wax bean was more resistant, injury being significant only when grown in the soil to which 8.5 parts per million of thallium sulphate were applied.

2. The injury to the leaves of the corn plants was of the intraveinal type, that of the soybean, wax bean, and buckwheat veinal, that of the rye and wheat general, and that of the alfalfa basal.

3. The extent of injury due to the thallium sulphate varied slightly in different soils. It was greatest in sandy loam, less in silt loam, and least in fibrous sedimentary peat.

4. Commercial fertilizer and lime did not lower the injurious action of the thallium sulphate.

5. Much larger quantities of thallium sulphate were required to reduce nitrification in soils than were found to injure plants.

6. Base exchange took place when thallium sulphate was added to sandy loam and silt loam soils, the amounts of aluminum and calcium in soil extracts being markedly increased.

7. Leaching thallium-treated soils with large quantities of water did not prevent the deleterious action of the thallium.

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CHANGES IN LEAVES DURING THE PERIOD PRECEDING FROST

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INTRODUCTION

The view that a considerable portion of the substance of a leaf is resorbed into the body of a perennial plant at a time just preceding the detachment of the leaf has attracted a great deal of interest.

Even if we avoid the temptation to regard this behavior as evidence of a wise provision of nature against loss of valuable material from the plant body and direct our attention to such questions as the extent of this transfer, the time at which it occurs, the behavior of different species, and the most suitable methods of obtaining accuracy in measuring these changes, the problem, although losing much of its speculative interest, nevertheless remains an attractive one.

The literature of the subject was reviewed thoroughly in recent articles by Murneek and Logan (11), and by Michel-Durand (9), and the problem was summarized previously in the papers of Combes (2) and of Combes and Kohler (3). There is general agreement that the experiments show extensive losses from the leaf blades throughout the latter part of the growing season, and especially in the period preceding frost.

The behavior of the nitrogenous substances has been quite consistent in showing that from 20 to 75 per cent of the nitrogen of the leaves disappears from them by the time of leaf fall. Rippel (12) gives a list of 33 species of trees and shrubs from which the loss of nitrogen ranged from 52.1 to 81.0 per cent of the highest amount found in previous periods during the growing season. He speaks of a remarkable constancy of the "resorption coefficient" at about 70 per cent.

The situation with respect to the carbohydrate fraction in the leaf is less clear. Combes and Kohler (3) estimate that about 55 per cent of the soluble carbohydrates are lost; of this loss less than one-half is translocated backwards to the plant body, the other portion being used in respiration or removed by leaching from the leaf-blade. Michel-Durand (8) found that there was a loss of 15 per cent of the total carbohydrates (non-cellulose), and that all but traces of the starch had disappeared. But Harter (5) found rather high percentages of starch in the fallen leaves of several species. Leclerc du Sablon (6) gives results for six species of fruit trees which show only small and inconsistent changes in sugar, and losses in the insoluble carbohydrates amounting to only about 10 per cent of the highest values, with at least two out of six cases doubtful. Murneek and Logan (11) furnish more complete measurements of the carbohydrate changes in two varieties of apple, and, while reporting some evidence of removal of carbohydrates

from leaves preparatory to their normal dropping, they believe that "there is lack of information on the extent of and quantitative relationships in this phenomenon" (11, p. 9).

The bases that have been used for computing changes in constituents have been dry weight, leaf area, and the total amount in a certain number of leaves. It is obvious that the dry weight basis was unsuitable in the experiments in which the authors reported a loss in dry weight during the period of the analyses. The leaf area and total amount in a certain number of leaves should have given dependable conclusions provided care was taken that the selected leaves were comparable at the start, and that the differences found at the end of the test were not due to differences which existed initially at the time of the selection of samples.

The present experiments were restricted to the period just preceding frost, and samples were taken at shorter intervals than has been done in previous experiments. Since there were suggestions in the literature that this was a critical period, a more intensive study of it by obtaining samples every three to five days seemed likely to give a better record of the changes.

Three bases were used to compute the results, the dry weight, the total amount in 50 leaves, and the residual dry weight, a value obtained by subtracting the weight of the carbohydrates and the protein from the dry weight. It is shown how these three bases may be used as checks against each other, and how the results from the three may be combined so as to increase our confidence in the dependability of the data.

Of the two species used, the leaves of one of them, *Viburnum*, underwent autumnal coloration, while those of the other, lilac, remained dark green until leaf fall.

In general, only small changes in the constituents of the leaves of these two species were found. Previous reports of large losses were corroborated only in the case of the nitrogen in *Viburnum* leaves. Small and doubtful losses of nitrogen occurred in lilac. But as for other constituents, such as sugar and carbohydrate, an unexpected uniformity was found, no important change being observed in the entire period from September 24 to November 4.

METHODS

Two plants of *Viburnum dentatum* L. about eight feet high, and 16 of lilac (*Syringa vulgaris* L.) varying in height from five to eight feet were selected for the leaf samples. Fifty white paper tags were used for each species to mark opposite leaves which appeared to be equal in size. One leaf of each pair was removed at once, and the other tagged leaves were allowed to remain until three to five days later, at which time they also were collected for analysis. On the same or the following day, the tags were placed upon another set of paired leaves, which were to show the changes in the

leaves for another interval of three, four, or five days. Sampling began on September 24, 1932, and continued until November 4, at which time the occurrence of frosts prevented further sampling. In fact, more tender plants growing nearby showed frost injury previous to November 4, but the leaves of the *Viburnum*, which were red by November 1, and those of lilac, which are quite leathery and which remained green, probably could have been frosted without showing visible evidence of injury.

Samples were collected at approximately 9:00 a.m. on each sampling period. On days in which two successive samples were taken, e.g., on October 11, 15, etc., the second sample was taken about one hour after the first.

The fresh weights of the leaves could not be obtained in most cases because of the interference by rain, dew, or possibly guttation liquid. As soon as the leaves were collected the tissue was chopped into small pieces and was dropped into tared beakers containing boiling 95 per cent ethyl alcohol. Subsequently the alcohol was evaporated slowly on a steam bath and the beakers were placed in an electric vacuum oven regulated at 70° C. After the tissue ceased to lose weight under these conditions the dry weight values were recorded, and the tissue was ground with mortar and pestle until it would pass through a 60-mesh sieve. After again being dried, portions of this leaf powder were weighed out for analysis. The methods used in the analysis of the tissue were the same as those described previously (4). The term polysaccharides as used in this paper refers to the substances insoluble in 70 per cent alcohol and hydrolyzable by dilute acid (1, p. 95). They were calculated as starch, but they include all constituents that, upon hydrolysis with dilute acid, will form substances that reduce Fehling's solution. Total carbohydrate is the sum of sugar and polysaccharides. The total nitrogen value is the sum of the values for soluble and insoluble forms of nitrogen, 70 per cent alcohol being the solvent.

RESULTS

ANALYTICAL VALUES

The chemical analyses of the leaf powders calculated on the dry weight basis are shown in Tables I and II. These dry weight percentages do not tell us whether changes had occurred until we find out whether the dry weight itself had changed. A reduction in dry weight percentage value of a constituent during a sampling interval could occur under the following conditions: (a) constituent decreased and dry weight remained constant, (b) constituent remained constant and dry weight increased, (c) constituent decreased and dry weight also decreased, but to a less extent, (e) constituent increased and dry weight also increased, but to a greater extent.

We can obtain information as to whether the dry weight has changed by computing it on the basis of the residual dry weight. This value was

suggested as a basis for calculation by Mason and Maskell (7) who obtained the residual dry weight by subtracting the total carbohydrate from the dry weight. In the present experiments, since the total nitrogen in Tables I and II shows decreasing values, it seemed possible that changes were occurring in this fraction also, and that not only total carbohydrate but also the weight of the nitrogenous fraction should be subtracted from

TABLE I

ANALYSIS OF VIBURNUM LEAVES

Date 1932	Per cent of the dry weight							
	Poly-sacc.	Total sugar	Total carbo-hydr.	Insol. N	Sol. N	Total N	N × 6.25	Resid. dry wt.
Sept. 24	7.92	7.55	15.5	1.42	0.009	1.43	8.93	75.6
" 28	7.09	8.00	15.1	1.36	0.007	1.37	8.54	76.4
" 29	7.85	8.98	16.8	1.35	0.006	1.36	8.48	74.7
Oct. 3	8.66	8.50	17.2	1.39	0.006	1.40	8.73	74.1
" 4	8.79	8.06	16.9	1.32	0.006	1.33	8.29	74.9
" 8	8.29	8.29	16.6	1.30	0.005	1.31	8.16	75.3
" 8	8.57	8.91	17.5	1.24	0.006	1.25	7.79	74.7
" 11	9.28	7.79	17.1	1.29	0.006	1.30	8.10	74.8
" 11	8.99	7.93	16.9	1.20	0.007	1.21	7.54	75.5
" 15	8.23	9.04	17.3	1.20	0.006	1.21	7.54	75.2
" 15	8.51	10.20	18.7	1.22	0.008	1.23	7.68	74.6
" 19	8.27	8.44	16.7	1.15	0.007	1.16	7.23	76.1
" 19	7.92	7.78	15.7	1.12	0.009	1.12	7.06	77.2
" 22	7.14	8.18	15.3	1.09	0.010	1.10	6.88	77.8
" 22	7.82	8.19	16.0	1.17	0.007	1.18	7.36	76.6
" 26	7.45	7.43	14.9	1.04	0.009	1.05	6.56	78.6
" 26	7.16	8.43	15.6	1.08	0.009	1.09	6.81	77.6
" 31	7.08	9.43	16.5	1.01	0.011	1.02	6.38	77.1
" 31	7.23	8.26	15.5	0.94	0.010	0.95	5.94	78.6
Nov. 4	7.04	6.67	13.7	0.78	0.013	0.79	4.96	81.3

Note: The paired horizontal lines represent results from paired leaves, e.g., the sample on September 24 was obtained by taking one leaf and the one on September 28 the opposite leaf from each pair of leaves.

the dry weight. Accordingly the percentages of total nitrogen were multiplied by 6.25 in order to convert them into percentages of protein (1, p. 71). This method is only approximately correct, and is used only because the nature of the nitrogenous substances is not known. Probably the error involved does not influence seriously the results in the present case. The values for the residual dry weight in column 9, Tables I and II, were obtained, therefore, by subtracting the sum of the total carbohydrate per-

centage and 6.25 times the percentage of total nitrogen from 100. For example, in the top line of Table I, $75.6 = 100 - (15.5 + 8.93)$. The principle involved is that the residual dry weight represents an unchanging fraction under the conditions of the sampling, and that calculations on this basis should give a true measure of the changes in the other constituents. The percentages of certain constituents on the basis of the residual dry weight

TABLE II
ANALYSIS OF LILAC LEAVES

Date 1932	Per cent of the dry weight							
	Poly-sacc.	Total sugar	Total carbo-hydr.	Insol. N	Sol. N	Total N	N × 6.25	Resid. dry wt.
Sept. 24	13.3	5.81	19.1	1.94	0.19	2.13	13.3	67.6
" 28	12.0	6.23	18.2	1.97	0.21	2.18	13.6	68.2
" 29	12.0	6.36	18.4	1.99	0.20	2.19	13.7	67.9
Oct. 3	12.0	6.60	18.6	1.99	0.20	2.19	13.7	67.7
" 4	12.6	6.26	18.8	1.94	0.20	2.14	13.4	67.8
" 8	11.6	7.59	19.2	1.98	0.21	2.19	13.7	67.1
" 8	11.1	7.31	18.4	2.04	0.22	2.26	14.1	67.5
" 11	11.5	6.63	18.2	1.97	0.19	2.16	13.5	68.3
" 11	12.3	6.12	18.5	1.95	0.21	2.16	13.5	68.1
" 15	11.1	8.75	19.8	1.90	0.21	2.11	13.2	67.0
" 15	13.3	8.93	22.2	1.94	0.20	2.14	13.4	64.4
" 19	13.3	6.68	20.0	1.93	0.28	2.20	13.8	66.3
" 19	11.2	6.73	17.9	1.90	0.23	2.12	13.3	68.8
" 22	9.9	7.45	17.3	1.90	0.24	2.14	13.3	69.3
" 22	10.8	5.84	16.7	1.96	0.20	2.15	13.4	69.9
" 26	10.2	7.29	17.5	1.88	0.22	2.10	13.1	69.4
" 26	12.6	7.60	20.2	1.82	0.21	2.03	12.7	67.1
" 31	11.9	9.17	21.0	1.77	0.21	1.98	12.4	66.6
" 31	12.1	9.29	21.4	1.79	0.21	2.00	12.5	66.1
Nov. 4	10.5	8.89	19.4	1.83	0.23	2.06	12.9	67.7

are shown in Table III. The values were obtained from Tables I and II by dividing each percentage by the corresponding value in column 9. Thus, in the first line in Table I for polysaccharides $7.92 \div 75.6 = 0.105$, or 10.5 per cent. The dry weight itself can be calculated on the residual-dry-weight basis, e.g., in the same case, $100 \div 75.6 = 1.32$, or 132 per cent. The dry weight values calculated in this way are shown for *Viburnum* and lilac in columns 2 and 6 in Table III. It is seen that on this basis the dry weight values have been nearly constant throughout the period of sampling. There is no evidence whatsoever of loss of dry weight in the lilac leaves, and with

the *Viburnum* only the sample on November 4 shows any indication of an important loss of dry weight.

We can obtain further evidence as to whether the dry weight, and also the residual dry weight, have been constant during the sampling period by reference to Table IV, which shows the total amounts in the 50 leaves which were taken at each sampling period. The data are arranged in paired

TABLE III

ANALYSES OF VIBURNUM AND LILAC LEAVES

Date 1932	Per cent of the residual dry weight							
	Viburnum leaves				Lilac leaves			
	Dry wt.	Poly-sacc.	Total sugar	Total N	Dry wt.	Poly-sacc.	Total sugar	Total N
Sept. 24	132	10.5	10.0	1.89	148	19.6	8.59	3.15
" 28	131	9.3	10.5	1.79	147	17.6	9.14	3.19
" 29	134	10.5	12.0	1.82	147	17.7	9.36	3.22
Oct. 3	135	11.7	11.5	1.88	148	17.7	9.74	3.23
" 4	134	11.7	10.8	1.77	147	18.5	9.23	3.15
" 8	133	11.0	11.0	1.73	149	17.2	11.30	3.26
" 8	134	11.5	11.9	1.67	148	16.4	10.82	3.35
" 11	134	12.4	10.4	1.73	146	16.9	9.70	3.16
" 11	132	11.9	10.5	1.60	147	18.1	9.00	3.17
" 15	133	10.9	12.0	1.60	149	16.5	13.05	3.15
" 15	134	11.4	13.7	1.65	155	20.6	13.86	3.32
" 19	131	10.9	11.1	1.52	151	20.0	10.07	3.32
" 19	129	10.3	10.1	1.46	145	16.3	9.78	3.08
" 22	129	9.2	10.5	1.41	144	14.3	10.74	3.08
" 22	130	10.2	10.7	1.54	143	15.5	8.35	3.08
" 26	127	9.5	9.5	1.34	144	14.7	10.50	3.03
" 26	129	9.2	10.9	1.40	149	18.8	11.32	3.03
" 31	130	9.2	12.2	1.32	150	17.8	13.77	2.97
" 31	127	9.2	10.5	1.21	151	18.3	14.05	3.02
Nov. 4	123	8.7	8.2	0.98	148	15.5	13.12	3.04

horizontal lines, the first line of each pair showing the total amounts of each constituent in the 50 twin-leaves taken at the beginning of the experimental interval, and the second line of each pair shows the amounts in the opposite 50 leaves which remained upon the plant and were removed at the end of three to five days. These values for each constituent at the beginning and at the end of each interval show the gain or loss in grams of the constituent during the interval. Whether this absolute gain or loss is a true measure depends upon whether the twin-leaves represented

closely agreeing pairs at the time they were selected as samples. Information on this point can be found in columns 6 and 11, Table IV, which show the residual values obtained by subtracting from the dry weight the total carbohydrate (sum of polysaccharides and sugar) and 6.25 times the total nitrogen. If the paired leaves were closely agreeing twins, and if the only changes in the dry weight occurred in the substances represented by

TABLE IV

TWIN-LEAF TOTALS FOR VIBURNUM AND LILAC LEAVES

Date 1932	Total amount in 50 twin-leaves									
	Viburnum leaves					Lilac leaves				
	Dry wt. g.	Poly-sacc. g.	Total sugar g.	Total N mg.	Resid. dry wt. g.	Dry wt. g.	Poly-sacc. g.	Total sugar g.	Total N mg.	Resid. dry wt. g.
Sept. 24	8.79	3.05	0.66	134	6.64	15.00	1.99	0.87	320	10.14
" 28	8.71	3.14	0.70	126	6.64	15.10	1.81	0.94	328	10.29
" 29	7.23	2.50	0.65	103	5.39	13.81	1.66	0.88	302	9.38
Oct. 3	7.30	2.53	0.62	108	5.41	14.39	1.72	0.95	315	9.74
" 4	7.26	2.43	0.59	101	5.43	11.98	1.50	0.75	256	8.13
" 8	7.14	2.38	0.59	98	5.37	11.62	1.35	0.88	255	7.80
" 8	6.81	2.18	0.61	90	5.08	11.85	1.31	0.87	267	8.01
" 11	6.95	2.30	0.54	95	5.19	11.37	1.31	0.75	245	7.77
" 11	7.08	2.34	0.56	92	5.34	13.63	1.68	0.83	294	9.28
" 15	7.10	2.43	0.64	91	5.34	13.63	1.51	1.19	287	9.13
15	8.41	2.74	0.86	110	6.18	12.11	1.61	1.08	259	7.80
" 19	7.69	2.65	0.65	95	5.83	11.99	1.59	0.80	264	7.95
" 19	9.89	3.76	0.77	119	7.64	16.75	1.87	1.13	355	11.53
" 22	9.93	3.64	0.81	119	7.72	16.04	1.59	1.20	343	11.12
" 22	8.64	3.42	0.71	108	6.62	13.69	1.48	0.80	295	9.57
" 26	8.64	3.16	0.64	99	6.79	13.07	1.33	0.95	275	9.06
" 26	9.51	3.43	0.80	112	7.37	13.74	1.73	1.04	279	9.22
" 31	9.80	3.46	0.92	110	7.55	13.60	1.61	1.25	269	9.06
" 31	9.50	3.35	0.79	99	7.46	12.00	1.46	1.12	240	7.93
Nov. 4	9.09	3.41	0.61	84	7.39	12.62	1.33	1.12	260	8.55

total carbohydrate and 6.25 times the total nitrogen, the paired values in columns 6 and 11 in Table IV should be equal. In the case of the *Viburnum* the residual dry weights agree very well, the average deviation between the two values for each pair being only about 1.5 per cent of the average residual dry weight. In the case of the lilac, the agreement is much less satisfactory, the deviation between pairs being about 3.3 per cent of the average value; and in the sampling period from October 22 to October 26 the deviation of the residual-dry-weight values from each other was

5.4 per cent. These considerations show that the sampling was not satisfactory in the case of the lilac, and that the gains or losses in absolute amounts of material during a sampling interval for this species might not show the situation correctly, because of this failure to obtain twin-leaves which agreed closely as to size or as to composition.

Column 2, Table IV, shows that no consistent differences in the pairs of values were obtained, and that no important change in the dry weight of the *Viburnum* leaves took place during the entire period from September 24 to 31. Possibly there was a loss from October 31 to November 4. The values in column 7, for lilac, show dry weight losses in six out of ten sampling periods, but these decreases are probably only apparent losses, and are due to lack of agreement between the pairs of leaves selected for the samples. This is shown by the residual-dry-weight values in column 11. It will be noted that, in the intervals during which the dry weights (column 7) showed large decreases, the corresponding residual-dry-weight values (column 11) show high values for the initial sample of the period and low values for the sample at the end. In such cases, even if no change in dry weight occurred, the values would show calculated decreases. From this point of view, the lilac data also fail to show any important change in the dry weight of the leaves during the period from September 24 to November 4.

It is concluded that the dry weight and the residual dry weight of the leaves of both species were essentially constant during the entire period of sampling, and that the changes in the percentages of the constituents on these two bases represent true measures of the changes in the leaves during that period. The total amounts in the 50 leaves for each sample also represent a valid basis for computation in the case of *Viburnum*, but the sampling error was so large in the case of lilac that care would need to be used in forming conclusions merely from the changes of the total amounts in 50 leaves.

PREPARATION OF GRAPHS

The analytical values in Tables I, II, III, and IV have been arranged for certain constituents in the form of graphs, see Figures 1 and 2. Instead of plotting the dry weight, residual dry weight, and twin-leaf values on separate graphs, an attempt was made to combine the observations by these three methods into a single graph, in such a manner that the absolute values, although numerically different, would occupy on the plotting paper distances proportional to the absolute values. Thus, in Figure 1 for total nitrogen the ordinates at the left of the graph were arranged for plotting both the dry weight and residual-dry-weight percentages. The average value of all of the dry weight percentages from September 24 to November 4 was 1.19, and that for the residual-dry-weight percentages was 1.57; these

two values were placed opposite on the graph. The scale division for the dry weight values is seen to be $1.39-1.19=0.20$, and the scale range for the residual dry weight to correspond to this range for dry weight was made equal to $1.57\div 1.19\times 0.20=0.26$, and the scale reading $1.57+0.26=1.83$ was placed opposite 1.39. The zero values for the two are now at the same level on the graph (in all cases much below the values shown on the graph), and one scale division on the graph represents an amount of dry weight or residual-dry-weight percentage proportional to the absolute values of each.

The values for each constituent on the dry weight basis were plotted as open circles, and those on the residual-dry-weight basis as closed circles; if the two values were identical or so nearly the same that they could not be separated on the plotting scale, the value was plotted as a circle half-open and half-closed.

Since two samples of leaves representing different pairs of twin-leaves were taken on October 8, 11, 15, 19, 22, 26, and 31, the results from them should permit us to obtain information regarding the sampling error. The error of the chemical analysis is small compared with the error in the samples of leaves. But the differences in the values plotted on the same day in Figures 1 and 2 represent the total error due both to sampling and analysis. Since the values for both the dry weight and residual dry weight were plotted so as to make the units of the plotting scale proportional to the absolute values of each constituent, we can obtain a value for the standard deviation in terms of the units of the plotting scale. There were available seven groups of four values, each representing the plotting values in units of the graph paper for each constituent, being the values for October 8, 11, 15, 19, 22, 26, and 31. Using the formula given by Morris and Wesp (10, p. 57) the standard deviation (*S.D.*) of a single measurement was computed from these quadruplicate values, and the value so obtained is shown by the arrows in the central vertical line at the left of Figures 1 and 2. The arrow points were placed above and below the average value of each constituent for the entire period at a distance which was equal to the *S. D.* of a single determination measured in units of the plotting scale.

Figures 1 and 2 also show the twin-leaf values (total amounts in 50 leaves), and in order to arrange the twin-leaf totals so that they could be plotted the following procedure was used. The total amount in the first sample on September 24 was set equal to 100; the value of the September 28 sample of opposite leaves was expressed as a percentage of this value. For example, the total nitrogen for *Viburnum* on September 24 was 134 mg. and for September 28 it was 126. The nitrogen, therefore, dropped from 100 to 94. Between September 29 and October 3 the nitrogen changed from 103 to 108; that is, the nitrogen increased 4.9 per cent, and if we may

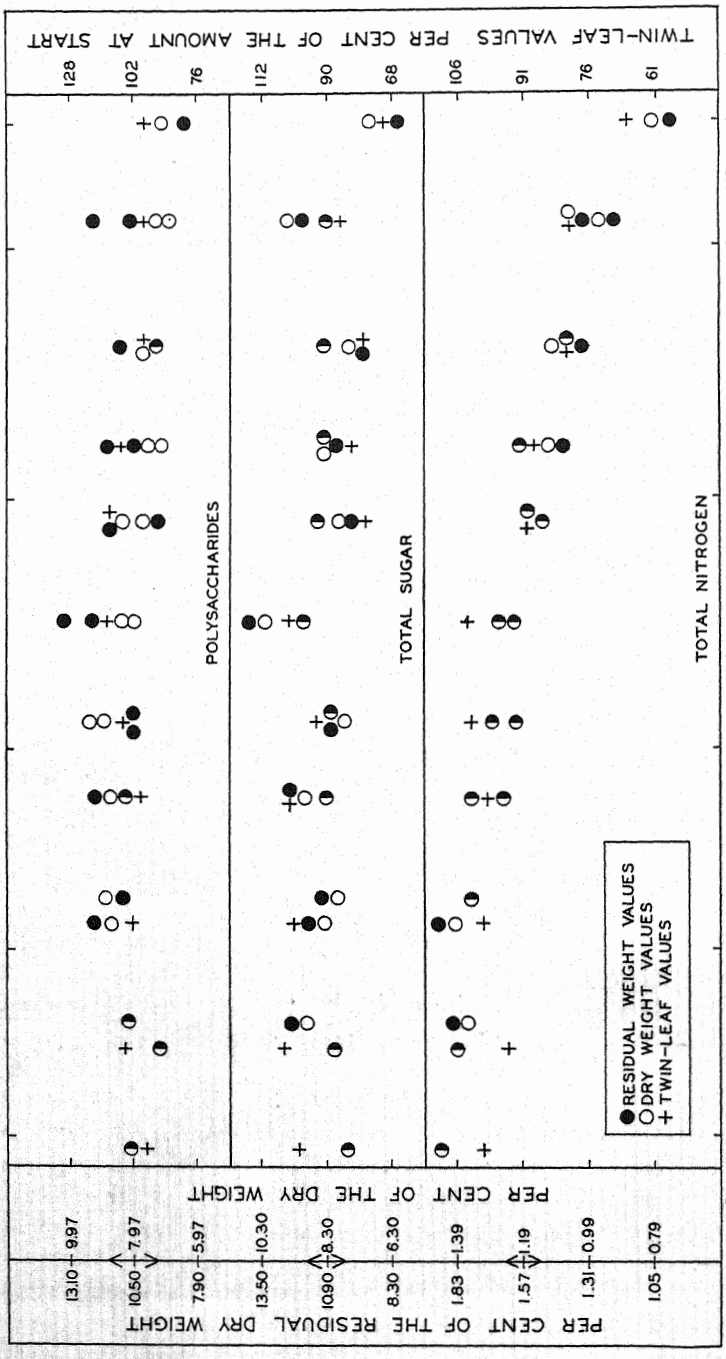


FIGURE 1. Composition of leaves of *Viburnum dentatum* L. during the period from September 24 to November 4, 1932.

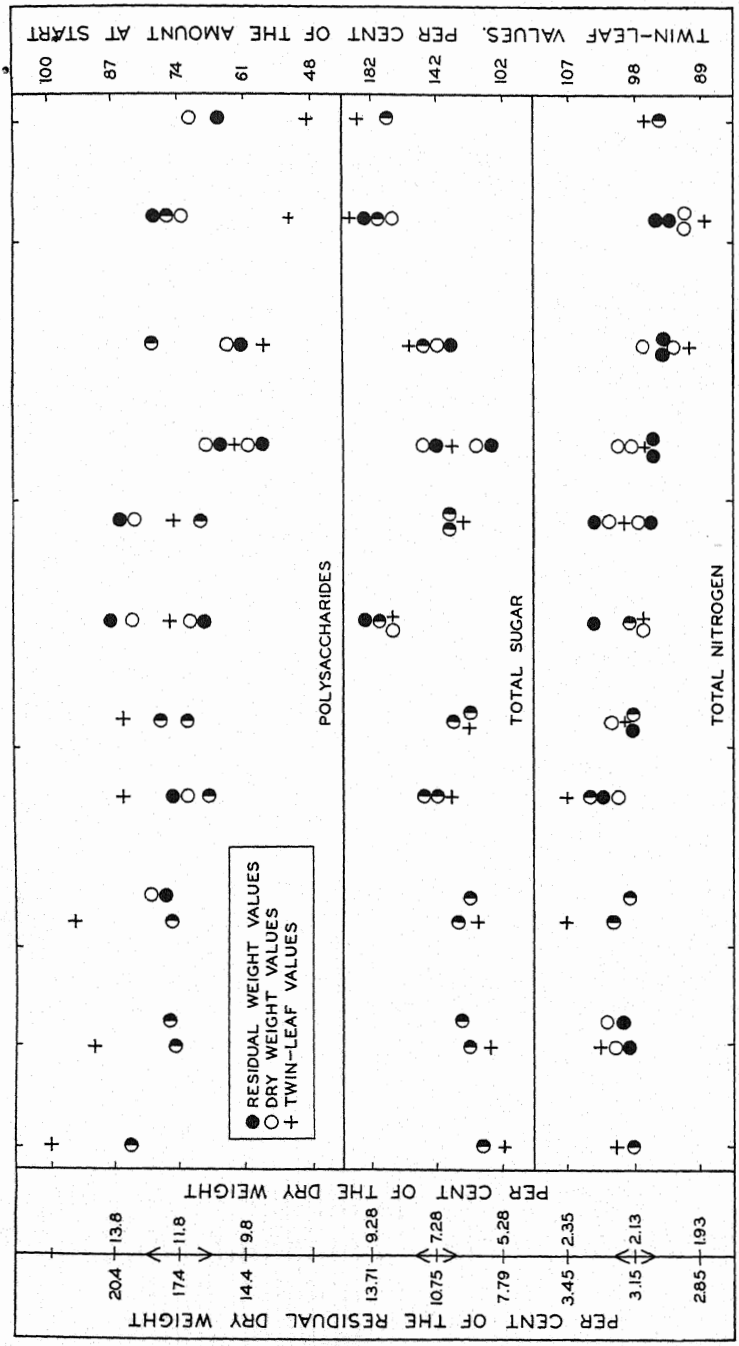


FIGURE 2. Composition of leaves of *Syringa vulgaris* L. during the period from September 24 to November 4, 1932.

be permitted to use the September 28 values as a new base, the nitrogen increased from 94 to 99. By this step-wise procedure, values for the entire period of sampling were obtained in terms of the percentage of the initial amount in sample on September 24. Beginning with October 8, it will be noted that two samples of leaves were obtained at each sampling period, one lot to complete the sample of twin-leaf pairs taken on the previous date, and the other to represent the initial sample of twin-leaves for another sampling interval. Consequently, it is only on September 29 and October 4 that the value for the preceding day must be used as a basis for computing the change in the succeeding interval. However, since in most cases only small changes in any constituent occurred even during an interval of many days, it seems that the error caused by assuming no change from September 28 to September 29, and from October 3 to October 4 has been very small.

After obtaining the twin-leaf values on September 28, October 3, October 8, etc., in terms of the percentage of the amount present on September 24, the average of these values was computed and placed on the plotting scale at the right-hand side of the figure in a position directly opposite the averages for the dry weight and residual-dry-weight percentages. That is, in Figure 1 for total nitrogen the average of the twin-leaf percentages was 91 and this value was plotted opposite 1.19 and 1.57, the corresponding dry weight and residual-dry-weight percentages. The scale reading for the twin-leaf values should be $91 \div 1.19 \times 0.2 = 15$, and the scale reading $91 + 15 = 106$ is plotted opposite 1.39 and 1.83. The twin-leaf values obtained in this way were plotted as plus signs (+) on the graphs.

CHANGES IN CONSTITUENTS

Total nitrogen. The most important change observed in these experiments was in the nitrogen of the *Viburnum* leaves. Figure 1 and Tables I, III, and IV show a consistent and continuous decrease in the nitrogen of the leaves. Especially was this true from about October 15 to November 4. It was on about October 10 that the leaves began to develop a reddish color. The decrease in the nitrogen of the *Viburnum* leaves was about 35 to 40 per cent. In the case of the lilac, however, this comparatively large decrease in nitrogen was not observed (Fig. 2 and Tables II, III, and IV). From September 24 until October 22 the nitrogen values were practically constant. There may have been a decrease in nitrogen between October 22 and October 31 but, if so, the decrease was probably not more than about 5 per cent.

Total sugar. In *Viburnum*, the total sugar remained nearly constant throughout the period of sampling. High values were obtained in the October 15 sample. In lilac, the sugar values tended to increase throughout the period. As with *Viburnum* a high value was obtained on October 15. The

sugar values fluctuated sharply in the period from October 8 to October 20, but the curves show that the same fluctuations occurred simultaneously for both *Viburnum* and lilac. Possibly greater fluctuations should be expected in sugar values than in those for other more stable constituents.

Polysaccharides. The polysaccharides were nearly constant throughout the period of sampling. The values for *Viburnum*, Figure 1, at all times were close to the average value for the period. It is true that most of the values are higher than the average in the first half of October, and lower during the second half, but the differences are small and cannot be considered significant. With lilac, the situation is less clear, because of the poor agreement between the results of analyses of leaves sampled on the same day. But the dry weight and residual-dry-weight values do not deviate from the average values significantly throughout the period. It is true that the twin-leaf values show a distinct decrease from the beginning to the end of the test. If, however, we examine the polysaccharide values in column 8, Table IV, and compare them with the corresponding residual dry weights in column 11, it is seen that high initial values and low final values in the different intervals are usually correlated with high initial and low final values of the residual dry weights, indicating that the differences were due mainly to inequalities that existed in the twin-leaf samples at the time of their selection, and were not due to changes during the period of the test. If the polysaccharide values in column 8 are expressed as a percentage of the corresponding residual values in column 11, these values when plotted in Figure 2 give the same curve as that for the residual dry weight.

Total carbohydrates. Curves for these are not shown but they were prepared, and showed nearly constant values throughout the period of observation. This conclusion is supported by the data in Tables I, II, III, and IV. There was no important change in the amounts of total carbohydrate in the leaves of either species in the period from September 24 to November 4.

DISCUSSION

Although the experiments were undertaken for the purpose of measuring changes in the leaves, the results emphasize how small the changes were, and how few of the constituents were affected. In both *Viburnum* and lilac the dry weight, sugar, and polysaccharides did not show any important change during the entire period from September 24 to November 4. Distinct and unmistakable decreases were found only in nitrogen, and, in this case, only the *Viburnum* results should be emphasized as the nitrogen losses in lilac were small and of doubtful significance. If, as has been assumed, or perhaps even demonstrated, the substances which disappear from the leaves upon the approach of frost are translocated, in part, at

least, backwards into the storage tissues of the plant, these experiments indicate a selective translocation, in which the nitrogenous substances are preferentially or possibly even exclusively involved. It might be expected that carbohydrates, representing energy sources, should be selected for storage in the roots and stems, but, if this has been the case in the present experiments, an approximately equal amount of carbohydrates has been synthesized to replace those translocated.

Since there was a decrease in the nitrogen in the leaves of *Viburnum*, there should have been a corresponding decrease in the dry weight. However, the nitrogen calculated as protein by the factor 6.25 represents less than 10 per cent of the dry weight, and a change in nitrogen amounting to 40 per cent would change the dry weight by only 4 per cent. It is difficult to establish with certainty a change of such an amount.

The present experiments show that, in general, the total amounts of material in 50 twin-leaves served as a fairly accurate index of the changes in the leaves. However, the polysaccharide data for lilac show that a conclusion on this basis alone could become very misleading, as a result of errors due to inequalities in the samples of leaves at the time of their collection. In this particular case the differences between the values at the beginning and at the end of the sampling period were not the result of changes during the interval, but were differences that existed from the beginning. But if these inequalities in tissue were found in equal numbers of leaves obtained by a sampling method in which the leaves were selected in pairs for the purpose of establishing uniformity, how much larger errors may have been made in some of the experiments previously reported upon! In some cases the data are expressed as the amounts per 1000 leaves. If approximately 1000 leaves were used perhaps the totals should be dependable, but if a smaller number of leaves were taken and the totals calculated to the basis of 1000, we cannot feel certain that the sampling procedure was satisfactory. It is not enough that equal numbers of leaves were taken; the extent to which uniformity in the samples was attained would need to be established and described in some detail.

No attempt is made to generalize upon the basis of the results of the present experiments. Only two species were used, and the indications are that species vary in relation to the changes in composition in the period preceding frost. Previous reports have not usually recognized this possibility in the response of different species. Furthermore, the present experiments are for one year only, and may represent the behavior under the particular conditions that prevailed at that time. There was evidence in the experiments of Murneek and Logan (11) that the response of the same species may be different in different years. Thus, the starch in the apple leaves showed gains between October 7 and November 14 in 1927, but during a similar period in 1928 no gains were found.

The *Viburnum* results show a rather sharp drop in the value of all constituents from the October 31 to November 4 sample. This may be the aftermath of frost injury. Frosts had occurred in other locations nearby at periods previous to November 4, but the *Viburnum* leaves were nearly completely red in color by that time, and the question whether they had been frosted could not be decided by the appearance of the leaves. Possibly the low value on November 4 may be due mainly to leaching of constituents from injured tissue.

SUMMARY

Samples of 50 pairs of opposite leaves of *Viburnum dentatum* and *Syringa vulgaris* were selected to measure the changes in leaves at intervals of three to five days from September 24 to November 4, at which time the experiment was ended because of frost. One leaf of each pair was taken at the beginning and the other was left on the plant until the end of each interval, there being ten such intervals during the experimental period.

Dry weight, sugars, polysaccharides (alcohol-insoluble substances hydrolyzable with dilute acid), and nitrogen were determined, and calculations were made upon three bases: per cent of the dry weight, per cent of the residual dry weight (obtained by subtracting from the dry weight the sum of the carbohydrates and 6.25 times the nitrogen), and the total amounts of constituents in 50 leaves.

The dry weight of the leaves was nearly constant throughout the period of sampling, and no important change was observed in total carbohydrate (sum of sugar and polysaccharide). Previous reports of extensive losses of substances from leaves during the interval preceding frost (autumnal migration) were confirmed only for the nitrogenous substances in these species, and, even in this case, only the *Viburnum* results should be emphasized, as the nitrogen losses from lilac were small and of doubtful significance.

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COMPARATIVE ANATOMICAL STUDIES OF DOROTHY PERKINS AND AMERICAN PILLAR ROSES. I. ANATOMY OF CANES. II. ORIGIN AND DEVELOPMENT OF ADVENTITIOUS ROOTS IN CUTTINGS

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In order to study the internal changes resulting in the formation of adventitious roots by stem cuttings it was necessary to understand the anatomy of the cuttings and to know what tissues were involved in the initiation of such roots. The anatomical observations reported here were made in connection with microchemical work on cuttings of Dorothy Perkins (*Rosa wichuraiana* × Mme. Gabriel Luizet) and American Pillar (*R. wichuraiana* × *R. setigera*) roses to determine any differences between them which might lead to an understanding of their differences in rooting behavior (Carlson, 1).

Vegetative stems (canes) of Dorothy Perkins rose attain a greater length and are smaller in diameter and more flexible than those of American Pillar. A bud at each node will produce a flower shoot during the second growing season.

Early in the spring cuttings made from the canes of the two roses show a difference in rooting response. Dorothy Perkins cuttings root from the base of developing shoots and not from the base of the piece of cane. American Pillar cuttings do not root from the bases of shoots but may, after a month or two, root from the base of the piece of cane. Exceptions to this response may occur; for example, an occasional shoot of Dorothy Perkins does not produce roots, and an occasional shoot of American Pillar may produce roots at the base.

A similar response is shown when cuttings are made of current season's shoots, three to four inches long, with a small piece (mallet) of the cane attached (Hitchcock and Zimmerman, 5). These investigators also found that cuttings of both roses cut exactly at the base or with a heel of the cane attached rooted readily. Such cuttings were taken between May and August when the current season's shoots had made a good growth.

This study was confined to the anatomy of the canes and to the anatomical changes occurring when the cuttings of canes taken in March and April were placed in rooting conditions.

MATERIAL AND METHODS

Material for the study of developing canes and buds was collected at intervals during the season of 1926 from the gardens of the Boyce Thomp-

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son Institute for Plant Research. A large number of nodes and buds were examined in various stages of development from free-hand and microtome transverse and longitudinal sections.

Mature canes of each rose were divided into equal numbers of pieces and transverse sections of comparable regions were studied. The bases, the regions midway between base and tip, etc., were considered to be comparable in development in the two roses. The vascular systems were also studied by placing canes in a dye, usually eosin, and then dissecting or following serial transverse sections.

Cuttings, 10 to 12 inches long, were made at intervals during March and April of 1926 and 1927. These were placed with their basal ends in water and were covered with bell jars through which a current of air was passed. The water was changed frequently and the cuttings were kept in good condition from three to six weeks. Ten such series were made.

The branches which developed from buds on the cuttings of Dorothy Perkins rose furnished the material for the study of the formation of a basal enlargement and the origin and development of the adventitious roots. Similar regions of cuttings of American Pillar rose were studied for comparison with Dorothy Perkins. Young shoots were removed from the cuttings at intervals of one or two days, beginning with the third and continuing until the twelfth day. In order to secure the entire base of each shoot, a wedge-shaped portion of the main stem was removed with it. Any remaining bud scales were removed to facilitate penetration of the fixing agent. Basal pieces of these shoots, three to six mm. long, were prepared for sectioning in the usual manner. Serial transverse and longitudinal sections 10 to 15μ in thickness were made. The sections were stained with safranin and crystal violet. Most of the roots traverse the cortex at right angles to the long axis of the branch, hence in transverse sections of the stem most of the root primordia and roots[2] are cut longitudinally. When one of these roots was located, all of the sections in which it appeared were counted. The section midway between the one in which it first appeared and the one in which it could be seen last was taken as the median section of the root. Only those sections which were median-longitudinal were used for the figures.

OBSERVATIONS

Structure of canes. During and at the close of the first year's growth, comparable regions of the canes of the two roses show some differences in structure. American Pillar canes have a greater diameter at points of equal maturity than Dorothy Perkins canes. This is due to the larger size of cells in the cortex and pith and to the larger number of cells in the xylem and pith. The cuticle on the epidermis of the American Pillar stem is much

[2] These terms are used as defined in a previous article (2, p. 120).

thicker, in comparable regions, than the cuticle of the Dorothy Perkins. The cortex, in both roses, consists of two types of cells, collenchymatous and parenchymatous. The collenchyma lies just inside the epidermis and is interrupted where stomata occur. It is a region varying from four to eight cells in radial dimension. The walls of the collenchyma cells are thickened, the tangential walls being thicker than the radial walls. Many of the cells of the collenchyma are filled with tannin; others contain crystals of calcium oxalate. The parenchyma cells of the cortex are quite uniformly oval in cross section and their walls are thicker than is typical for cortical parenchyma cells. The cells of the innermost layer of the cortex are smaller and thinner-walled, but otherwise similar to the other cortical cells. The question arises as to whether or not this layer is an endodermis. It forms a distinct boundary between the cortex and the stele. Its cells usually contain starch; single grains in the young parts of the stems, large complex ones in the more mature regions. No thickenings of the nature of Casparian strips or dots were observed. This layer will be referred to as the endodermis. The pericycle consists of many rows of cells which become sclerenchymatous opposite the vascular bundles, and remain parenchymatous across the primary rays.

The vascular bundles are distinct and vary considerably in size. Three leaf traces "leave" the ring of bundles just below each node and "pass into" the petiole of the leaf. Leaf traces extend lengthwise for many internodes as individual bundles before they fuse with neighboring bundles in the vascular ring. The primary tissues comprising the bundles are remarkably similar in the two roses.

Cambial activity begins early. In comparable regions of the stems there is always more secondary xylem in the American Pillar than in the Dorothy Perkins. This feature accounts largely for the fact that American Pillar canes have a greater diameter and are always more rigid and woody than those of Dorothy Perkins.

The primary rays between the vascular bundles are multiseriate (one to six cells wide). The interfascicular cambium across the rays adds to the radial dimension of the rays by producing, toward the outside, cells which remain parenchymatous and, toward the inside, cells which become thick-walled and pitted. An uninterrupted ray may extend longitudinally through the stem for many internodes. The secondary rays are usually uniseriate. They consist of thick-walled pitted cells in the region of the secondary xylem and parenchymatous cells in the region of the secondary phloem. Starch is abundant in those cells of the ray which lie inside the cambium.

A slight difference in the structure of the mature canes of the two roses appears in the proportion of the two types of cells which constitute the pith. Small thick-walled cells, like those in the rays, occur bounding the

pith region and scattered in groups throughout the pith. These groups extend vertically in rows. They are separated by large thin-walled, empty cells, which are larger in American Pillar than in Dorothy Perkins. The groups of small cells are, therefore, closer together and the pith region is smaller and more compact in Dorothy Perkins than in American Pillar.

The petioles of the leaves girdle the stem for half its circumference, i.e., the petioles have wing-like stipules on either side. This is true of both roses.

At any particular time during the first season's growth, the average buds on American Pillar canes are larger and the bud scales are longer, thicker, and more numerous than those of Dorothy Perkins. Most of the cells of the outer scales are filled with tannin while others contain crystals of calcium oxalate. No differences in the structure of the stem tips of the buds of the two roses were noted except that the pith region is larger and the size of the pith cells greater in American Pillar.

When placed in water the buds on cuttings enlarge. The buds of Dorothy Perkins usually develop into shoots more rapidly than those of American Pillar.

A transverse section taken a few internodes back from the tip of a young developing shoot of American Pillar (Fig. 1) shows young leaves half sheathing the stem. Each leaf contains three vascular bundles. The three leaf traces in the cortex pass into the leaf at the next node above this section. The epidermis has a very thin cuticle and the walls of collenchyma cells are just beginning to thicken. The pith consists of parenchymatous cells not yet fully differentiated. The vascular cylinder consists of a large number of bundles (in this case about 54) which vary in size, some being single while others seem to be made up of several bundles joined together. The bundle marked *a* in Figure 1 is shown in detail in Figure 4 B. The endodermis (*e*) is plainly evident because of the presence of starch grains, and the pericycle (*p*) is just beginning to differentiate. The primary phloem and xylem appear in three groups. The bundles are separated by rays two to four cells wide, across which the interfascicular cambium is formed later.

Transverse sections taken a comparable distance back of the tip of young shoots of Dorothy Perkins are very similar to American Pillar in structure and arrangement of tissues. Except for the smaller diameter and the smaller number of bundles in the vascular cylinder it would be difficult to distinguish between them.

The bases of the shoots on cuttings of the two roses, however, show a distinct difference in structure. The under, or abaxial, side of a shoot on a Dorothy Perkins cutting begins to swell just above the place of attachment to the main stem. The swelling may continue around the branch in both directions until it completely surrounds the developing shoot. Adventitious roots arise from this swelling, forming a halo about the base

of the shoot. When the swelling is confined to the under side of the shoot, the adventitious roots appear only from the under side (1, Fig. 5). Occasionally no swelling develops and no roots arise from that shoot. In American Pillar cuttings no swelling and no adventitious roots appear on the bases of shoots.

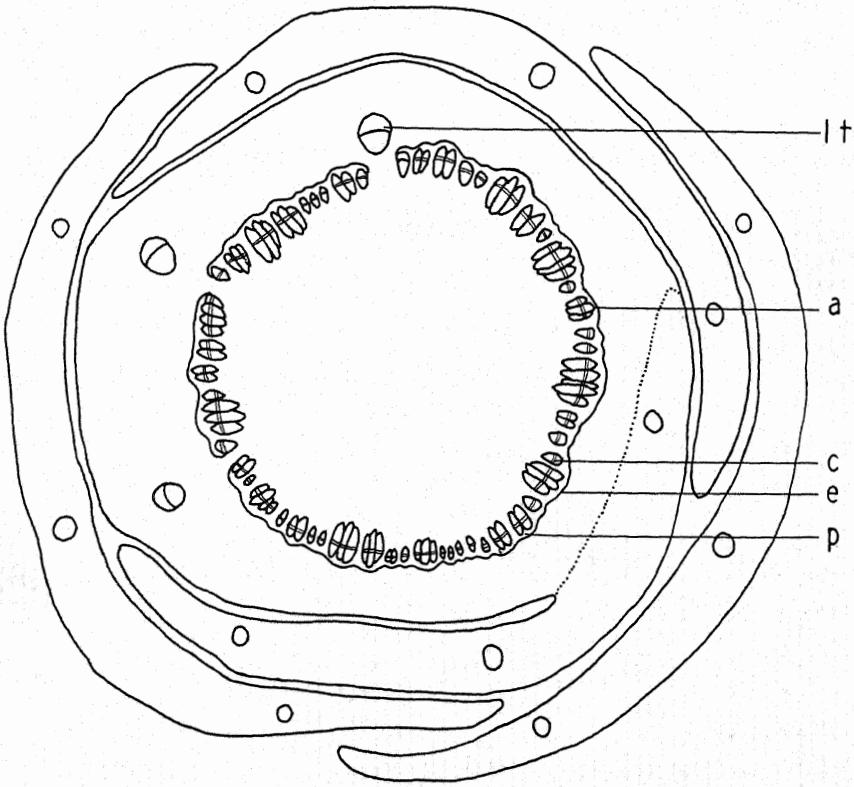


FIGURE 1. Diagram of a transverse section a few internodes back from the tip of a young shoot on a cutting of American Pillar rose. *e*, endodermis; *p*, pericycle; *c*, cambium; *lt*, leaf trace; *a*, a vascular bundle, the detailed structure of which is shown in Figure 4 B.

A study of the steps in the formation of the swelling at the base of shoots of Dorothy Perkins and a comparison of the bases of Dorothy Perkins shoots with and without swellings show that the enlargement is due to an extraordinary activity of the cambium.

The primary tissues are fairly mature in the basal region of the shoot and secondary tissues have been added by the cambium. In an early stage in the formation of the swelling, the vascular bundles become larger (radially) on the lower side than on the upper side of the shoot. This is shown in Figure 2 A. The increase in size is due to the addition of secondary

tissues, chiefly outside of the cambium. As the swelling continues around the shoot, this radial enlargement of the vascular bundles also continues (Fig. 2 B). When the swelling has completely encircled the base of the shoot the vascular cylinder appears as in Figure 3 B. If, as is rarely the case, the swelling is not produced, no such extraordinary increase in the vascular tissues occurs.

A comparison of the structure of comparable regions of the bases of shoots on American Pillar and Dorothy Perkins cuttings is shown in Figure 3 A and B. There is a small accessory bud on each side of the main bud, one a little lower than the other. These buds do not develop unless the

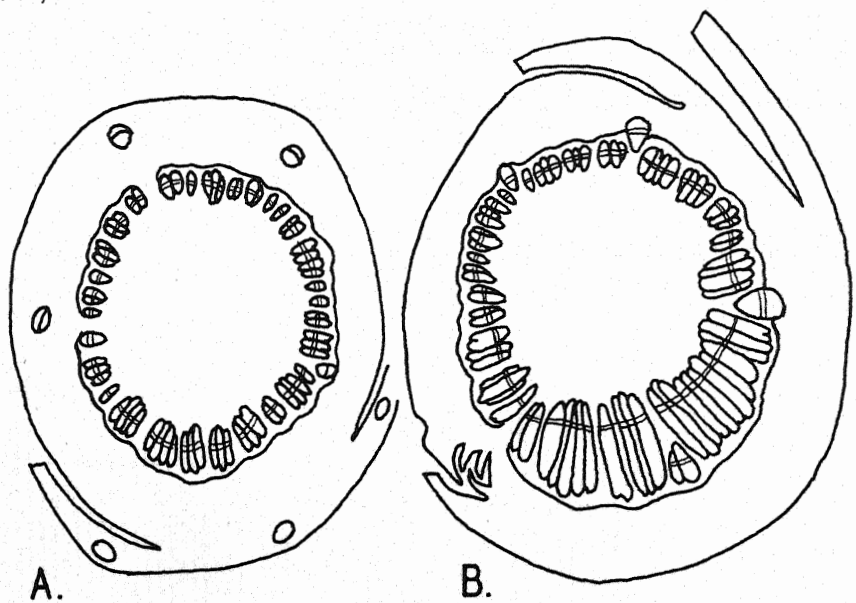


FIGURE 2. Transverse sections, showing two stages in the formation of the enlargement at the bases of shoots on cuttings of Dorothy Perkins rose. The enlargement is due to an extraordinary formation of secondary tissues, especially outside the cambium. A photomicrograph of a section through the fully formed enlargement is shown in Figure 3 B.

main bud is injured or killed. The sections of shoots shown in Figure 3 A and B, were cut through these accessory buds. The average shoot of American Pillar is larger than that of Dorothy Perkins, but in these figures the diameters appear to be about the same because the shoot of Dorothy Perkins was cut through the swelling. There is more pith in American Pillar but the vascular cylinder is narrower in radial dimension. The amount of xylem is approximately the same in both stems but there is about twice as much vascular tissue outside the cambium in Dorothy Perkins as in American Pillar. The pericycle and endodermis are immature but can be recognized.

The structure of the small bundle marked *x* in Figure 3 A is shown in Figure 4 A and the one marked *y* in Figure 3 B is shown in Figure 4 C. The bundle from the vascular cylinder of Dorothy Perkins is much larger, radially, than that of American Pillar. The difference in size of the two bundles is due to the greater size of cells and to a greater amount of secondary tissue produced in Dorothy Perkins. There is more of this tissue outside the cambium than inside, and much of it is parenchymatous.

The swelling on the bases of Dorothy Perkins shoots is, therefore, due to an extraordinary development of secondary tissue, especially outside the cambium, much of which does not immediately become xylem and

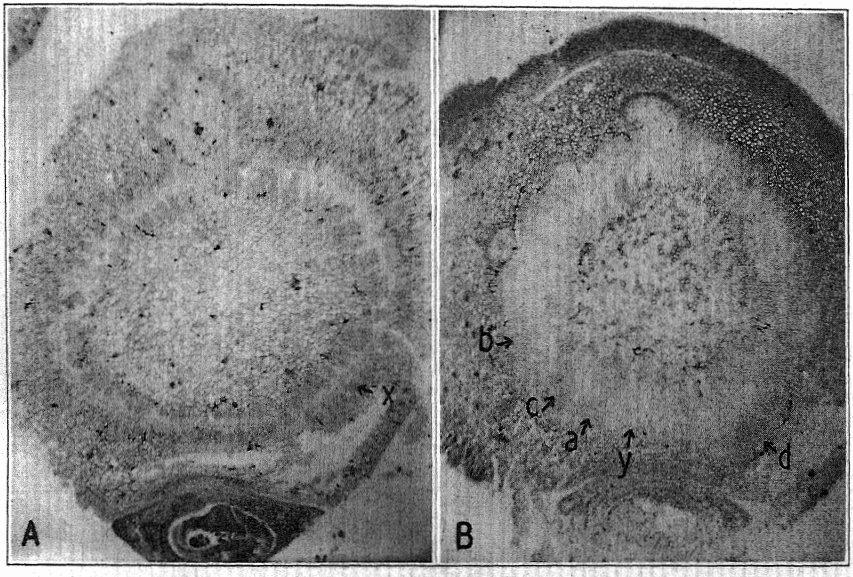


FIGURE 3. A and B through comparative regions of shoots of the same age. The vascular cylinder is wider (radially) in B than in A. (A) Transverse section through the base of a young shoot, attached to a piece of cane, of American Pillar. *x*, a typical vascular bundle, shown in detail in Figure 4 A. (B) Transverse section through the swelling at the base of a young shoot of Dorothy Perkins. *a*, *b*, *c*, and *d*, four primordia of roots, lettered in the order of their age, beginning with the youngest. *y*, a typical vascular bundle, shown in detail in Figure 4 C.

phloem. The cells of this undifferentiated region are arranged in fairly regular radial rows. They are large and thin-walled, with a central vacuole, a small amount of cytoplasm, and a fairly prominent oval or hemispherical nucleus which lies pressed against the cell wall (Fig. 4 C). An occasional mitotic figure indicates that these cells are capable of division.

The cambium is sometimes difficult to identify as a distinct layer of cells. In cross sections of shoots of Dorothy Perkins it appears as an ir-

regular line arching outward across a bundle and dipping inward across a ray. This can be seen in Figures 4 C and 5 C. In stems of American Pillar

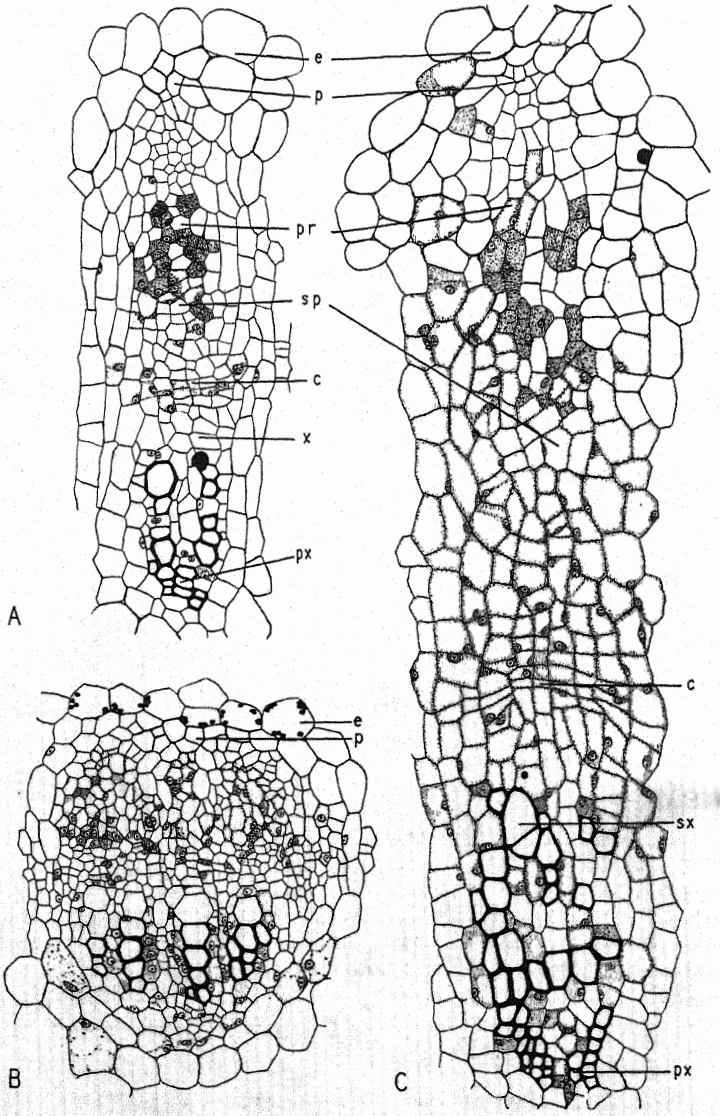


FIGURE 4. (A) Vascular bundle, *x* in Figure 3 A, from cross section taken near base of shoot of American Pillar. (B) Vascular bundle, *a* in Figure 1, from cross section taken near tip of shoot of American Pillar. (C) Vascular bundle, *y* in Figure 3 B, from cross section taken near base of shoot of Dorothy Perkins. Note the large amount of secondary tissue, especially secondary phloem parenchyma, as compared with A. Adventitious roots arise from this tissue. *e*, endodermis, or starch sheath; *p*, pericycle; *pr*, primary phloem; *px*, primary xylem; *sp*, secondary phloem; *sx*, secondary xylem; *c*, cambium. All drawings ×350.

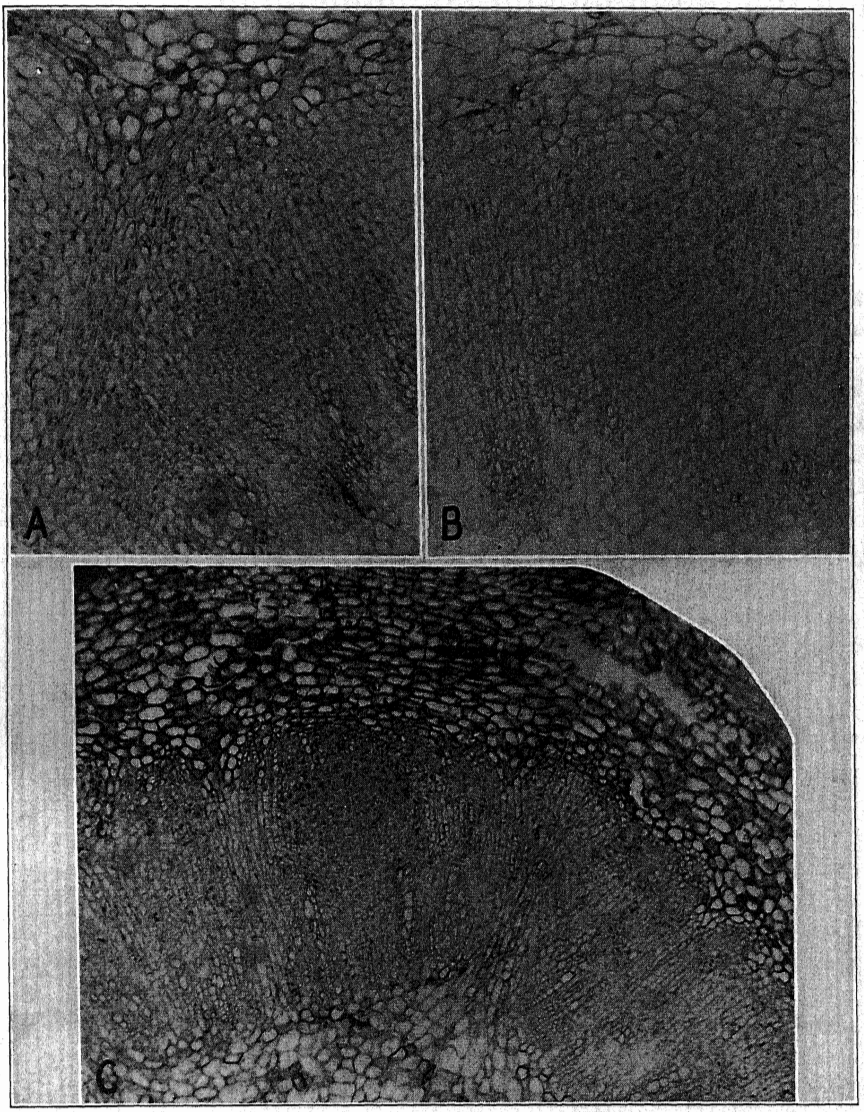


FIGURE 5. Portions of the vascular cylinder from cross sections of bases of shoots of Dorothy Perkins, showing root primordia. (A) Young root primordium originating in the secondary phloem parenchyma of a vascular bundle. The pericycle and the primary phloem are pushed aside by a larger root, a portion of which can be seen at the extreme left. (B) A young root primordium lying in a ray, within the pericycle and on a line joining the secondary phloem regions of adjacent bundles. (C) A much older root primordium, not yet differentiated into root tissues, lying entirely between the primary phloem and the cambium. The cells adjacent to the primordium are pushed slightly out of place by its enlargement.

the cambium is usually very prominent (Fig. 3 A). Here there is only a small amount of undifferentiated tissue on either side of the cambium. The tissue inside the cambium may be considered as undifferentiated secondary xylem and that outside the cambium as undifferentiated secondary phloem. The question arises as to what to call the tissues in similar positions in stems of Dorothy Perkins. Longitudinal sections show that the cells on either side of the cambium are not much elongated, that they are arranged in regular horizontal rows and that they are parenchymatous. It is assumed, therefore, that the tissue inside the cambium is secondary xylem parenchyma and that outside the cambium, secondary phloem parenchyma.

Origin and development of adventitious roots. In the parenchymatous secondary tissue of the vascular cylinder at the base of shoots on pieces of canes of Dorothy Perkins rose one finds, singly and in small groups, cells which contain dense cytoplasm, with several small vacuoles rather than a large central one, and a large, spherical nucleus. The nucleus usually lies in or near the center of the cell. A large nucleolus and much chromatin appear in the nuclei. These groups of cells are conspicuously different from the neighboring cells; they are definitely meristematic (Fig. 5 A, B and 7 A). Those cells which undergo the changes described above are considered the initials of a root primordium since all stages between such groups of cells and fully differentiated roots have been found.

All of the initial cells appear to be similar to each other. The cytoplasm becomes more dense and the nucleus more enlarged. The diameter of the nucleolus becomes two to four times that of the nucleoli in cells outside of the group. This is evident in Figure 6 A. The nucleolus is surrounded by a large clear area, the nuclear sap, and the chromatin lines the nuclear membrane. Soon the cells begin to divide and the group may be considered a primordium of a root. The group is approximately spherical at first. This is shown in a series of sections of the primordium which appear as slices of a sphere placed in a row in the order removed. The primordium is not bounded by a distinct layer of cells, but is obvious because of the marked contrast between the structure of its cells and the structure of the neighboring cells. There is no organization which would indicate which cells will develop into the various tissues of a root. The primordium increases in size by the division and by the enlargement of the cells of the group, and by the addition of cells adjacent to the group. That all cells of a primordium are capable of division is evidenced by the fact that nuclei in various stages of division are found scattered throughout any section of the primordium. Only a few dividing cells were seen in any one section, probably because none of the material happened to be fixed at a time when most of the cells of a group were dividing. Dividing cells are seen in Figure 7 B, C, and D.

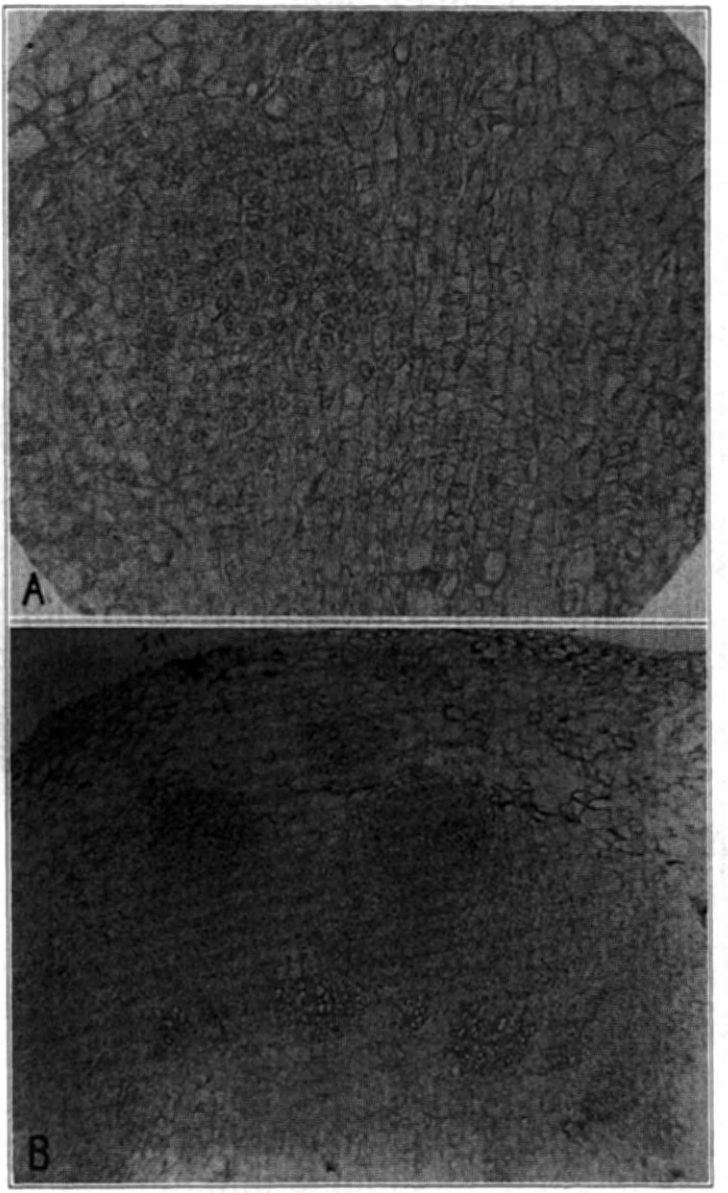


FIGURE 6. (A) A root primordium, enlarged to show the dense cytoplasm and large nuclei and nucleoli in cells of the primordium as compared with other cells of the vascular cylinder. This primordium is also shown in Figure 7 D. (B) Portion of the vascular cylinder of Dorothy Perkins shoot showing two large root primordia pushing out into the cortex. The endodermis still surrounds the primordia, neither of which is as yet differentiated into root tissues. A leaf trace is seen in the cortex.

FIGURE 7. Portions of the vascular cylinder from bases of shoots of Dorothy Perkins, showing origin and early development of root primordia. (A) Young root primordium originating in secondary phloem parenchyma of a bundle. (B) Slightly older root primordium arising inside of the pericycle and outside of the cambium of a bundle. (C) A young primordium, showing neighboring cells becoming meristematic. Several tracheids already differentiated. (D) An older root primordium which originated beside a vascular bundle, chiefly in the ray. A photomicrograph of this primordium is shown in Figure 6 A. All drawings ×150. Letters have the same meanings as in Figure 4.

Cells adjacent to a primordium begin to appear intermediate in structure between mature parenchyma cells and truly meristematic cells. When the cytoplasm of the neighboring cells has increased in amount and the nuclei and nucleoli have enlarged, these cells become a part of the primordium (Fig. 7 B, C, and D).

Consecutive stages in the enlargement of the primordia are shown in the successive illustrations of Figure 7 and in Figures 5 C and 6 B. Figure 6 A is a photograph of a section of the same root shown in Figure 7 D. As a primordium increases in size there may be considerable crowding of the adjacent tissues in the vascular cylinder (Fig. 5 C). The primordium in this figure is fairly large, but it still lies entirely within the vascular cylinder. It is beginning to push apart two patches of primary phloem and pericycle.

Soon the primordium begins to elongate along a radius of the stem and push outward (Fig. 6 B). The primary phloem and pericycle may be incorporated or pushed aside. The endodermis is stretched and increased in circumference by cell divisions, thereby covering the end of the primordium for a time, but it is eventually broken or dissolved. A space ahead of the more mature primordium indicates that it digests its way through the cortex.

By the time the primordium has extended about half way through the cortex it begins to differentiate into the regions and tissues, including the root cap, which make up the tip of a young root. The root increases in length and emerges at right angles to the long axis of the branch. It then turns downward.

Differentiation of xylem to connect with the xylem of the root may begin very early, as shown in Figure 7 C, or it may be delayed until differentiation of tissues begins in the root primordium.

The position of the intial groups of cells which give rise to a root primordium will now be considered. They lie in the vascular cylinder in tissue which is outside the cambium but was produced by it. This is shown in Figure 7 B. As stated above, the cambium is sometimes difficult to locate as a single definite layer of cells completely around the stem because there is so much immature tissue in the cambium zone. However, in cases where a distinct cambium is evident, the initial cells of the primordium always lie outside it. The primordium lies within the primary phloem, that is, the endodermis, the pericycle, and the primary phloem can be seen outside the young primordia of roots (Fig. 7 B). As the primordium enlarges, all the cells, including the cambium, in the vicinity of the original group may become a part of the root. The roots, therefore, originate from certain parenchymatous cells which are, by reason of their position, secondary phloem parenchyma.

A primordium may lie on a radial line between the primary xylem and the primary phloem, that is, in a vascular bundle (Figs. 5 A and 7 A and

B). Sometimes the primordium lies between two bundles, that is, in a ray (Figs. 5 B and 7 D). In such a case the primordium lies outside the interfascicular cambium and inside the pericycle, that is, on a circle joining those primordia which originate within the bundles. Primordia may occasionally be found in the outer part (phloem) of leaf traces, either before they have "left" the vascular cylinder or when they are "traversing" the cortex.

Primordia usually appear first on the lower side of a young branch and others are then formed around the branch in both directions. There is no definite order, however, in the time of origin of the roots. In some transverse sections all the primordia are of about the same age, while in others very young primordia are interspersed among fully differentiated roots. In Figure 3 B there are root primordia lettered in the order of their age. In longitudinal sections the oldest roots may be nearest the base of the branch, but this is by no means the general rule. Young and old roots appear promiscuously throughout the whole rooting region.

In the case under consideration, therefore, the primordia of roots originate from parenchymatous secondary tissue of bundles and rays and from leaf traces. The primordia begin as small meristematic groups of cells which increase in size by cell division and by incorporation of surrounding cells. They do not differentiate into the tissues of roots until they have pushed out into the cortex.

DISCUSSION

The comparison of the rooting behavior of shoots attached to pieces of canes of the two roses is striking in that the roots appear at the bases of shoots of Dorothy Perkins and not at the bases of shoots of American Pillar (1, 5, 11). In the present study the pieces of canes were placed in water and kept in a saturated atmosphere, but the same rooting response occurs if the pieces of canes are planted in sand or a mixture of peat moss and sand (5).

Swellings were noted at the bases of all shoots (attached to canes) of Dorothy Perkins which rooted and no such swellings appeared at the bases of shoots of American Pillar in tests made between December and April. Anatomical studies were made only of material collected during March and April. During this time at least, there is a specialized region of rooting in Dorothy Perkins shoots which is absent in American Pillar shoots. This region appears as a swelling which is formed by an unusual activity of the cambium in producing parenchymatous secondary tissue. No such activity of the cambium occurs in the comparable region of American Pillar shoots.

The problem of why rooting occurs on shoots attached to canes of Dorothy Perkins and does not occur on shoots attached to canes of Amer-

ican Pillar is merely pushed a step further back by this investigation. What causes the extraordinary activity of the cambium which precedes or accompanies the origin of roots in Dorothy Perkins or what prevents this activity in American Pillar?

Two possible answers previously suggested to explain the difference in rooting response might be applied to this question. In the case of the very young shoots, two and one-half to three cm. long, used in this study the leaves had just begun to unfold when roots appeared and the developing shoots and roots were dependent on reserve food stored in the canes. Further evidence on this point was furnished by the fact that etiolated shoots rooted as well as shoots in the light. The amount of reserve starch in Dorothy Perkins canes was in every case greater than that in American Pillar canes (1). The activity of the cambium in Dorothy Perkins may be correlated with the high content of starch in the canes.

According to Hitchcock and Zimmerman (5) rooting from bases of current season's shoots of American Pillar rose "was practically prevented by the presence of a mallet piece," that is, of a piece of the cane. This implies the presence of an inhibiting influence in the cane. Evidence for this point is furnished by the fact that both roses will root from the current season's shoots if removed from the canes by cuts made exactly at the base and placed in rooting conditions. These shoots were cut between May and August when they were three to five inches long and had made considerable growth of stem and leaves. The matter of reserve food is, therefore, not so important in this case.

It would be interesting to study anatomically the rooting of shoot cuttings removed from the canes to discover whether or not the changes resulting in their rooting are similar to those occurring in shoots attached to the canes.

It seems to be impossible at present to make generalizations about the tissues involved in the initiation of adventitious roots from stem cuttings. The cases recently investigated may be summarized as follows:

Herbaceous plants
- Young stems of *Portulaca oleracea* L. (Connard and Zimmerman, 3)
  - In the medullary rays, from interfascicular cambium
  - Within the bundle, tissues unknown (pericycle not involved)
  - Within the pith
- Young stems (internodes) of *Coleus blumei* (Carlson, 2)
  - In the rays, from the pericycle
  - From the bundle, tissues unknown

Woody or semi-woody plants
- Young stems of *Cotoneaster dammeri* Schneid. (Wolfe, 10)
  - In the bud gap, from parenchyma

Young branches of *Ribes nigrum*, *Salix*, and *Populus* (van der Lek, 6)
"In connection with the cambium" as a "continuation of a medullary ray"
Apple varieties which produce burrknots (Swingle, 8)
In the cambium
Stems of *Roripa austriaca* Spach. (Wilson, 9)
From epidermis and cortical cells in the axil of branch bud
Young shoots attached to canes of Dorothy Perkins rose
In the bundles, from secondary phloem parenchyma
In the rays, from ray parenchyma which was produced by the cambium

Adventitious roots may, therefore, originate from actively dividing tissue (cambium); from tissue potentially capable of division (pericycle); from parenchyma recently derived from the cambium; from mature primary parenchyma of the bud gap; from epidermis and cortical cells.

Eames and MacDaniels (4, p. 238) state that adventitious roots develop in the pericycle of stems and roots, or, in older axes, where the pericycle is no longer active, in the secondary phloem. Priestley and Swingle (7) also emphasize the pericyclic origin of roots on young stems, and the origin in the neighborhood of the cambium on older stems.

In the case of Dorothy Perkins rose the adventitious roots arise from branches which are from a few days to two weeks old. The primary tissues of these branches, notably the pericycle, collenchyma, and pith are immature in the region of root formation. Cambial activity has begun but the branches are in every sense young. In this instance, therefore, the adventitious roots arise from secondary phloem in young stems. It may be, however, that even though the pericycle of these stems is immature (not sclerenchymatous) it is "no longer active."

The visible structural changes occurring in the cells which become primordia of adventitious roots are clear. The cytoplasm becomes more abundant, the nucleus and nucleolus increase in size, the amount of cell sap decreases, and then the nuclei and cells divide.

The physiological changes which result in the structural changes in the cells initiating roots are little known. These physiological changes can be investigated microchemically if one can predict which cells in the stem are destined to become meristematic initials of a root. Such a prediction can be made only in cases where the origin of roots is definitely localized. Anatomical studies show that the rooting of shoot cuttings attached to canes of Dorothy Perkins rose and of *Cotoneaster dammeri* (Wolfe, 10) is localized in predictable regions and hence these plants seem to offer excellent material for such studies.

SUMMARY

1. The anatomy of developing canes and buds of Dorothy Perkins and American Pillar roses was studied.

2. American Pillar canes have a thicker cuticle, a larger number of vascular bundles, and a larger amount of xylem and pith than those of Dorothy Perkins. In other respects they are remarkably similar.

3. When given conditions favorable for rooting the shoots attached to pieces of canes of Dorothy Perkins rose root from a basal swelling. An occasional shoot produces no swelling and no roots at its base. American Pillar rose treated in the same way produces no swelling and no roots (except in rare instances) from the bases of shoots attached to pieces of canes.

4. The basal swelling is the result of the unusual development of parenchymatous secondary phloem.

5. Root primordia are initiated by small groups of cells in this parenchymatous secondary tissue, either within a bundle or between two bundles. A root occasionally arises in a leaf trace.

6. The initial cells change in structure from parenchymatous to meristematic and then begin to divide, forming a root primordium.

7. The root primordium increases in size by the enlargement and division of the cells of the initial group and by the incorporation of neighboring cells.

8. Differentiation into tissues of a root begins when the primordium has pushed into the cortex of the shoot.

9. The root elongates, emerges at right angles to the long axis of the shoot, and then turns downward.

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CHANGE IN THE GLUTATHIONE CONTENT OF POTATO TUBERS TREATED WITH CHEMICALS THAT BREAK THE REST PERIOD[1]

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In a previous paper (4) it was shown that the chemicals that are effective in breaking the rest period of potato tubers may be conveniently divided into two groups, namely sulphur compounds and non-sulphur compounds. It was shown that the non-sulphur chemicals increased the pH, iodine titration, and power of the juice to reduce methylene blue. Although it was realized that the iodine titration was an unsatisfactory measure of the sulphydryl content of the juice, due to the presence of non-sulphydryl substances capable of reducing iodine, it was suggested that the non-sulphur chemicals increased the sulphydryl content of the juice. This was supported by qualitative tests which indicated that ethylene chlorhydrin, the most effective non-sulphur chemical, increased the sulphydryl content of the juice as indicated by the nitroprusside test and by the power of the juice to reduce sulphur to hydrogen sulphide (6). It was obvious that further progress necessitated the development of a satisfactory method for estimating the sulphydryl content of tissues. Such a method, based on the reaction of sulphydryl compounds with sulphur to form hydrogen sulphide, has been developed (6). It was also important to find what sulphydryl compound was increased in the tubers by the treatments. This will be shown to be glutathione as has been reported in a preliminary note (5). The present paper deals mainly with the estimation of the glutathione content of potato tubers treated with various chemicals with the object of determining what relationship exists between the effectiveness of a chemical in breaking the rest period and its effect on the glutathione content of the tissue.

ISOLATION OF GLUTATHIONE FROM POTATO TUBERS TREATED WITH ETHYLENE CHLORHYDRIN

Cut pieces of freshly-harvested potato tubers (*Solanum tuberosum* L.) were treated with ethylene chlorhydrin, 40 cc. of the 40 per cent solution per liter, by the dip method and planted in moist soil for five days. After washing free of soil, the pieces were peeled, ground through a food chopper, the juice squeezed out through cheesecloth, and the starch centrifuged down. The juice was boiled to coagulate the proteins, cooled, and filtered through glass wool. Following the procedure of Hopkins (7) the cuprous salt of glutathione was isolated.

In one experiment 50 cc. of saturated lead acetate and 300 cc. of 10 per cent mercuric sulphate in 20 per cent sulphuric acid were added to 1500 cc.

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of boiled, filtered juice. After standing for two hours the precipitate was centrifuged down and suspended in 150 cc. of water. Hydrogen sulphide was passed through this suspension for four hours. The precipitated sulphides were centrifuged down, and the supernatant liquid filtered and acidified with 2 cc. of concentrated sulphuric acid. Hydrogen was then bubbled through the solution until all of the hydrogen sulphide was removed. This took about one and one-half hours. The solution was warmed to 40° C. and a suspension of cuprous oxide was added with care to avoid excess. After standing overnight the precipitated cuprous salt was centrifuged down and washed free from sulphates with water. Six washings were required. The precipitate was then washed twice with absolute alcohol and dried in a vacuum desiccator over sulphuric acid. On adding more cuprous oxide to the supernatant liquid an additional amount of the cuprous compound was obtained and treated in the same manner. The total yield was 870 mg. The copper content was 17.48 per cent; theoretical 17.26 per cent.

The cuprous salt prepared by the above method was suspended in water and decomposed with hydrogen sulphide. After filtering out the cupric sulphide, the hydrogen sulphide was removed in a stream of hydrogen and the filtrate evaporated in a vacuum desiccator over sulphuric acid. When reduced to a small volume, absolute alcohol was added and the glutathione allowed to crystallize. The yield from 395 mg. of the cuprous salt was 190 mg. of crystalline glutathione. An additional 70 mg. was obtained by bringing the mother liquor to dryness. The crystalline fraction was analyzed for nitrogen and sulphur. In the micro Kjeldahl determination, 23.04 mg. of the substance were equivalent to 10.8 cc. of N/50 H_2SO_4. Ignited in a Parr bomb with sodium peroxide, 0.1141 g. of the substance yielded 0.0862 g. $BaSO_4$. The nitrogen content was therefore 13.1 per cent and the sulphur content 10.38 per cent. The calculated values for $C_{10}H_{17}N_3SO_6$ are 13.7 and 10.42 respectively. By the same procedure 970 mg. of the cuprous salt yielded 650 mg. of glutathione. This was re-crystallized and the first fraction, which weighed 275 mg., was analyzed. The nitrogen content was 13.3 per cent and the sulphur content 10.34 per cent.

Using the same procedure, no glutathione could be isolated from the juice of untreated tubers, although qualitative tests indicated a small amount was present. When potatoes that had been stored for several months were treated with ethylene chlorhydrin, the cuprous salt could be isolated, but in smaller yield than with freshly-harvested tubers. In one experiment, one liter of juice yielded 280 mg. of the cuprous salt; in another 600 cc. yielded 60 mg. The copper content of the latter was 17.15 per cent.

ISOLATION OF ADENINE FROM POTATO TUBERS

When an excess of cuprous oxide was added to the supernatant liquid from which the cuprous salt of glutathione had been separated, a small,

rather gelatinous precipitate formed. This was separated, washed with water and alcohol, and dried. About 600 mg. of this precipitate were obtained from the combined experiments representing about 7 liters of juice. This precipitate was decomposed with hydrogen sulphide, freed from hydrogen sulphide in a stream of hydrogen, and evaporated down. The residue was taken up with dilute hydrochloric acid and evaporated again. Microscopic lath-shaped crystals formed. These were filtered off and washed with alcohol acidified with hydrochloric acid. The yield was 106 mg. The nitrogen content of this preparation was 31.7 per cent. This high value automatically excluded a large number of nitrogen compounds and made identification relatively easy. The purine bases as a class have a high nitrogen content, so this possibility was first investigated. Adenine was indicated by a positive Kossel test (1, p. 1021). The nitrogen content of adenine hydrochloride is 40.8 per cent. This discrepancy caused considerable difficulty until it was found that the preparation from potato was the dihydrochloride of adenine, a compound not listed by Abderhalden (1), although its existence was predicted by Wulff (10). The theoretical nitrogen content of adenine dihydrochloride is 33.7 per cent and the theoretical chlorine content 34.1 per cent. The existence of adenine dihydrochloride was shown by its preparation from a known sample of adenine sulphate. This preparation contained 34.0 per cent nitrogen and 33.0 per cent chlorine. Final identification of the potato preparation was made by preparing the gold chloride double salt, $C_5H_5N_5.2HCl.AuCl_3.H_2O$, and comparing its crystals microscopically with the same preparation from a known sample of adenine and with the excellent description given by Wulff (10). The presence of adenine in etiolated potato sprouts has been reported by Schweizer (9). He also reports a higher total purine content in sprouting tubers than in non-sprouting tubers. This suggests that it would be desirable to investigate the amounts of the various purines in treated and untreated potatoes.

QUANTITATIVE EXPERIMENTS

SOURCES OF POTATOES

The experiments were carried out during 1931 and 1932. The potatoes were from the following sources: Irish Cobbler potatoes from South Carolina early in June and from Maryland in July; Irish Cobbler and Bliss Triumph potatoes from the Institute gardens in August and second crop Irish Cobblers from New Jersey in November. During the winter a few experiments were made on immature potatoes harvested from flats in the greenhouse at the conclusion of experiments.

METHODS

The potatoes were treated according to the vapor, dip, and soak methods of Denny (2). The vapor treatments were in all cases of 24-hour dura-

tion and whole tubers were used. Cut pieces were used in the dip method and the treatments were for 24 hours. All the soak treatments were for one hour and cut pieces were used. In all cases one-eye pieces were planted in flats until time for analysis. The pieces not used for analysis were planted in flats, 12 pieces per flat. In the 1931 experiments the effect on growth was estimated by taking the weight of tops after about two months. In the 1932 experiments the effect on growth was estimated by counting the number of sprouts above ground twice each week. At the conclusion of the experiment the number of intact pieces was determined and from these data the number of days for one-half of the intact pieces to come above ground was found. The weight of tops was also taken as in the 1931 experiments, but these values will not be reported since they merely confirmed the results obtained by taking the time for one-half of the pieces to put sprouts above ground.

Analysis for glutathione was made on 50 grams of the tissue by the sulphur-reduction method. This method is based on the reaction of glutathione with sulphur to form hydrogen sulphide. The hydrogen sulphide is converted to methylene blue and determined colorimetrically. The details of this method have been published (6). In all cases the sample taken contained portions from at least 12 pieces. After the samples had been taken for the glutathione determinations, the tissue was ground through a food chopper, the juice squeezed out through cheesecloth, and the pH determined with the quinhydrone electrode.

THE EFFECT OF VARIOUS CHEMICAL TREATMENTS ON THE GLUTATHIONE CONTENT

The effect of various chemical treatments on the glutathione content of dormant potato tubers is shown in Table I. The analyses reported in this table were made on peeled pieces, with the exception of the analyses made on the immature potatoes from the greenhouse. The callus tissue was removed from these by cutting away a thin slice with a scalpel. The results obtained with these are essentially the same as with the peeled tubers. The largest increases in glutathione content were produced by the ethylene chlorhydrin treatments, as was also the largest pH change. With ethylene chlorhydrin the dip treatment was more effective in increasing the glutathione content and pH than the vapor treatment. Ethyl alcohol produced glutathione changes that, with the exception of the 15-day sample of the potatoes from New Jersey, were smaller than those obtained with ethylene chlorhydrin. The response of this lot of potatoes was unusual in many ways. In no other case has a similar increase been observed between the one-week and two-seek samplings. The pH response with these tubers was also exceptional, since in previous (4) and in subsequent experiments ethyl alcohol produced an increase in pH. It will be noted that the pH of

the juice of the untreated tubers of this lot is higher than usually found. Miller (8) also reports exceptional results with this lot of potatoes with regard to their catalase and peroxidase response. Acetaldehyde and chloral hydrate, produced increases in glutathione of about the same magnitude as ethyl alcohol. Hydrogen cyanide produced significant increases in glutathione that were, however, smaller than those produced by the other non-sulphur chemicals. The sulphur chemicals investigated were thiourea and potassium thiocyanate. These have been shown by Denny (2) to be among the most effective chemicals in breaking the rest period of the potato, being equal to ethylene chlorhydrin in this respect. Both potassium thiocyanate and thiourea treatments brought about small but significant increases in the glutathione content of the tubers.

In general, Table I shows that all the chemicals investigated increased the glutathione content of the tubers, but that the non-sulphur chemicals as a group produced the largest increases. Among the non-sulphur chemicals there appears to be a relation between the effectiveness in breaking the rest period and the effectiveness in increasing the glutathione content. Ethylene chlorhydrin, which is the most effective non-sulphur chemical in breaking dormancy, produced the largest increase in glutathione. Judging from the data and also from previous experiments with these chemicals, ethyl alcohol, acetaldehyde, and chloral hydrate are of approximately equal effectiveness in breaking dormancy, but considerably inferior to ethylene chlorhydrin. Hydrocyanic acid appears to be slightly inferior in effectiveness to this group of chemicals. However, it seems to be more effective in breaking dormancy than might be expected from its capacity to increase the glutathione content.

COMPARISON OF VARIOUS CHEMICALS WITH ETHYLENE CHLORHYDRIN

The above experiments suggested that the only way to compare quantitatively two chemicals with respect to their effect on the glutathione content and on growth was to include both chemicals in the same series, using the same potatoes, analyzing at the same time, and following the growth response on a sufficient number of pieces to give a reasonably accurate estimate of the growth response. In most cases 48 pieces were used for following the growth response, the effectiveness of the chemicals being measured by the time for one-half of the intact pieces to show sprouts above ground. In all cases the sample used for glutathione contained portions of at least 12 pieces. The callus layer was cut from the pieces with a scalpel, but they were not peeled since it was thought that peeling might introduce an error. Ethylene chlorhydrin was chosen for the standard chemical with which to compare the others. Since it was known to be more effective than the other chemicals in increasing the glutathione content and in breaking the rest period, suboptimal concentrations were

TABLE I

EFFECT OF VARIOUS CHEMICAL TREATMENTS ON THE GLUTATHIONE CONTENT OF IRISH COBBLER POTATO TUBERS

Source of tubers	Treatment			Days after treatment	pH of juice	Mg. glutathione per 100 g. fresh tissue	Wt. of sprouts after 2 mo., grams
	Chemical used	Amount per liter	Method				
South Carolina	Ethylene chlorhydrin*	40 cc.	Dip	6	7.29	39.6	382
		20 "	"	6	7.13	35.2	139
		10 "	"	6	7.03	30.0	93
		0 "	"	6	6.22	8.6	15
South Carolina	Ethylene chlorhydrin*	1.0 cc.	Vapor	7	6.61	12.3	69
		0.5 "	"	7	6.53	12.0	43
		0.25 "	"	7	6.34	14.3	9
		0.0 "	"	7	6.15	6.4	7
Institute	Ethylene chlorhydrin*	1.0 cc.	Vapor	4	6.42	15.0	291
		0.33 "	"	4	6.24	12.0	164
		0.11 "	"	4	6.31	11.3	67
		0.0 "	"	4	6.14	8.6	47
Institute, same series as above	Ethylene chlorhydrin*	1.0 cc.	Vapor	13	6.39	17.2	291
		0.33 "	"	13	6.15	11.3	164
		0.11 "	"	13	6.15	10.8	67
		0.0 "	"	13	6.07	10.0	47
Greenhouse	Ethylene chlorhydrin*	1.0 cc.	Vapor	6		19.7	130
		0.0 "	"	6		6.1	0
South Carolina	Ethyl alcohol	80 cc.	Dip	7	6.39	11.8	65
		40 "	"	7	6.42	12.0	40
		20 "	"	7	6.32	9.3	13
		0 "	"	7	6.24	6.9	10
New Jersey	Ethyl alcohol	4 cc.	Vapor	6	6.42	13.8	151
		3 "	"	6	6.42	16.7	97
		1 "	"	6	6.46	10.0	0
		0 "	"	6	6.42	7.4	0
New Jersey, same series as above	Ethyl alcohol	4 cc.	Vapor	15	6.56	28.3	151
		3 "	"	15	6.56	28.5	97
		1 "	"	15	6.56	12.5	0
		0 "	"	15	6.61	8.4	0
Greenhouse	Ethyl alcohol	80 cc.	Dip	7		11.1	92
		0 "	"	7		4.4	13
Institute	Acetaldehyde	30 cc.	Dip	5	6.31	14.7	218
		20 "	"	5	6.31	16.2	59
		10 "	"	5	6.17	13.0	86
		0 "	"	5	6.14	7.9	37
Institute	Acetaldehyde	0.5 cc.	Vapor	5	6.49	18.2	177
		0.25 "	"	5	6.20	15.5	119
		0.0 "	"	5	6.05	10.3	49
New Jersey	Chloral hydrate	10 g.	Soak	7	6.39	13.5	139
		5 "	"	7	6.39	12.0	62
		2.5 "	"	7	6.39	9.1	80
		0 "	"	7	6.41	7.6	7
Greenhouse	Chloral hydrate	10 g.	Soak	12		22.1	87
		0 "	"	12		12.3	12

* 40 per cent solution.

TABLE I (*Continued*)

Source of tubers	Treatment			Days after treatment	pH of juice	Mg. glutathione per 100 g. fresh tissue	Wt. of sprouts after 2 mo., grams
	Chemical used	Amount per liter	Method				
South Carolina	Hydrogen cyanide	0.33 g.**	Vapor	6	6.31	10.6	68
		0.16 "	"	6	6.39	10.8	75
		0.08 "	"	6	6.34	9.8	60
		0.00 "	"	6	6.34	8.6	19
Institute	Hydrogen cyanide	0.33 g.**	Vapor	7	6.22	12.3	85
		0.16 "	"	7	6.19	11.8	58
		0.00 "	"	7	5.97	10.8	43
Institute	Hydrogen cyanide	0.33 g.**	Vapor	6		12.8	307
		0.16 "	"	6		11.8	265
		0.08 "	"	6		10.3	115
		0.00 "	"	6		9.8	116
Institute, same series as above	Hydrogen cyanide	0.33 g.**	Vapor	12	6.19	11.0	307
		0.16 "	"	12	6.20	9.8	265
		0.08 "	"	12	6.14	10.1	115
		0.00 "	"	12	6.05	7.2	116
South Carolina	Thiourea	20 g.	Soak	8		13.0	213
		10 "	"	8		12.0	106
		5 "	"	8		10.6	68
		0 "	"	8		7.6	3
Institute	Thiourea	20 g.	Soak	6	6.15	10.3	216
		10 "	"	6	6.19	11.3	275
		5 "	"	6	6.19	10.1	207
		0 "	"	6	6.10	6.1	64
South Carolina	Potassium thiocyanate	20 g.	Soak	7		9.1	275
		10 "	"	7		7.1	413
		5 "	"	7		6.4	187
		0 "	"	7		7.1	13
Institute	Potassium thiocyanate	20 g.	Soak	6	6.09	6.9	
		10 "	"	6	6.10	7.9	
		5 "	"	6	6.05	6.9	
		0 "	"	6	6.05	5.9	
New Jersey	Potassium thiocyanate	20 g.	Soak	6	6.31	5.6	150
		10 "	"	6	6.31	5.9	180
		5 "	"	6	6.29	3.9	108
		0 "	"	6	6.22	3.2	0
New Jersey, same series as above	Potassium thiocyanate	20 g.	Soak	20	6.20	9.1	150
		10 "	"	20	6.20	8.6	180
		5 "	"	20	6.31	7.1	108
		0 "	"	20	6.37	5.9	0
Greenhouse	Potassium thiocyanate	20 g.	Soak	6	5.90	10.1	180
		10 "	"	6	5.81	8.7	—
		5 "	"	6	5.87	8.1	—
		0 "	"	6	5.87	7.6	0

** "Cyanogas," 50 per cent calcium cyanide.

used since it was desired to obtain approximately the same glutathione response with both chemicals and then follow the growth responses. Conversely, it was intended to obtain lots of potatoes with the same growth response and compare their glutathione content. It was also planned to obtain curves by plotting the growth response against the glutathione content in order to see if the curve obtained with the chemical under investigation agreed with the curve obtained with ethylene chlorhydrin.

Ethyl alcohol. The results of an experiment in which ethyl alcohol was compared with ethylene chlorhydrin with respect to its effect on the glutathione content and on the growth of the tubers is shown in Table II.

TABLE II

A COMPARISON OF ETHYL ALCOHOL AND ETHYLENE CHLORHYDRIN WITH RESPECT TO THEIR EFFECT ON THE GROWTH AND ON THE GLUTATIONE CONTENT OF DORMANT IRISH COBBLER POTATOES

24 hour vapor treatments	pH of juice			Mg. glutathione per 100 g. tissue			No. of intact pieces*	Days for ½ above ground
	Days after treatment			Days after treatment				
	5	10	12	5	10	12		
Ethylene chlorhydrin,** 0.6 cc. per l.	6.68	6.54	6.47	24.6	27.0	17.0	48	23
Ethylene chlorhydrin,** 0.3 cc. per l.	6.68	6.37	6.61	27.3	21.9	19.0	45	29
Check, closed container	6.05	6.05	6.09	5.4	6.9	6.1	48	52
Ethyl alcohol, 3 cc. per l.	6.32	6.37	6.37	13.3	14.0	12.3	28	26
Ethyl alcohol, 1 cc. per l.	6.26	6.12	6.36	10.3	13.8	10.8	45	29
Check, closed container	6.19	6.03	6.22	5.4	7.1	7.1	47	66

* 48 pieces were planted in each lot.
** 40 per cent solution.

The potatoes used in this experiment were from South Carolina. The 0.6 cc. per liter chlorhydrin treatment is somewhat less than that usually found most effective, 1 cc. per liter being usually the most favorable, while the 0.3 cc. per liter treatment is decidedly suboptimal. The alcohol treatments were approximately equal to this treatment in promoting the sprouting of the tubers. The most interesting point shown is that although the 1 cc. per liter alcohol treatment and the 0.3 cc. per liter ethylene chlorhydrin treatment were approximately equally effective in promoting sprouting, the tubers treated with 0.3 cc. ethylene chlorhydrin per liter contained about twice as much glutathione. The data in Table II are shown graphically in Figure 1 A where the time for one-half of the pieces to come above ground is plotted against the average glutathione content of the tubers. Ethyl alcohol was more effective than would be predicted from its effect in increasing the glutathione content, basing the prediction on the data obtained with ethylene chlorhydrin.

The results of an experiment with Bliss Triumph potatoes from the Institute garden are shown in Table III. In this experiment, a considerable number of the pieces rotted, but it is believed that this did not seriously

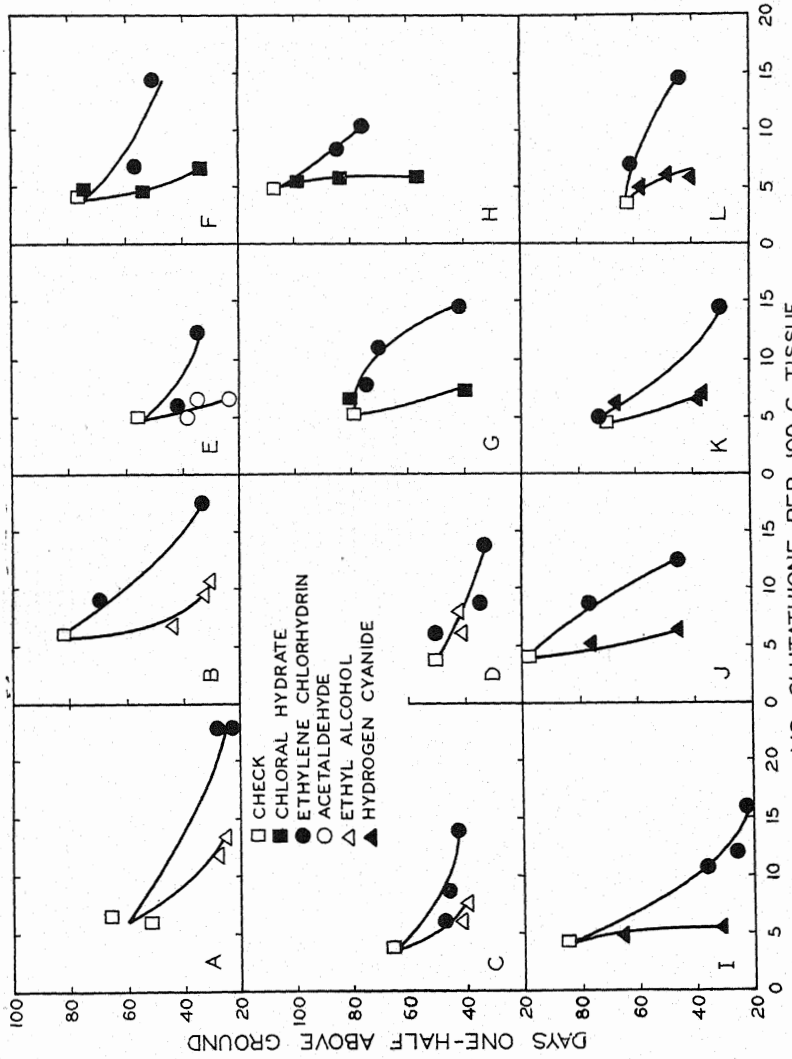


FIGURE 1. A comparison of different chemicals with respect to their effect on the growth and glutathione content of dormant potato tubers.

affect the results. Although the 3 cc. and 2 cc. ethyl alcohol treatments were in this case as effective as the 1 cc. per liter ethylene chlorhydrin treatment, the ethylene chlorhydrin treatment brought about a much larger increase in the glutathione content. It is also of interest that although the 2 cc. per liter alcohol treatment and the 0.15 cc. per liter

chlorhydrin treatment produced approximately the same increase in glutathione, the alcohol series was one-half above ground in 33 days, while the 0.15 cc. per liter chlorhydrin treatment required 69 days. The data in Table III are plotted in Figure 1 B. The glutathione values used are the

TABLE III

A COMPARISON OF ETHYL ALCOHOL AND ETHYLENE CHLORHYDRIN WITH RESPECT TO THEIR EFFECT ON THE GROWTH AND ON THE GLUTATHIONE CONTENT OF DORMANT BLISS TRIUMPH POTATO TUBERS

24 hour vapor treatments	pH of juice			Mg. glutathione per 100 g. tissue			No. of intact pieces*	Days for ½ above ground
	Days after treatment			Days after treatment				
	3	7	13	3	7	13		
Ethyl alcohol, 3 cc. per l...		6.27	6.31		9.1	12.3	41	25
Ethyl alcohol, 2 cc. per l...	6.25	6.24	6.22	5.3	9.3	9.8	35	33
Ethyl alcohol, 1 cc. per l...	6.14	6.17	6.12	5.4	7.1	6.4	37	44
Check, closed container...	6.10	6.03	6.12	5.1	5.7	6.3	25	82
Ethylene chlorhydrin,** 0.15 cc. per l.....	6.22	6.14	6.14	7.9	8.5	9.2	45	69
Ethylene chlorhydrin,** 1 cc. per l.....		6.34	6.39		17.5	17.5	42	33

* 48 pieces were planted in each lot.
** 40 per cent solution.

average of the 7-day and 13-day analyses. The results are essentially the same as those obtained with the Irish Cobbler potatoes from South Carolina, ethyl alcohol being more effective in promoting sprouting of the dormant tubers than one would expect from its effect on the glutathione content.

These results with ethyl alcohol suggested the possibility that some factor in addition to glutathione was effective in the case of ethyl alcohol. If this factor is related to the amount of injury produced, its effect might be minimized by injuring all the treatments equally by cutting away the callus layer at intervals. Other experiments had shown that this cutting produced a significant hastening of germination of tubers otherwise untreated and it was thought that cutting away the callus might produce a condition in which closer agreement would be shown between the glutathione content and the sprouting of the tubers. The results of this experiment are shown in Table IV. The potatoes used were 1932 second crop Irish Cobblers from New Jersey. The treatments were made in the usual manner, but the pieces planted were divided into two lots, and the callus cut off of the pieces of one lot at intervals of 7, 14, and 21 days. The glutathione analyses were made after 7 days and 14 days. The callus had been cut once on the tubers taken from the 14-day sample. The data in Table IV show that the callus cutting hastened the germination in the

check and in two of the chlorhydrin treatments but not with the alcohol treatments. The results of this experiment are shown in Figure 1 C and D. The ethyl alcohol and ethylene chlorhydrin curves agree better in Figure 1 D where the callus was cut than in Figure 1 C where the pieces were

TABLE IV

A COMPARISON OF ETHYL ALCOHOL AND ETHYLENE CHLORHYDRIN WITH RESPECT TO THEIR EFFECT ON THE GROWTH AND ON THE GLUTATHIONE CONTENT OF DORMANT IRISH COBBLER POTATOES, SHOWING THE EFFECT OF REMOVING THE CALLUS

24 hour vapor treatments	pH of juice		Mg. glutathione per 100 g. tissue		Callus not removed		Callus removed	
	Days after treatment		Days after treatment		No. of intact pieces*	Days for ½ above ground	No. of intact pieces*	Days for ½ above ground
	7	14	7	14				
Ethyl alcohol, 3 cc. per l.	5.97	5.95	8.1	7.2	27	41	30	42
Ethyl alcohol, 1 cc. per l.	5.93	5.95	5.8	6.0	21	42	34	41
Check, closed container.	5.92	5.85	3.7	3.6	26	66	31	50
Ethylene chlorhydrin,** 0.08 cc. per l.	5.92	5.87	6.2	5.8	32	48	32	50
Ethylene chlorhydrin,** 0.33 cc. per l.	5.97	5.92	10.2	7.2	30	48	35	34
Ethylene chlorhydrin,** 1 cc. per l.	6.10	5.97	17.2	10.2	31	43	33	33

* 36 pieces were planted.
** 40 per cent solution.

handled as usual. Additional experiments in which the callus was cut would be required to establish this agreement conclusively.

Since preliminary experiments showed that ethyl alcohol was somewhat effective in breaking the dormancy of gladiolus corms, and ethylene chlorhydrin has been shown by Denny (3) to be very effective in breaking the rest period in gladiolus, these chemicals were compared on gladiolus corms (*Gladiolus* sp.) of the varieties Alice Tiplady and Halley. The results are given in Table V. Attempts to isolate glutathione from gladiolus corms have so far been unsuccessful. Therefore it is not known whether or not the sulphydryl compound in gladiolus is glutathione. For this reason the sulphydryl values are given in terms of milli-equivalents of SH per 100 grams of tissue. The analysis of the Alice Tiplady series was made 16 days after the beginning of the treatments, the analysis of the Halley series 13 days after the beginning of the treatments. Since only 16 corms were planted, the days for one-half above ground are estimated somewhat less accurately than with potatoes. The results, however, indicate that the relationship between sulphydryl content and the sprouting response is closer in the case of gladiolus than with potatoes. In the Alice Tiplady series the strongest alcohol treatments and the strongest ethylene chlor-

TABLE V

A COMPARISON OF ETHYL ALCOHOL AND ETHYLENE CHLORHYDRIN WITH RESPECT TO THEIR EFFECT ON THE GROWTH AND ON THE GLUTATHIONE CONTENT OF GLADIOLUS CORMS

Treatment			Alice Tiplady			Halley		
Chemical used	cc. per liter	Days exposed	Milli-equiv. SH per 100 g. tissue	No. of intact corms*	Days ½ above ground	Milli-equiv. SH per 100 g. tissue	No. of intact corms*	Days ½ above ground
Ethyl alcohol.....	4	3	0.026	15	40	0.028	14	46
Ethyl alcohol.....	2	2	0.019	16	73	0.020	15	46
Ethyl alcohol.....	1	1	0.015	16	89	0.013	8	>90
Check, untreated..	0	0	0.018	16	77	0.007	13	>90
Ethylene chlorhydrin**.....	0.33	1	0.018	16	68	0.008	11	46
Ethylene chlorhydrin**.....	3	3	0.033	16	32	0.028	16	33

* 16 corms were treated.
** 40 per cent solution.

hydrin treatments produced approximately the same sulphydryl change and approximately the same sprouting response. In the Halley series, with the exception of the 0.33 cc. per liter chlorhydrin treatment, the agreement between the sprouting response and the glutathione content is fair.

Acetaldehyde. The results of an experiment in which acetaldehyde and ethylene chlorhydrin vapor treatments were compared with respect to their effect on glutathione content and on growth are shown in Table VI. The potatoes used were Irish Cobblers from the Institute garden. Although the 0.33 cc. per liter chlorhydrin treatment produced a much larger increase in glutathione than the 0.26 cc. per liter acetaldehyde treatment, the acetaldehyde treatment sprouted sooner than the chlorhydrin treat-

TABLE VI

A COMPARISON OF ACETALDEHYDE AND ETHYLENE CHLORHYDRIN WITH RESPECT TO THEIR EFFECT ON THE GROWTH AND ON THE GLUTATHIONE CONTENT OF DORMANT IRISH COBBLER POTATO TUBERS

24 hour vapor treatments	pH of juice 7 days after treatment	Mg. glutathione per 100 g. tissue		No. of intact pieces*	Days for ½ above ground
		Days after treatment			
		7	14		
Acetaldehyde, 0.26 cc. per l.....	6.19	7.1	5.9	48	24
Acetaldehyde, 0.13 cc. per l.....	6.10	6.1	6.5	48	34
Acetaldehyde, 0.07 cc. per l.....	6.07	5.1	5.5	47	39
Check, closed container.....	6.00	4.5	4.8	46	56
Ethylene chlorhydrin,** 0.08 cc. per l..	6.05	6.1	5.9	48	41
Ethylene chlorhydrin,** 0.33 cc. per l..	6.22	14.4	10.2	48	34

* 48 pieces were planted.
** 40 per cent solution.

ment. The table also shows that although the 0.13 cc. per liter acetaldehyde treatment and the 0.33 cc. per liter chlorhydrin treatment produced the same sprouting response, the tubers treated with 0.33 cc. per liter of ethylene chlorhydrin contained twice as much glutathione. In Figure 1 E the number of days for one-half of the sprouts to come above ground are plotted against the average glutathione content of the tissue. The chlorhydrin and acetaldehyde curves do not agree, acetaldehyde being more effective than one would predict from its effect on the glutathione content.

Chloral hydrate. Chloral hydrate was investigated since previous experiments had indicated that it was somewhat effective in breaking the rest period of the potato and also increased the glutathione content. It contains three chlorine and three hydroxyl groups, while ethylene chlorhydrin contains only one chlorine and one hydroxyl group. The results of three experiments in which chloral hydrate and ethylene chlorhydrin soak treatments were compared on different lots of Irish Cobbler potato tubers are shown in Table VII. Chloral hydrate and ethylene chlorhydrin are of approximately

TABLE VII

A COMPARISON OF CHLORAL HYDRATE AND ETHYLENE CHLORHYDRIN WITH RESPECT TO THEIR EFFECT ON THE GROWTH AND ON THE GLUTATHIONE CONTENT OF DORMANT IRISH COBBLER POTATO TUBERS

1 hour soak treatments	pH of juice 7 days after treatment	Mg. glutathione per 100 g. tissue		No. of intact pieces*	Days for ½ above ground
		Days after treatment			
		7	14		
Ethylene chlorhydrin,** 3 cc. per l.	6.39	18.6	11.3	48	50
Ethylene chlorhydrin,** 1 cc. per l.	6.22	7.9	5.4	45	57
Check, water	6.17	4.7	3.0	48	77
Chloral hydrate, 1 g. per l.	6.09	5.4	3.7	44	76
Chloral hydrate, 2 g. per l.	6.10	4.9	4.2	47	54
Chloral hydrate, 5 g. per l.	6.27	8.9	4.3	46	33
Chloral hydrate, 5 g. per l.	6.20	7.4	6.9	46	40
Chloral hydrate, 3 g. per l.	6.07	7.4	7.4	47	80
Check, water	6.00	4.8	5.4	48	79
Ethylene chlorhydrin,** 1 cc. per l.	6.03	8.2	7.5	48	75
Ethylene chlorhydrin,** 2 cc. per l.	6.07	12.3	10.0	48	70
Ethylene chlorhydrin,** 4 cc. per l.	6.15	15.6	13.2	48	41
Chloral hydrate, 4 g. per l.		5.4	5.9	39	56
Chloral hydrate, 2 g. per l.		5.1	6.4	43	84
Chloral hydrate, 1 g. per l.		4.6	5.6	46	95
Check, water		3.8	5.4	48	107
Ethylene chlorhydrin,** 1 cc. per l.		7.4	9.3	42	85
Ethylene chlorhydrin,** 3 cc. per l.		10.3	—	36	56

* 48 pieces were planted.
** 40 per cent solution.

equal effectiveness when treatments are made by the soak method. However, the soak method is not an effective way of making ethylene chlorhydrin treatments, the dip or vapor method being much more satisfactory. For the purpose of the experiment it was necessary to keep the conditions as nearly comparable as possible and since chloral hydrate could not be used as a vapor, it was necessary to apply ethylene chlorhydrin by the soak method. Potatoes from South Carolina were used in the first series, potatoes from the Institute gardens in the second series, and potatoes from Maryland in the third series. The number of days for one-half of the pieces to come up are plotted against the average of the 7-day and 14-day glutathione values in Figure 1 F, G, and H. Chloral hydrate is more effective in promoting sprouting than would be expected from its effectiveness in increasing the glutathione content, using the ethylene chlorhydrin curves as a basis for comparison.

Hydrogen cyanide. The results of an experiment in which hydrogen cyanide was compared with ethylene chlorhydrin on Bliss Triumph potatoes from the Institute garden are shown in Table VIII. The data

TABLE VIII

A COMPARISON OF HYDROGEN CYANIDE AND ETHYLENE CHLORHYDRIN WITH RESPECT TO THEIR EFFECT ON THE GROWTH AND ON THE GLUTATHIONE CONTENT OF DORMANT BLISS TRIUMPH POTATOES

24 hour vapor treatments	pH of juice 7 days after treatment	Mg. glutathione per 100 g. tissue		No. of intact pieces*	Days for ½ above ground
		Days after treatment			
		3	7		
"Cyanogas,"** 0.08 g. per l.	6.14	5.1	5.4	56	31
"Cyanogas,"** 0.04 g. per l.	6.07	4.9	4.9	58	66
Check, closed container	6.00	4.6	4.1	57	84
Ethylene chlorhydrin,† 0.08 cc. per l.	6.29	10.4	11.0	60	35
Ethylene chlorhydrin,† 0.16 cc. per l.	6.31	10.4	13.5	59	26
Ethylene chlorhydrin,† 0.32 cc. per l.	6.39	15.2	16.0	58	22

* 60 pieces were treated in each lot.
** 50 per cent calcium cyanide.
† 40 per cent solution.

show that although the 0.08 g. per liter "cyanogas" treatment was approximately as effective as the 0.08 cc. per liter ethylene chlorhydrin treatment, the ethylene chlorhydrin-treated tubers contained twice as much glutathione. The data in Table VIII are plotted in Figure 1 I. The hydrogen cyanide curve and the ethylene chlorhydrin curve do not even approach agreement. Experiments with Irish Cobbler potatoes are reported in Table IX. In the first series the potatoes were obtained from Maryland, in the second series from the Institute gardens. The results of the first

TABLE IX

A COMPARISON OF HYDROGEN CYANIDE AND ETHYLENE CHLORHYDRIN WITH RESPECT TO THEIR EFFECT ON THE GROWTH AND ON THE GLUTATHIONE CONTENT OF DORMANT IRISH COBBLER POTATO TUBERS

24 hour vapor treatments	pH of juice 14 days after treatment	Mg. glutathione per 100 g. tissue			No. of intact pieces*	Days for ½ above ground
		Days after treatment				
		7	10	14		
"Cyanogas,"** 0.08 g. per l.	6.22	5.4	6.6	6.9	36	47
"Cyanogas,"** 0.04 g. per l.	6.10	4.7	5.2	5.4	44	76
Check, closed container	6.03	3.9	4.4	4.4	48	98
Ethylene chlorhydrin,† 0.08 cc. per l.	6.17	8.1	7.9	9.1	48	77
Ethylene chlorhydrin,† 0.33 cc. per l.	6.29	11.3	12.3	13.0	42	45
"Cyanogas,"** 0.16 g. per l.	6.19	8.0		6.0	39	37
"Cyanogas,"** 0.08 g. per l.	6.14	7.2		5.7	47	38
"Cyanogas,"** 0.04 g. per l.	6.14	6.2		5.5	46	68
Check, closed container	6.07	4.4		4.6	47	70
Ethylene chlorhydrin,† 0.08 cc. per l.	6.05	5.0		5.2	47	72
Ethylene chlorhydrin,† 1 cc. per l.	6.24	17.0		12.0	47	31

* 48 pieces were planted.
** 50 per cent calcium cyanide.
† 40 per cent solution.

series are of especial interest, since two cases are presented where tubers of widely different glutathione content showed the same sprouting response. The data in Table IX are plotted in Figure 1 J and K. Hydrogen cyanide is more effective in breaking dormancy than would be expected from its effectiveness in increasing the glutathione content of the tubers.

TABLE X

A COMPARISON OF HYDROGEN CYANIDE AND ETHYLENE CHLORHYDRIN WITH RESPECT TO THEIR EFFECT ON THE GROWTH AND ON THE GLUTATHIONE CONTENT OF DORMANT IRISH COBBLER POTATOES SHOWING THE EFFECT OF REMOVING THE CALLUS

24 hour vapor treatments	pH of juice		Mg. glutathione per 100 g. tissue		Callus not removed		Callus removed	
	Days after treatment		Days after treatment		No. of intact pieces*	Days for ½ above ground	No. of intact pieces*	Days for ½ above ground
	7	14	7	14				
"Cyanogas,"** 0.16 g. per l.	6.07	5.88	6.5	4.7	43	45	42	41
"Cyanogas,"** 0.08 g. per l.	6.05	5.88	6.7	5.0	36	66	47	48
"Cyanogas,"** 0.04 g. per l.	6.03	5.88	5.6	4.5	43	72	42	59
Check, closed container	5.92	5.80	3.2	4.0	42	72	38	62
Ethylene chlorhydrin,† 0.33 cc. per l.	6.10	5.88	6.6	7.7	41	61	48	62
Ethylene chlorhydrin,† 1 cc. per l.	6.17	5.98	15.1	14.0	34	52	49	44

* 48 pieces were planted.
** 50 per cent calcium cyanide.
† 40 per cent solution.

Since fair agreement between the ethyl alcohol and the ethylene chlorhydrin curves was obtained in an experiment in which the callus was cut from the pieces at intervals, a similar test was made in which hydrocyanic acid and ethylene chlorhydrin were compared under these conditions. The results of this experiment are given in Table X. The potatoes used were 1932 second crop Irish Cobblers from New Jersey. The pieces for growth data were divided into two lots and the callus cut from the pieces of one lot at intervals of 7, 14, and 21 days. Cutting the callus did not bring about an agreement between the hydrogen cyanide curve and the ethylene chlorhydrin curve. This is shown in Figure 1 L, where the number of days for the treatments in which the callus was cut to show one-half above ground are plotted against the average glutathione values.

CORRELATION BETWEEN THE INCREASE IN THE GLUTATHIONE CONTENT OF THE TISSUE AND THE CHANGE IN THE pH OF THE JUICE

Since it had been shown that there was a correlation between the increase in the iodine titration of the juice and the increase in pH (4), it was thought desirable to see if there was a correlation between the increase in glutathione and the increase in pH. This was especially necessary, since it was pointed out that the iodine titration was an unsatisfactory measure of the glutathione content of tissues, inasmuch as the iodine reaction is only partly due to glutathione (6). For this reason the pH of the expressed juice was determined in most of the experiments. These data have been included in the foregoing tables. With the exception of one lot of potatoes, second crop Irish Cobblers obtained from New Jersey in 1931, a correlation was evident between the capacity of a chemical to increase the glutathione content of the tissue and its capacity to increase the pH of the juice. In order to show this better the data have been summarized in Figure 2, where the increase in the glutathione content has been plotted against the change in pH. Only the data from the experiments with the non-sulphur chemicals have been included, since little change in either pH or glutathione was produced by the thiourea or potassium thiocyanate treatments. The values obtained from the 1931 second crop Irish Cobbler potatoes from New Jersey have also been omitted, as have also a few points near the origin, which could not be included on account of crowding. There is a good correlation between the increase in glutathione and the increase in pH. This correlation is independent of the chemical used, with the possible exception of hydrogen cyanide, which appears to be slightly more effective in increasing the pH than in increasing the glutathione content. The reason for this correlation is believed to be due to the utilization of sulphuric acid in the synthesis of glutathione by the potato. The effect of ethylene

chlorhydrin, potassium thiocyanate, and hydrocyanic acid treatments on the sulphuric acid content of the expressed juice of different lots of recently harvested Bliss Triumph potato tubers is shown in Table XI. It will be seen

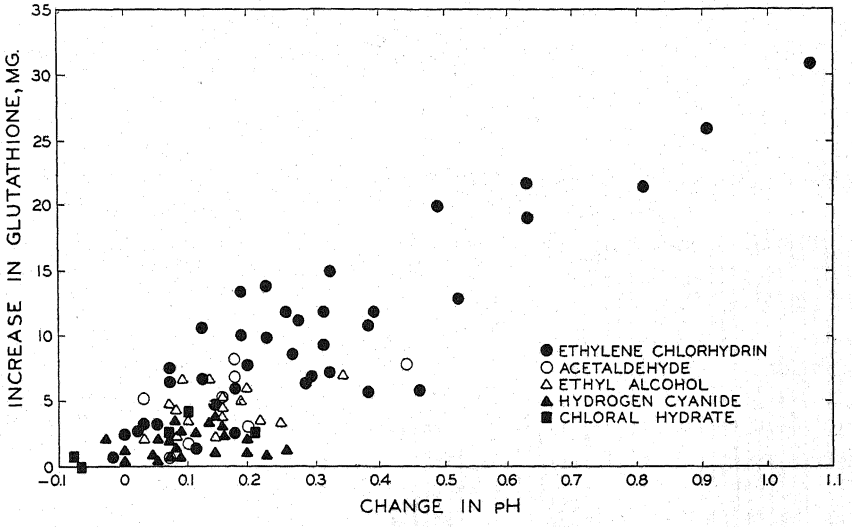


FIGURE 2. The correlation between the increase in the glutathione content of the tissue and the change in the pH of the expressed juice.

TABLE XI

THE EFFECT OF VARIOUS CHEMICAL TREATMENTS ON THE SULPHURIC ACID CONTENT OF THE EXPRESSED JUICE OF RECENTLY-HARVESTED BLISS TRIUMPH POTATO TUBERS

Chemical used for treatment	Method	Days after treatment	SO_4 content of 100 cc. of juice as cc. N/10 H_2SO_4	
			Treated	Check
Ethylene chlorhydrin, 40 cc. per l.	Dip	10	5.7	9.1
Ethylene chlorhydrin, 40 cc. per l.	Dip	11	5.2	9.2
Potassium thiocyanate, 10 g. per l.	Soak	8	12.3	11.3
"Cyanogas," 0.08 g. per l.	Vapor	7	12.0	12.7
Potassium thiocyanate, 10 g. per l.	Soak	6	11.9	11.4
"Cyanogas," 0.08 g. per l.	Vapor	5	10.5	10.3
Ethylene chlorhydrin, 40 cc. per l.	Dip	7	5.2	10.8

that ethylene chlorhydrin treatments decreased the sulphuric acid content of the expressed juice. More detailed experiments on this point will be reported in a subsequent paper. Present indications are that the sulphuric acid change accounts for approximately one-third of the pH change produced by ethylene chlorhydrin.

DISCUSSION

The foregoing experiments show that all the chemical treatments effective in breaking dormancy of potato tubers so far investigated bring about some increase in the glutathione content of the tissue. However, a quantitative relationship could not be shown between the capacity of a treatment to increase the glutathione content of the tissue and its capacity to break dormancy. The experiments also show that the rule that chemicals effective in breaking dormancy are either sulphur compounds or increase the glutathione content of the tissue does not hold quantitatively, since a quantitative relation between the breaking of dormancy and the capacity to increase glutathione does not hold even among the non-sulphur chemicals. It is true, however, that the most favorable treatments with non-sulphur chemicals bring about large changes in the glutathione content of the tissue, ethylene chlorhydrin producing increases of about 200 per cent, ethyl alcohol 100 per cent, acetaldehyde 80 per cent, and chloral hydrate 60 per cent. Hydrocyanic acid, however, can best be classified as an exception, since the most favorable treatments increase the glutathione content only 30 per cent, while the effectiveness of hydrogen cyanide in breaking dormancy approximates that of ethyl alcohol and acetaldehyde. It was at first thought possible that hydrogen cyanide was an exception because of some error that this treatment introduced into the glutathione estimation. Another possibility was that glutathione was formed but for some reason remained in the oxidized form which the method of estimation did not include. In order to check these points, an attempt was made to isolate glutathione from tubers treated with hydrocyanic acid, but none could be isolated, indicating that the increase in glutathione content could not have been large. Another point which indicates that the glutathione increase with hydrocyanic acid was not underestimated is that, as shown in Table XI, hydrocyanic acid treatments did not appreciably decrease the sulphuric acid content of the juice. If it is true that the decrease in the sulphuric acid content of the juice is due to the utilization of sulphuric acid in the synthesis of glutathione, then treatments producing large increases in glutathione should also decrease the sulphuric acid content of the juice. Therefore, the fact that hydrogen cyanide treatments produce no significant decrease in the sulphuric acid content of the juice offers additional support to the correctness of the glutathione analyses of tubers treated with hydrogen cyanide.

Aside from this lack of quantitative agreement between glutathione content and the breaking of dormancy, certain points lend support to the idea that glutathione plays a rôle in the case of the non-sulphur chemicals. The three chemicals found most effective by Denny (2) are thiourea, potassium thiocyanate, and ethylene chlorhydrin. The first two contain sulphur in the divalent form and the third brings about a large increase

in the glutathione content of the tissue. It has also been shown by Miller (8) that a number of other compounds containing sulphur in the divalent form are effective in breaking the dormancy of potato tubers. The oxygen analogs of thiourea and potassium thiocyanate are relatively ineffective (4). Hydrocyanic acid, which at present must be regarded as an exception, has some properties in common with the sulphur chemicals. One of these is its ability to prevent the darkening of potato juice when exposed to air. Another property in common with the sulphur chemicals is its ability to reduce iodine in neutral or slightly alkaline solution. The results obtained with gladiolus corms may also be cited in support of the idea that sulphydryl plays a rôle in the breaking of dormancy by non-sulphur chemicals, since the two chemicals that have been found to break their dormancy also increase the sulphydryl content of the corms.

SUMMARY

1. Glutathione was isolated from the juice of potato tubers that had been treated with ethylene chlorhydrin.

2. Adenine was isolated from potato juice.

3. As determined by the sulphur-reduction method, all effective chemical treatments so far investigated produced a significant increase in the glutathione content of the tissue of dormant potato tubers. However, a quantitative relation could not be shown between the effectiveness of a chemical treatment and its capacity to increase glutathione.

4. In general, non-sulphur chemicals were more effective in increasing the glutathione content of the tissue than sulphur chemicals. Hydrocyanic acid can best be regarded as an exception to the rule that chemicals effective in breaking dormancy are either chemicals containing sulphur in the divalent form or chemicals which produce large increases in the glutathione content of the tissue.

5. Ethylene chlorhydrin and ethyl alcohol treatments of gladiolus corms increased the sulphydryl content of the tissue.

6. There is a correlation between the capacity of a chemical to increase the glutathione content of potato tubers and its capacity to increase the pH of the expressed juice.

7. Ethylene chlorhydrin treatments of potato tubers brought about a decrease in the sulphuric acid content of the expressed juice.

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INITIATION AND STIMULATION OF ADVENTITIOUS ROOTS CAUSED BY UNSATURATED HYDROCARBON GASES

P. W. ZIMMERMAN AND A. E. HITCHCOCK

Initiation and stimulation of adventitious roots was recently accomplished by exposure of growing plants to a pure chemical, carbon monoxide gas (2). Since reporting that work three other chemicals, ethylene, acetylene, and propylene, also were found to be effective. The purpose of the present paper is to report the results obtained from treatment of plants with these three gases.

MATERIALS AND METHODS

The ethylene, acetylene, and propylene used in the experiments were especially made by the Union Carbide and Carbon Corporation or its subsidiaries. According to the analyses furnished by the companies the composition was as follows:

Ethylene		*Acetylene*		*Propylene*	
Ethylene	97.2%	Acetylene	99.8%	Propylene	97.3%
Oxygen	0.5%	Air	0.2%	Propane	2.6%
Absorbed by KOH	0.3%			Ethane	0.1%
Nitrogen	2.0%				

The plants used were grown in pots in the greenhouses. Hardwood cuttings were taken from trees or shrubs and placed in beakers containing about an inch of water. The plants and cuttings were exposed to the gases either under bell jars or in large Wardian cases. Controls were kept in comparable conditions without the gas. The concentrations used varied from 0.0001 per cent to 1.0 per cent.

RESULTS

Out of 27 species and varieties of plants exposed to ethylene, acetylene, and propylene, 15 showed definitely some kind of rooting response (Table I). The list of those showing positive responses is being constantly increased as new species are tested.

The effect of the three different chemicals on plants varied with the species, the age of the tissues, the concentration of the gases, and the length of exposures. The most evident responses were as follows: (a) initiation of roots from young stem tissues, (b) stimulation of preexisting root primordia, (c) root formation on the under side of leaves, (d) change in orientation of roots to gravity, (e) induced root hairs.

The gas was effective over a wide range of concentrations, though the

TABLE I

INITIATION AND STIMULATION OF ROOTS WITH UNSATURATED HYDROCARBON GASES

Species	Gas	Initiation of roots	Stimulation of roots	Especial nodal rooting	Change of orientation to gravity	Roots on leaves	Root hairs induced
Balsam	Acetylene	+		+			
	Propylene	+		+			
	Ethylene	+		+			
Begonia	Acetylene	+	+				
	Propylene	+	+		+		
Bryophyllum	Acetylene	+		+			
	Propylene	+		+			
	Ethylene	+		+			
Coleus	Acetylene	+		+			+
	Propylene	+		+			+
	Ethylene	+		+			+
Cosmos (common)	Acetylene	+			+		+
	Propylene	+			+		+
	Ethylene	+			+		+
Cosmos (sulphur)	Acetylene	+		+	+	+	+
	Propylene	+		+		+	+
	Ethylene	+		+		+	+
Fuchsia	Acetylene	+		+			
	Propylene	+		+			
	Ethylene	+		+			
Galinsoga	Acetylene	+					
Heliotrope	Acetylene	+				+	+
	Propylene	+				+	+
	Ethylene	+				+	+
Hydrangea	Acetylene	+					
	Propylene	+					
Marigold	Acetylene	+	+		+	+	+
	Propylene	+	+		+	+	+
	Ethylene	+	+		+	+	+
Pop corn	Acetylene	+			+		
Tobacco	Acetylene	+			+		
	Propylene	+					
	Ethylene	+			+		
Tomato	Acetylene	+	+			+	+
	Propylene	+	+				+
	Ethylene	+	+				+
Willow	Acetylene		+				
	Propylene		+				
	Ethylene		+				

Note: blank spaces indicate that under the conditions of the experiments the response was negative.

higher the concentration the greater the injury to leaves. The most effective ranges for the different gases were as follows: (a) ethylene, 0.2 per cent to 0.001 per cent; (b) acetylene, 1.0 per cent to 0.1 per cent; (c) propylene, 1.0 per cent to 0.1 per cent. Due to the large number of variables, the optimum concentrations for inducing root initials were not determined.

INDUCED ROOT INITIALS

The following 15 species of plants have been induced to grow roots from young stem tissue: *Begonia semperflorens* Link & Otto, *Bryophyllum pinnatum* Kurz., *Coleus blumei* Benth., *Cosmos bipinnatus* Cav., *C. sulphureus* Cav., *Fuchsia hybrida* Voss., *Galinsoga parviflora* Cav., heliotrope (*Heliotropium peruvianum* L.), *Hydrangea macrophylla* DC., balsam (*Impatiens balsamina* L.), tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L. var. Burley), willow (*Salix babylonica* L.), marigold (*Tagetes erecta* L. and *T. patula* L.), pop corn (*Zea mays* L. var. *everta* Bailey).

The following four species produced roots from leaves: heliotrope, cosmos, tomato, and marigold.

Roots from the Region of Elongation

Tobacco. Ethylene, acetylene, and propylene induced roots to form on young tobacco stems within ten days after the treatment was started. The portion of the stems which produced roots was the region of elongation at the time the experiments started (Fig. 1). Control plants grew occasional roots near the base of the stem but not from the young portion. The treated plants continued to grow in height while in the gas, but the zone of roots did not extend over the newly produced stem. The new leaves were well supplied with starch as shown by microchemical tests. The necessary balance could be restored by growing the plants for a few days under normal conditions, in which case they responded to retreatment by producing a second band of roots (Fig. 2). Three successive bands of roots were produced by tobacco plants handled in this way. The response was consistent enough to show that, grown under normal conditions, there was something about the region of elongation which made it susceptible to the gas. However, regions of elongation formed while the plants were in the gas were not susceptible. No explanation can as yet be offered for this difference.

Hydrangea. Actively growing plants of *Hydrangea macrophylla* were induced to root in 20 to 25 days after treatment with 0.25 per cent acetylene (Fig. 3). During the period of treatment the plants were exposed to the gas for two days at a time and then rested for two days before retreating. With this method only the old leaves were lost during the experiment and some new leaves were produced. The roots came from young tissue just back of the stem tip representing a part of the region of elongation at the time the treatment was started. Neither the new stem tissue

grown after the experiment was started nor the old stem were forced to root. The response to the gas was similar to that of tobacco though the time required was twice as long. As contrasted with tobacco and hydran-

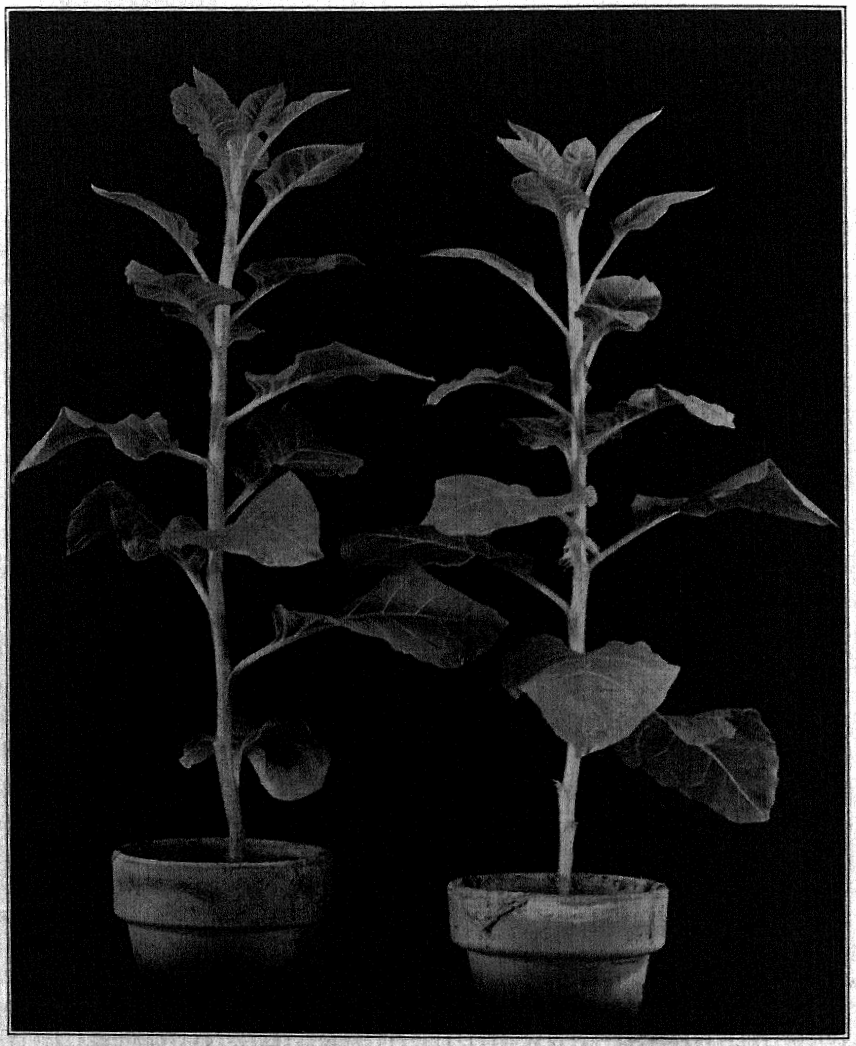


FIGURE 1. Tobacco (*Nicotiana tabacum*) showing initiation of adventitious roots from intermittent exposures to ethylene gas. Left, control plant. Right, plant exposed to 0.1 per cent ethylene as follows: December 23 to 24, December 27 to 29, December 30 to 31. The photograph was taken on January 6, 14 days after the experiment began.

gea, many other species were induced to root, not only from the region of elongation but also from other regions along the stem.



FIGURE 2. Tobacco (*Nicotiana tabacum*) showing root initiation induced by acetylene. Left, control plant. Right, plant exposed to 0.25 per cent acetylene at intervals from November 10 to November 21, then rested for 8 days, preparatory for retreatment at intervals from November 29 to December 15, at which time the photograph was taken. The lower zone of roots appeared on November 21 and the upper on December 10.

Propylene induced roots on hydrangea stems similar to acetylene but the time required was ten days longer. However, since only two plants were used in this experiment the difference might have been due to individual variation.

Hydrangea was not included in the list of plants treated with ethylene.

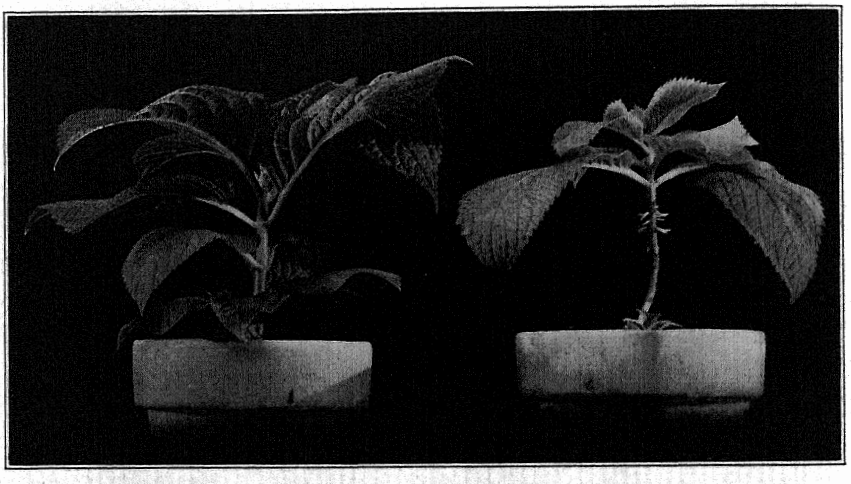


FIGURE 3. Hydrangea showing adventitious roots induced by acetylene. Left, control plant. Right, plant treated at three-day intervals with 0.25 per cent acetylene from October 18 to November 16, when photograph was taken. Roots were first seen on November 10.

Roots Induced Somewhat Generally over the Stem

Coleus. Adventitious roots of coleus plants were induced by ethylene, acetylene, and propylene gases. The first roots usually appeared near the lower nodes within 12 days after treatment started but a few days later internodal rooting was evident from near the tip to the base of the stem. Figure 4 shows the appearance of a coleus plant after 20 days of intermittent treatments with one part of ethylene to 5000 of air. Only occasional roots appeared on control plants and these were near basal nodes.

Tomato. Figure 5, A and B, shows the characteristic response of tomato plants exposed to the three different hydrocarbon gases. A given concentration of gas was effective whether the exposure lasted for one day or ten days. With the long exposures, however, the root initials were induced to arise from the younger tissue, sometimes appearing within a half inch of the growing tip. As compared with continuous exposure, intermittent exposures, as one or two days in gas and an equal number of days of rest, caused less injury to leaves and were equally effective for inducing roots. Ethylene, when used for continuous, long treatment, caused much injury and plants thereafter gave a poor rooting response.

High concentrations caused considerable injury to leaves if the period of exposure was more than 48 hours. With 0.1 per cent ethylene, best rooting responses were obtained with 24 hours of treatment and then 24 hours of rest until the plants had a total of 96 hours in the gas. With 1.0 per cent acetylene and 1.0 per cent propylene, intermittent treatments of



FIGURE 4. Adventitious roots of coleus induced by ethylene gas. Left, control plant. Right, plant treated with 0.02 per cent ethylene for 20 days. First roots appeared after six days at lower nodes. Young tissue near tips of stems produced roots within 15 days.

48-hour periods were most effective. Due to the many variables involved, it has not been possible to find optimum concentrations or periods for exposure.

Tomatoes showed varietal differences in response to gas treatments. For example, Bonny Best rooted readily and frequently control plants

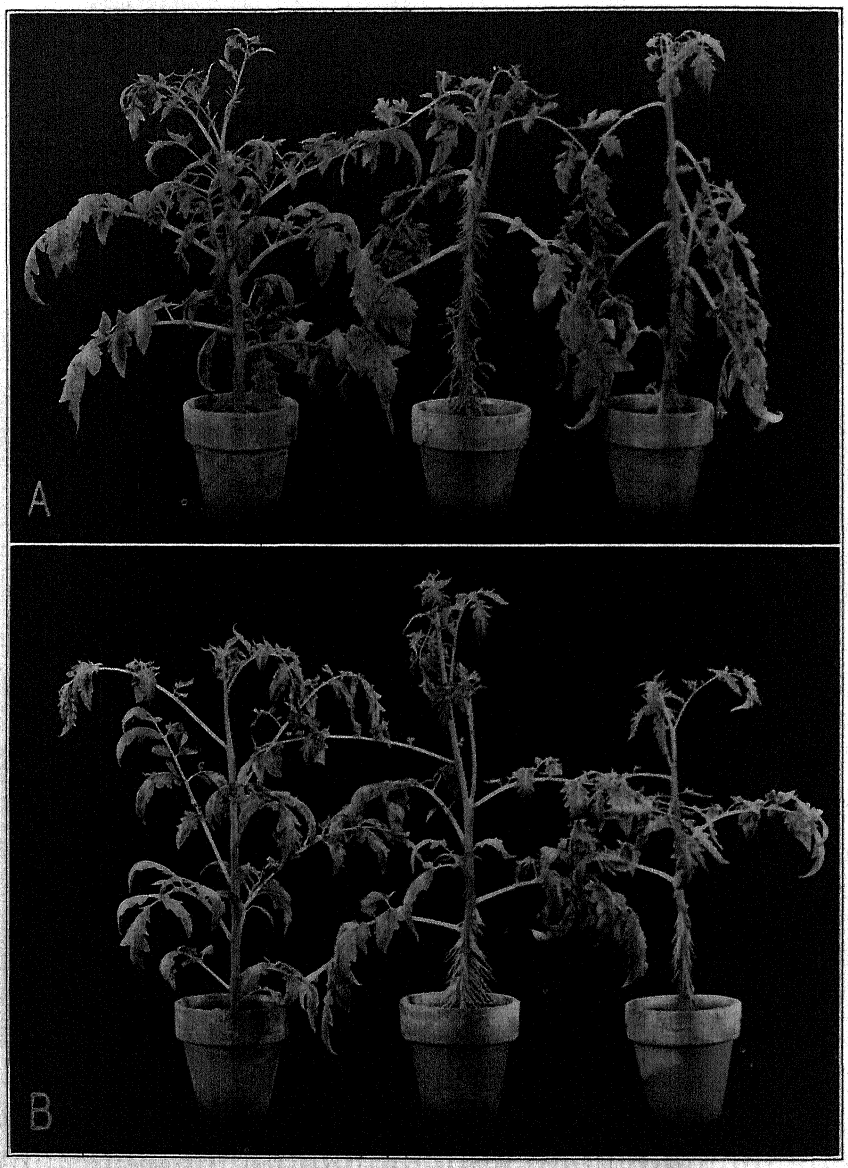


FIGURE 5. Adventitious roots produced by tomato plants after exposure to unsaturated hydrocarbon gases. (A) Left, control. Middle, treated with 0.25 per cent acetylene at two periods, February 14 to 18, and February 20 to 24. Photographed on February 25. Right, treated with 0.25 per cent propylene as described for acetylene. (B) Left, control. Middle, treated with 0.1 per cent acetylene from January 4 to 9, and photographed on January 12. Right, treated with 0.001 per cent ethylene January 4 to 9 and photographed January 12.

showed a considerable number of roots by the close of an experiment. Marglobe rooted with less ease but an occasional check was found with roots. The most satisfactory varieties found for this work were Dwarf Giant and Magnus.

Marigold. The following varieties of marigold were used in the experiments: (a) African marigold, *Tagetes erecta* L., varieties Lemon, Prince of Orange, Lemon Alldouble, Orange Alldouble, Orange Ball, El Dorado, Sulphurea; (b) French marigold, *Tagetes patula* L., variety Tall French.

Among the African types the variety called Orange Ball was the most satisfactory for studying induced rooting by hydrocarbon gases. While the other varieties could be used satisfactorily, control plants frequently formed roots from the lower portion of the stem. Controls of variety Orange Ball seldom rooted during the course of the experiments. French varieties did not form roots unless treated with one of the gases in question.

Individual plants in four-inch pots or groups of five in eight-inch pots were used in the experiments. In height the plants ranged from 5 to 24

TABLE II

COMPARATIVE EFFECT OF THE THREE DIFFERENT GASES FOR INDUCING ADVENTITIOUS ROOTS ON STEMS OF MARIGOLD

Variety and number of plants used	Kind and concentration of gas	Period of exposure to gas in days	Rating according to estimated number of roots	Location of roots along stem in average inches from base
Tagetes erecta var. Lemon Alldouble 5 plants in eachs et	Acetylene 0.2%	3	1st	5.0
	Acetylene 0.1%	3	4th	4.2
	Ethylene 1.0%	3	3rd	3.4
	Ethylene 0.2%	3	2nd	4.2
	Ethylene 0.02%	3	3rd	3.8
	Check		Last*	2.5
Tagetes erecta var. Sulphurea 5 plants in each set	Ethylene 0.02%	3	1st	6.6
	Ethylene 0.2%	3	2nd	6.8
	Ethylene 1.0%	3	2nd	7.1
	Acetylene 0.1%	3	3rd	7.2
	Check		Last*	3.7
Tagetes erecta var. Orange Alldouble 5 plants in each set	Acetylene 0.2%	2	1st	5.6
	Acetylene 0.1%	2	5th	5.3
	Ethylene 0.02%	2	3rd	5.2
	Ethylene 0.2%	2	2nd	5.6
	Ethylene 1.0%	2	4th	5.6
	Check		Last*	2.8

* The word "last" was used to indicate a greater difference than would be inferred by the next figure in the series. Checks usually produced comparatively few scattered roots.

inches. The young plants were considered most satisfactory because they did not form adventitious roots under ordinary conditions. When such

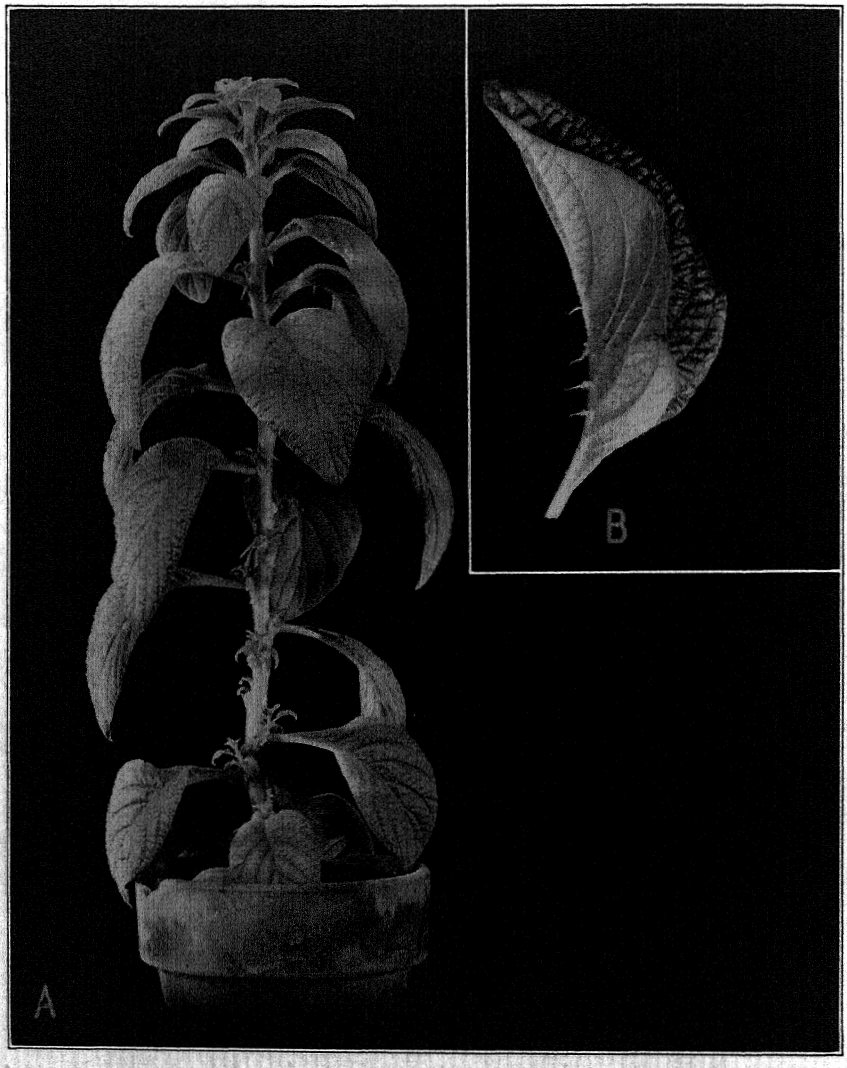


FIGURE 6. (A) Heliotrope plant with induced roots on stem and leaves due to treatment with 0.1 per cent ethylene at two-day intervals from February 7 to 25. (B) An enlarged leaf of heliotrope to show the position of roots induced by treatment with 0.25 per cent propylene at two-day intervals from February 14 to 25.

plants were treated the roots appeared first near the base, then far up the stem within an inch of the tip after five to ten days of intermittent treat-

ments. Table II gives an indication of the effectiveness of short period treatments.

Heliotrope. Actively growing heliotrope, six to ten inches in height, responded to the three gases by producing roots sparsely from a young portion of the stem an inch or more back of the growing point and from the under surface of leaves (Fig. 6). The first roots were usually associated with hypertrophies at the base of leaves but later they appeared promiscuously. Heliotrope response resembled somewhat that of tobacco though the latter produced comparatively many more roots.

Leaves of heliotrope also rooted concurrently with the stems, 10 to 15 days after the first treatment (Fig. 6). The oldest and youngest leaves never rooted. Usually eight to ten leaves over a region of three inches on the stem back of the original tip were caused to initiate roots. Leaves produced by the plant during treatment seldom formed roots.

Heliotrope is especially interesting in comparison with other plants because only a few cells on the stem were susceptible to the gas, thereby producing only a few roots. It is hard to understand how a stem that appears homogenous could have so few cells with the capacity to respond to the gas stimulus.

Cosmos. Cosmos sulphureus produced roots around the nodes when exposed to ethylene, acetylene, or propylene gases. The effective concentration ranges used were as follows: (a) ethylene, 1.0 per cent to 0.02 per cent; (b) acetylene, 1.0 per cent to 0.1 per cent; (c) propylene, 1.0 per cent to 0.1 per cent. No attempts were made to determine optimum concentrations or period of treatment. The ranges given above could probably be extended in both directions.

Pop corn. Pop corn treated for five days with 0.2 per cent acetylene produced prop roots from five nodes above the ground, whereas controls showed a start of roots from only the basal node.

Fuchsia, balsam, galinsoga, begonia, and bryophyllum. All five of these species showed induced rooting of varying degrees when exposed to the three different hydrocarbons, but not enough tests were made to justify making a detailed report at this time.

Rooting of Leaves

During the course of these experiments, the following types were found to root from the lower side of leaves: tomato, cosmos, marigold, and heliotrope. Rooting of leaves was by no means constant but it occurred often enough to justify the conclusion that it was a gas-induced response. The controls never formed roots on leaves. The most nearly constant response occurred when rapidly growing heliotrope plants were treated with any of the three unsaturated hydrocarbon gases. Table III shows the number of leaves per plant forming roots. Figure 6 shows a heliotrope plant from

which rooting occurred on both leaves and stem. The first roots of leaves came from the midrib just below secondary veins, 10 to 15 days after the treatment started. After 20 to 30 days of intermittent treatment, roots frequently arose from secondary veins. The oldest and youngest leaves never produced roots. The most susceptible were on the region of the stem tip at the time the treatment started. In a few cases roots were induced on leaves grown while the plants were in treatment.

TABLE III

ROOTS INDUCED ON HELIOTROPE LEAVES DURING EXPOSURE TO UNSATURATED HYDROCARBON GASES

Number of plants	Period of treatment in days	Concentration of gas in per cent	Number of leaves on plant	Number leaves rooted	Position of leaves on stem
1	30	Ethylene 0.001	20	10	Near region of tip at time treatment started
1	20	"	20	6	"
1	31	"	25	5	"
3	31	"	*	0	
1	14	Ethylene 0.1	15	9	Near middle zone of stem
1	20	"	22	15	Near original tip
1	31	"	28	16	"
2	31	"	*	**	"
3	31	"	*	0	
1	20	Propylene 0.25	23	18	
1	20	"	21	11	
1	20	"	18	6	
2	32	Acetylene 0.25	*	**	
4	32	"	*	0	
8	32	Control	*	0	

* Leaves were not counted.
** Leaves rooted but not counted.

Marigold plants were variable in response, sometimes 75 per cent of the leaves on a plant rooting, and often none. Cosmos and tomato seldom produced roots on leaves.

STIMULATION OF EXISTING ROOT PRIMORDIA

Hardwood Cuttings

Many species normally produce root primordia in the stem cortex without special treatment. These, as a rule, remain dormant unless the stem is removed from the plant and treated as a cutting. Attention was called by van der Lek (1) to such "root germs" in *Salix*, *Ribes*, and *Vitis*.

In an earlier paper (2) it was shown that carbon monoxide stimulated preexisting root primordia. Since that report it has been found also that acetylene, propylene, illuminating gas, and ethylene stimulate latent root primordia. Figure 7 shows comparative root growth of willow cuttings 11 days after a five-day treatment was started. The concentrations of the

gases used in this experiment were not all near the optimum for forcing. The ethylene at 0.1 per cent was far too strong. In later tests 0.01 per cent

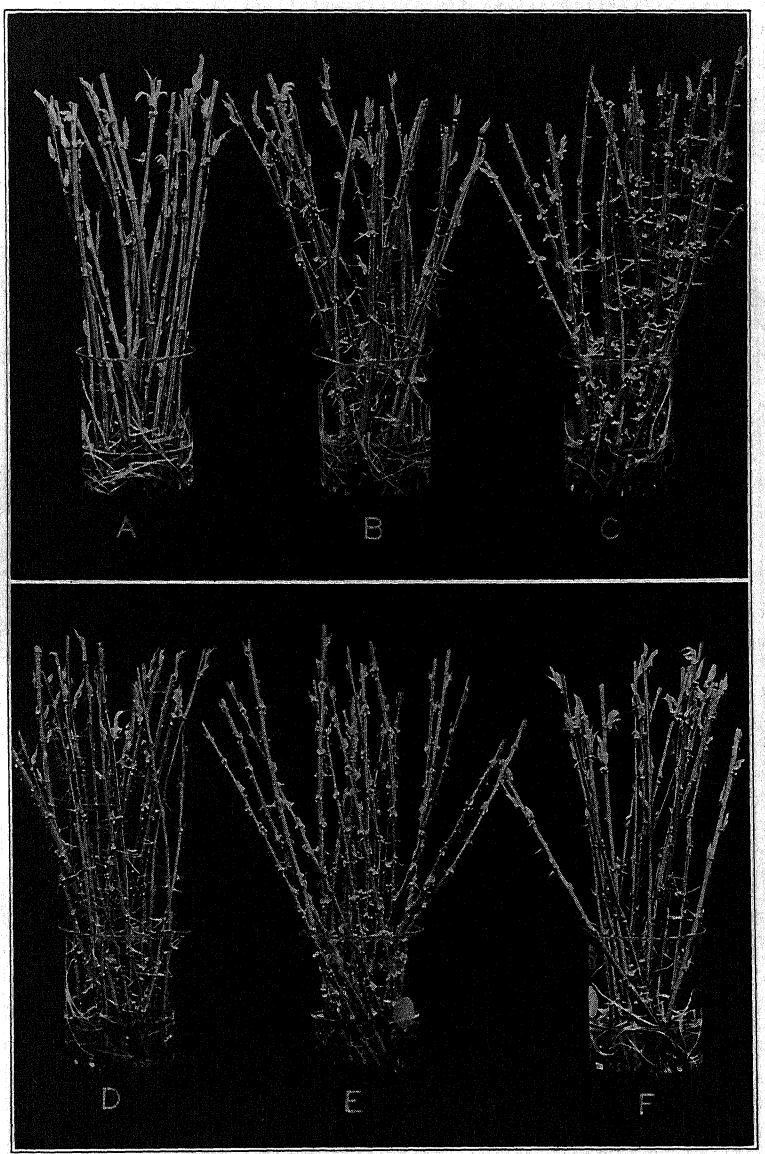


FIGURE 7. Root stimulation from exposure of willow cutting to five different gases from January 26 to 31. Photographed February 2. (A) Control; (B) 1.0 per cent acetylene; (C) 1.0 per cent propylene; (D) 0.1 per cent illuminating gas; (E) 0.1 per cent ethylene; (F) 2.0 per cent carbon monoxide.

ethylene proved to be nearly as effective as propylene and more effective than acetylene or carbon monoxide. The final rating in these experiments based on the estimated number of roots for the best concentrations is as follows: 0.5 per cent propylene, first; 0.01 per cent ethylene, second; 0.5 per cent acetylene, third; 0.1 per cent illuminating gas, fourth; and the control last with roots only on the portion of the stems under water.

In the experiment illustrated in Figure 7 the rating was as follows: propylene, first; illuminating gas, second; acetylene, third; ethylene, fourth; carbon monoxide, fifth. The control produced roots only on that part of the stem which was under water.

Not enough woody types have been tested to determine to what extent these gases can be used to hasten rooting of horticultural species. At present the method offers greater promise of success with actively growing plants than with dormant ones.

Herbaceous Species

Herbaceous types like tomato, marigold, etc., often form adventitious root primordia along the stems as the plants approach maturity. When such plants were used in the experiments the controls frequently produced a number of roots toward the basal part of the stem but the treated plants produced roots more readily and more abundantly (Fig. 5, A and B). Stimulation of latent root primordia seemed likely though it is possible that some initials were also induced.

ROOT HAIRS INDUCED BY GAS

Roots produced while the plants were kept continuously in gas had many more root hairs than those growing on controls. Plants which were exposed to the gas long enough to have roots initiated before being transferred to control conditions grew roots with very few hairs. Figure 8 (left) shows a part of a plant with roots that were produced under such conditions. Figure 8 (right) shows the same set of roots 48 hours after a retreatment with acetylene. Root hairs were induced in large numbers over the region of elongation. This response was, in some respects, comparable to the initiation of roots over the region of elongation on treated tobacco and hydrangea stems (Figs. 1, 2, and 3). Similar responses were obtained with other species and ethylene and propylene were equally effective. Similar effects from carbon monoxide were previously reported (2).

ORIENTATION OF ROOTS

Adventitious roots growing from the stem of a potted marigold plant have a striking uniformity in their normal orientation to gravity as shown in Figure 8 (left). Figure 8 (right) shows how in addition to inducing root hairs acetylene changes the direction of growth of the roots. The uniformity

of shift in direction of growth is almost as striking as the uniformity in normal orientation to gravity shown in Figure 8 (left). Ethylene and propyl-

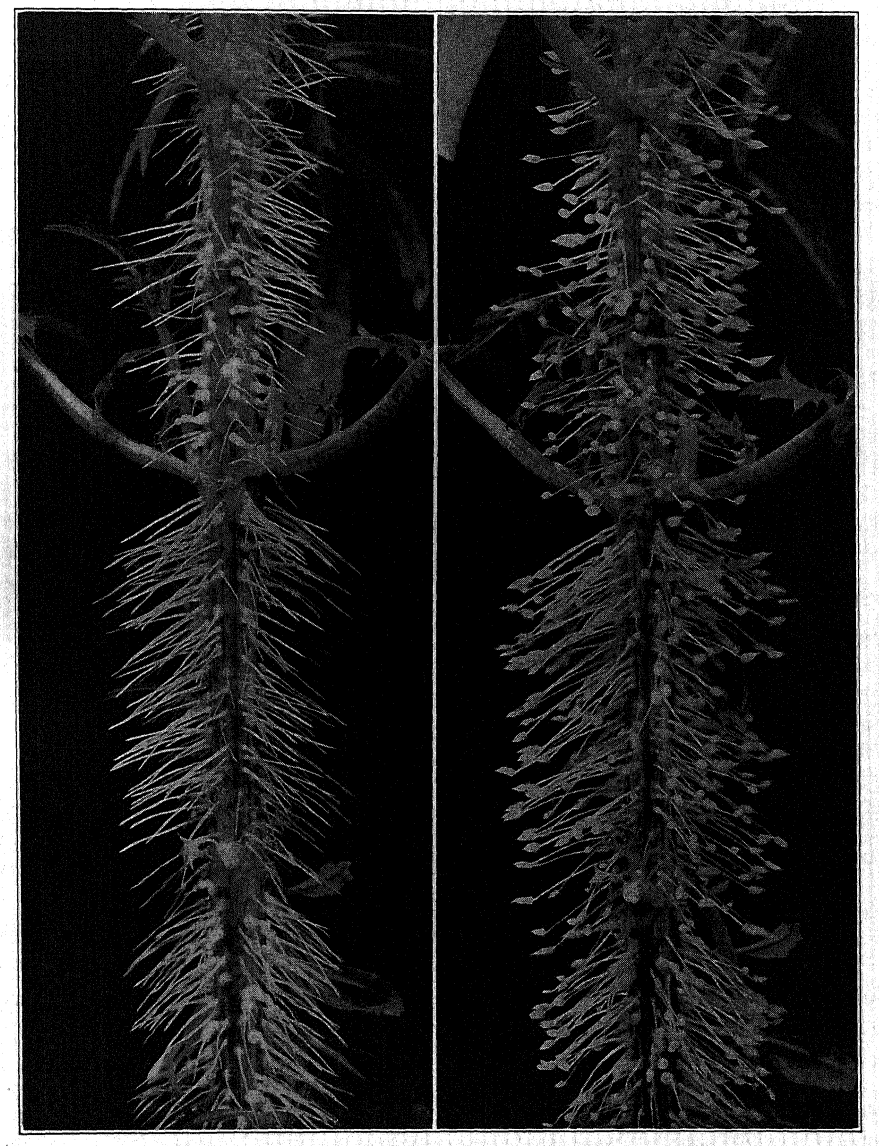


FIGURE 8. African marigold to show the effect of acetylene on orientation of roots to gravity and on formation of root hairs. Left, normal growth of roots six days after a three-day exposure to the gas. Right, the same roots after a 48-hour exposure to 0.25 per cent acetylene. Root hairs were induced and the roots changed their direction of growth.

ene used over a wide range of concentrations were fully as effective as acetylene.

The change in orientation of roots described for marigold was observed

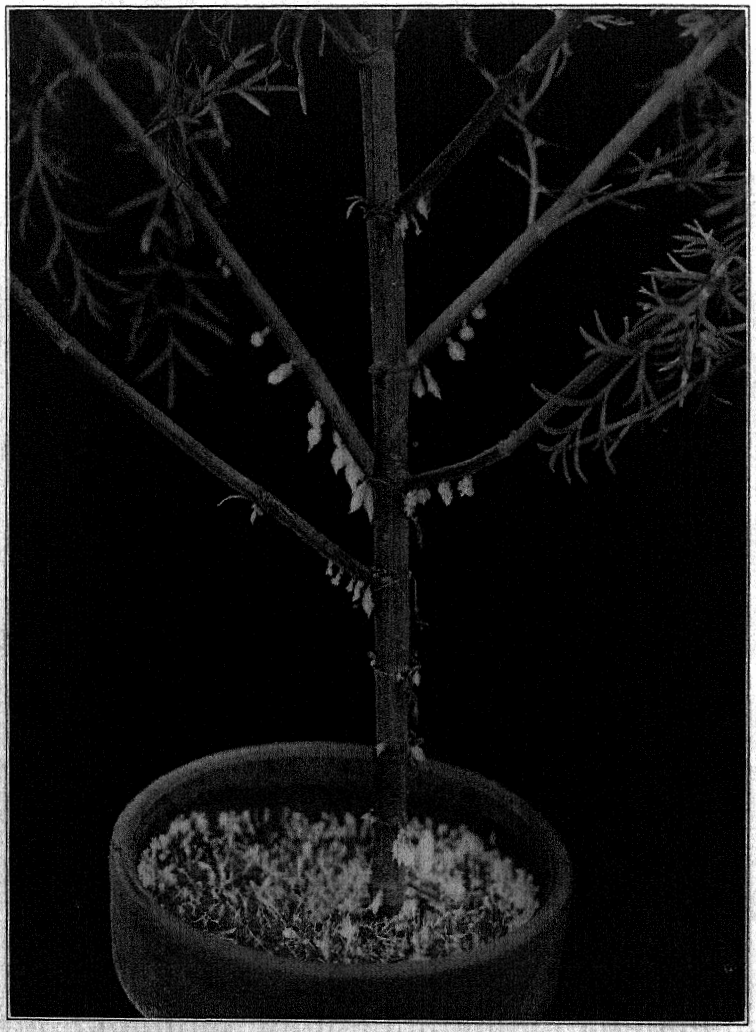


FIGURE 9. Reversal of direction of root growth caused by 0.5 per cent acetylene gas. The plant was treated for 36 hours, rested 48 hours, and then again treated for 48 hours after which time the photograph was taken. Roots came up out of the soil after the first treatment and then when the plant was reexposed to the gas many root hairs formed. Root hair effect is also seen on adventitious roots formed on the stems.

also in a number of other species, particularly tobacco. The tobacco roots turned upward, forming approximately a right angle within 48 hours of

exposure to the gas. After several days of treatment the tips showed considerable turning and twisting.



FIGURE 10. Branch roots produced on adventitious roots of African marigold during exposure to 0.2 per cent ethylene at two-day intervals from March 18 to April 3.

Potted plants, having many roots near the surface of the ground at the time they were placed in a gas chamber, sent roots up out of the ground,

indicating a change in normal orientation of soil roots to gravity (Fig. 9). It is not known how soil roots of varieties in general respond when subjected to gas.

SECONDARY ROOTS INDUCED

Plants which were treated and rested at two-day intervals produced adventitious roots which branched readily. This response may be interpreted as secondary roots induced on adventitious roots. Figure 10 shows the results of treating a marigold plant with 0.2 per cent ethylene at two-day intervals for 16 days. Acetylene and propylene, like ethylene, were effective over a wide range of concentrations.

SPECIFICITY OF THE THREE GASES

The three gases in question induced root initials to form on stems and on adventitious roots. They stimulated also preexisting root primordia but so far there has been no indication that these chemicals can induce shoots. Latent buds of some species were forced into growth, causing an abnormally large number of branches on plants. This, in some respects, resembles stimulation of preexisting root primordia as shown in Figure 7 for willow. Since no vegetative shoots have been induced by ethylene, acetylene, or propylene, it appears that these chemicals are specific for the initiation of roots.

EFFECT OF UNSATURATED HYDROCARBONS ON SHOOTS

During the course of these experiments many results other than effects on roots have been noted. For example, all three of the gases cause the following: (a) epinasty of leaves, (b) injury and abscission of leaves and flowers, (c) unusual proliferation of lenticular tissue, (d) retardation of stem elongation, and (e) modification in size of leaves produced while plants are in the gases.

The details of these observations will be reported in a later paper.

SUMMARY

1. The unsaturated hydrocarbon gases, ethylene, acetylene, and propylene, caused definite rooting responses in 15 species of plants.

2. Root initiation was induced in 15 species by one or more of the three gases.

3. *Hydrangea macrophylla* and *Nicotiana tabacum* responded to the gases by producing roots from only a short zone back of the growing point, representing approximately the region of elongation at the time the plants were started in the experiment.

4. When induced adventitious roots were allowed to grow in a control case for 24 to 48 hours and were then transferred to atmospheres of ethyl-

ene, propylene, or acetylene, root hairs were produced in abundance from the region of elongation.

5. The gases stimulated the growth of latent root primordia in hardwood cuttings of types like willow.

6. Leaves of four species formed roots while the plants were being exposed to the gases.

7. Six species showed a tendency to root at nodes.

8. The three gases caused a change in the normal orientation of roots to gravity in seven species of plants.

9. Secondary roots were produced from adventitious roots by intermittent gas treatments at two-day intervals.

10. Since the three chemicals did not induce shoots to form, it was concluded that these gases are specific for adventitious root formation.

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CARBON DIOXIDE STORAGE. III. THE INFLUENCE OF CARBON DIOXIDE ON THE OXYGEN UPTAKE BY FRUITS AND VEGETABLES[1]

NORWOOD C. THORNTON[2]

Carbon dioxide, as one of the end products of respiration in plants, might be expected, upon accumulation, to exert an inhibiting influence on the respiration of the tissues. In fact, this has been the prevailing conception of the problem since the time of de Saussure's (10) "Recherches chimiques sur la végétation" published in 1804. Many researches have been carried out to test experimentally the influence of carbon dioxide upon the respiration of plants. These have almost invariably shown a retarding influence. On the other hand the accelerating influence of carbon dioxide on the rate of breathing of animals has been demonstrated many times with practical application in the medical profession.

Mangin (8) seems to have been the first to make quantitative measurements upon the effect of carbon dioxide on the respiration of tubers and germinating seed. He depended upon the accumulation of carbon dioxide within closed containers to bring about the desired results. Disregarding the effect of the reduction in the oxygen concentration he found that up to 5 per cent carbon dioxide reduced the respiration of flax, radish, garden cress, barley, pea, and carrot seed and tubers of Jerusalem artichoke. Mangin pointed out that the respiratory quotient increased, indicating a decrease in oxygen uptake, to the greatest extent with the fatty seed and least with the tubers as the concentration of carbon dioxide was increased.

Kidd (6) studied the effect of various concentrations of carbon dioxide upon the anaerobic and aerobic respiration of pea and white mustard seed as well as cherry laurel leaves. The oxygen uptake and carbon dioxide output as well as changes in pressure were measured during various periods of storage in closed flasks at 16° to 20° C. From the results obtained he concluded that the degree of depression of respiration appeared to be proportional to the square root of the concentration of carbon dioxide over a range from 0 to 50 per cent at one atmosphere pressure. At higher concentrations of carbon dioxide the effect was less marked.

Stiles and Leach (12, p. 45, 46), citing the work of Kidd, stated in their recent monograph on respiration in plants that "Increased concentra-

[1] Part I of thesis presented to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The writer is indebted to Dr. O. F. Curtis, Dr. L. Knudson, and Dr. E. F. Hopkins of the Department of Plant Physiology, to Dr. H. C. Thompson of the Department of Vegetable Crops of Cornell University, Ithaca, New York, and to the members of the staff of the Boyce Thompson Institute for helpful suggestions and criticisms throughout the course of this work.

tion of carbon dioxide in the atmosphere brings about very marked depression in the respiratory process." They further suggest that the accumulation of carbon dioxide in the tissues may frequently be a limiting factor in the respiration intensity.

Bailey and Gurjar (1) stored wheat at 37.8° C. in closed containers for various periods and analyzed the atmosphere for carbon dioxide. They reported that the carbon dioxide produced per 100 grams of dry matter gradually decreased from 4.1 mg. to 1.1 mg. with 12 days of storage. However, they did not take into consideration the effect of the decreasing concentration of oxygen, within the chambers, upon the rate of the carbon dioxide production.

Willaman and Beaumont (14), using the aeration method, studied the effect of accumulating carbon dioxide upon the respiration of apple twigs at 0° C., potato tubers at 22° C., and wheat seed at 40° C. They concluded that the rate of carbon dioxide production under these conditions decreased with time. The authors disregarded the oxygen supply to the tissue during the period of accumulation of carbon dioxide through respiration. Upon aeration of the storage atmosphere after a period of accumulation the output of carbon dioxide was found to be far greater than that of the previous period. The theories suggested by them to account for this result are presented in the discussion of this paper.

Kidd and West (7) studied the average rate of respiration of apples in air and carbon dioxide storage at 7° and 8° C. by determining the loss in weight of dry matter. They found that the rate of respiration was approximately halved with storage in 9 to 12 per cent carbon dioxide with 12 to 9 per cent of oxygen. Their data suggest that approximately one-third of the lowering may have been due to the reduction in the oxygen supply to the fruit. This estimation was made from the measurements of carbon dioxide produced by the apples in the presence of 20, 10, and 5 per cent of oxygen.

Overholser, Hardy, and Locklin (9) determined, by analyses of the storage atmosphere, the respiration rate of immature and mature strawberries held in accumulating carbon dioxide. With concentrations of from 7 to 12 per cent carbon dioxide they found no depressing effect during long respiration intervals or when slightly greater increases of carbon dioxide occurred within the respiration chamber. The data show in fact a slightly higher rate of carbon dioxide output under these conditions. The average respiration ratio was below unity, indicating that there was probably an absence of anaerobic respiration.

Gonzalez (5) employed the aeration method to study the respiration of the chico (*Achras zapota* L.) at 28.5°C. During the experiment storage in carbon dioxide was obtained by passing the gas into the respiration chamber without further aeration for either 13- or 38-hour periods. The carbon

dioxide produced by respiration of the chico before and after the storage periods was determined by drawing air through the chamber and collecting the gas in barium hydroxide. With this procedure he reports a diminution of as much as 50 per cent in the rate of respiration of chico. His data given in Tables 3 and 4, pages 345 and 346 respectively, show a slight depressing effect with short period and a maximum effect with the longer period of storage in carbon dioxide. Following the removal of the carbon dioxide atmosphere the retardation in the rate of respiration was prolonged for approximately one day, after which time the rate increased until it became approximately that of the control.

In this paper the oxygen uptake as well as the carbon dioxide output by various plant tissues held in a confined atmosphere was determined by means of gas analyses. The object of this study was to obtain in general a knowledge of the effect of carbon dioxide on respiration of plant tissues as measured by the oxygen uptake. For this purpose experiments were carried on for various periods of time over a wide range of concentrations of carbon dioxide rather than for specific intervals with a definite amount of the gas. The results show the response of the various plant tissues to storage in 0 to 75 per cent of carbon dioxide. The oxygen uptake and carbon dioxide production were increased in the case of the potato, decreased in the case of the asparagus, and were not significantly altered in the case of carrot. Other tissues showed changes intermediate between those obtained with the potato and the asparagus.

MATERIALS AND METHODS

The plant tissues studied were as follows: potato (*Solanum tuberosum* L.) tubers; onion (*Allium cepa* L.) bulbs; tulip (*Tulipa gesneriana* L.) bulbs; beet (*Beta vulgaris* L.) roots; carrot (*Daucus carota* L.) roots; asparagus (*Asparagus officinalis* L.) shoots; bush lima bean (*Phaseolus lunatus* L.); banana (*Musa paradisiaca* L. var. *sapientum* Kuntze and var. *champa* Baker) fruit; and strawberry (*Fragaria chiloensis* Duchesne) fruit. The fruits and vegetables, with the exception of lima bean grown in the Institute garden and bananas received from the United Fruit Company,[3] were obtained from a local wholesale dealer. In any one experiment the same number of pieces of tissue of approximately the same weight was used in the control as well as in the lots treated with carbon dioxide. The quantity of plant tissue used and the duration of study varied with each experiment; therefore, such information will be given with the discussion of results.

During the carbon dioxide treatment the plant tissue was placed in

[3] I am indebted to Mr. J. M. Kelley of the Fruit Dispatch Company and to Mr. G. L. Poland of the United Fruit Research Laboratory for furnishing the bananas used in this work.

either an 8- or 18-liter tin container having a V-shaped depression into which the cover fitted. Upon assembling the container the V-shaped depression was filled with melted paraffin mixed with approximately 5 per cent of vaseline. The object of softening the paraffin was to obtain a sealing mixture that would not, upon cooling, crack away from the metal. After the sealing mixture hardened, a hot iron was passed over it to insure a smooth bubble-free contact with the surface of the metal. Since the carbon dioxide was found to pass through a thin layer of paraffin the thickness of the seal on these containers was increased to 1.5 cm. Passing through and sealed to the top of the container were two copper tubes, one extending to the bottom of the container and the other just through the top. Into each of the copper tubes was passed a glass capillary tube with a stopcock at the outer end. The capillary glass tubing was sealed into the copper tubing with paraffin. Attached to the lid of the container in a position to swing free was a piece of tin (4 cm. $\times$ 8 cm.) for the purpose of stirring the gas by rotating the container just before each sampling for analysis. The gas samples were drawn from the bottom of the container for convenience only, since analyses from the top and bottom gave the same results. The tissues were held 2.5 cm. from the bottom of the container by a 1 cm. galvanized iron screen to facilitate a movement of air in the container.

The gas mixtures were made from stock cylinders of the three gases, carbon dioxide, oxygen, and nitrogen, by passing the desired percentage of gas into a 20-liter bottle previously filled with water. The carbon dioxide used was the product of fermentation in a process connected with the refinement of cane sugar and the manufacture of ethyl alcohol. Carbon dioxide from this source was compared with that produced by the action of sulphuric acid on pure sodium carbonate. The results of the tests showed that there was no significant difference in the rate of oxygen uptake by potatoes held in the carbon dioxide from the two sources. In all cases, except those especially mentioned in the results, 20 per cent oxygen was used with a variation in the proportion of carbon dioxide and nitrogen to make 100 per cent. After the desired mixture had been made up the outlet of the bottle was attached, by rubber tubing, to the tube extending to the bottom of the container and the gas passed into and through the container by displacement from the bottle with water.

It was found difficult to prepare a mixture of gases which would produce a precise percentage of gases within the container at the start of an experiment. It was more convenient to prepare a mixture which was approximately the one that was desired, and then to determine the exact percentage of gases at the beginning of the experiment by gas analytical methods. It is for this reason that the concentrations of carbon dioxide used in the experiments are not exactly the same; in many cases they differ by only a small percentage.

The assembling of the apparatus and production of the gas mixtures was carried out at room temperature and then the container holding the tissue was placed in a constant temperature room held at 25° C. Previous to the setting up of the experiment the plant tissue was held from one to three days at 25° C. or until a sufficient time had elapsed for it to be adjusted to this temperature of storage. The assembled apparatus with the gas mixture was held at 25° C. for two hours before sampling for gas analyses. The gas pressure, when developed due to change in temperature, was allowed to equalize with the atmosphere by opening the stopcocks. After the original duplicate gas samples were obtained the stopcocks were closed until a future date when another gas sample was desired. During 96 to 144 hours of storage, especially with potatoes, in the presence of 50 to 70 per cent of carbon dioxide, there developed a reduction in pressure within the sealed container. With an attached manometer this reduction in pressure was found to be equivalent to 2 to 4 mm. of mercury. In comparative experiments it was found that the error brought about by this reduction in pressure amounted to less than 1 per cent which was very small as compared with the changes obtained in the rate of oxygen uptake.

The gas analyses were carried out according to Dennis and Nichols (4) using an Orsat apparatus having a 100 cc. burette graduated in 0.2 for measuring the gas. Purified mercury with a small layer of water for saturating the gas was used as the confining medium. Absorption of the gases was obtained by passing the carbon dioxide into approximately 33 per cent sodium hydroxide and the oxygen into alkaline pyrogallol. The absorption pipettes were filled with glass tubes to increase the absorption area. The accuracy of the analyses as determined from 55 pairs of duplicate determinations of the oxygen content of gases ranging from 17.6 to 24 per cent in oxygen gave an average difference between duplicate determinations of 0.11 per cent and a maximum of 0.2 per cent of the oxygen present.

The data presented, unless stated otherwise, have been obtained by converting cubic centimeters of oxygen uptake per kilogram of tissue per hour to milligrams by the use of the factor 1.31. This factor is the weight in milligrams of 1 cc. of oxygen under the conditions of the experiment.

As already shown the error in the gas analyses was very small. The error requiring attention is that shown by duplicate lots of tissue under the same conditions. To estimate the amount of this error 33 pairs of duplicate determinations were made with various tissues used in these experiments. The actual measurements as well as the deviation of the duplicates from each other in per cent of their mean are given in Table I. From these data the standard deviation of a single determination was calculated by the formula:

$$S.\ D.\ \text{of single det.}\ \sigma = 1/\sqrt{2}\sqrt{\Sigma d^2/N-1}$$

In this formula Σd^2 equals the sum of the squares of the differences between duplicates, and N equals the number of pairs of duplicates. From the data in Table I it is found that the standard deviation of a single determination

TABLE I

VARIATION IN DUPLICATE SAMPLES OF TISSUE

Tissue	Mg. O_2/kg./hr. by duplicate lots		Per cent total deviation between duplicates	Tissue	Mg. O_2/kg./hr. by duplicate lots		Per cent total deviation beween duplicates
	a	b			a	b	
Potato	8.79	8.62	1.9	Carrot	29.2	29.1	0.3
	7.55	7.31	3.2		30.7	30.6	0.3
	8.74	8.23	6.0		38.3	36.2	5.6
	7.22	6.96	3.7				
	8.07	7.60	6.0	Asparagus	149	146	2.0
	7.77	7.76	0.0		198	187	5.7
	8.49	7.89	7.3		313	277	12.2
	7.60	7.56	0.5		260	244	6.3
	7.83	7.60	3.0				
	8.29	7.87	5.2	Banana	69	67	2.9
	11.80	11.70	0.8		154	144	6.7
					118	106	10.7
Onion	12.3	11.4	7.6		47	46	2.1
	22.7	22.1	2.8		58	47	21.0
					52	51	1.9
Tulip	28.1	28.0	0.3				
				Strawberry	174	141	20.9
Beet	43.0	38.4	11.3		77	73	5.3
	38.3	33.1	14.5		111	105	5.5
	28.5	27.3	4.3				

is 5.5 per cent of the amount of oxygen uptake. The standard deviation of the difference between two measurements would then be $\sqrt{2} \times 5.5$ or 7.77 per cent. This value is shown by arrows in Figures 1 and 2.

In the experiments reported in this paper special precautions were taken that the oxygen supply to the controls did not fall below 14 per cent by volume. This was brought about by a regulation of the length of the storage period after preliminary tests to determine the rate of oxygen uptake by the tissue under investigation. The lots exposed to concentrations of carbon dioxide which increased the rate were not always maintained at oxygen concentrations of 14 per cent or more. The oxygen within the container fell to approximately 10 per cent in some cases in which the increase in oxygen uptake due to carbon dioxide was large.

The accumulation of carbon dioxide from respiration within the closed containers varied with the tissue, the storage period, and the concentration of carbon dioxide used as was observed in previous work (13, p. 240). The concentrations of carbon dioxide given in the tables represent the amount

present at the beginning of the experiment. In the case of the control tissues the carbon dioxide from respiration amounted to from 2 to 6 per cent depending upon the storage period. With the tissues treated with 60 per cent of carbon dioxide the increase in the carbon dioxide content varied greatly over a range from 2 to 12 per cent during the periods of storage. With intermediate concentrations of carbon dioxide the increase in carbon dioxide was equal, in most cases, to the decrease in oxygen. However, no definite statement can be made in regard to these measurements since at times very good correlation was obtained between the oxygen uptake and the carbon dioxide output, while at other times the correlation was not close.

Comparative respiration studies with carrots and potatoes made by using the aeration method, collection of carbon dioxide in barium hydroxide, and the present method of gas analysis gave results of fairly good agreement. However, with rapidly respiring tissue such as ripe bananas and fresh asparagus shoots the aeration method gave results that were from 20 to 30 mg. of CO_2 per kg. per hr. higher than those by the gas analysis method. Further tests were not made with the aeration method since it is very difficult to employ this method to measure the output of relatively small amounts of carbon dioxide by plant tissue in the presence of large amounts of added carbon dioxide.

Since the experiments were carried out for different lengths of time it is preferable to examine individually each experiment, comparing the results of each treatment with those of the control for that experiment.

Although most of the experiments are not directly comparable, an attempt has been made to combine the observations from the various tests, in order to obtain a curve showing approximately the relation between the concentration of carbon dioxide and the percentage gain or loss in the oxygen uptake. In preparing these curves the "moving average" method of Camp (3, p. 102) was used, 3 being taken as the value of "k." The percentage gains or losses of treated as compared with the control were calculated for all the experimental values in Tables II, IV, VI, VII, IX, X, and XV. The weighted average of these percentage gains or losses for each of three contiguous carbon dioxide values was obtained and was plotted as an ordinate over the weighted average of the carbon dioxide concentrations that gave these values. It is in this way that the curves in Figures 1 and 2 were prepared.

EXPERIMENTAL RESULTS

POTATO

Since potatoes at various stages of storage, after harvesting, have different rates of respiration, it was considered desirable to make a study of dormant, non-dormant, and sprouting tubers. For the test with non-

dormant and sprouting tubers Green Mountain potatoes of the Maine crop of 1931 that had been held in storage until February and March 1932 were used. The dormant potatoes, Irish Cobbler, were obtained from South Carolina immediately after harvest. Firm tubers weighing from 70 to 100 grams each were selected for the experiments.

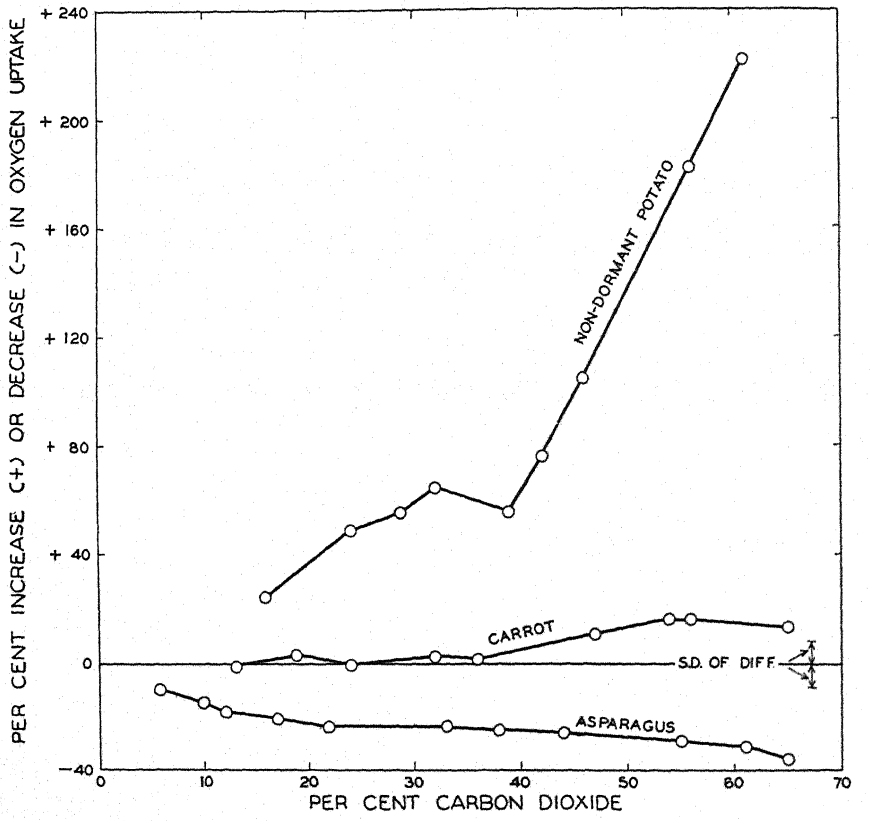


FIGURE 1. Effect of carbon dioxide upon the oxygen uptake by various tissues. The controls are plotted at zero. The curves show percentage increases or decreases in oxygen uptake by tissues treated with carbon dioxide at 25° C.

The effect of carbon dioxide upon the oxygen uptake by non-dormant potatoes is shown in Table II. The initial concentration of carbon dioxide within the containers varied from 8 to 64 per cent. It will be noted that at all concentrations tested carbon dioxide increased the rate of oxygen uptake. The percentage increase in the rate of treated over the control lots was greatest at about 50 to 60 per cent carbon dioxide, the increase being about 150 to 250 per cent. There were definite increases at 17 and 26 per cent carbon dioxide, and there was some evidence of an increase at 8 per

TABLE II

OXYGEN UPTAKE BY NON-DORMANT POTATOES (VARIETY GREEN MOUNTAIN) AT 25° C. IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour											
			Per cent of carbon dioxide within the container											
			0	8	17	26	30	33	36	42	52	57	59	64
1	160	87	9.4							12.4				
2	135	45	13.6	13.4		16.1				22.7				40.4
		69	11.4	12.9		13.7				17.2				37.3
		92	9.5	12.3		12.7				13.6				33.3
		140	7.3	10.6		11.1				11.3				30.0
3	140	48	16.0				24.8							
		72	11.8				24.4							
		96	10.6				20.8							
		120	10.3				18.9							
		216	7.5				11.8							
4	320	45	12.6					17.7						
		67	9.4					13.5						
5	311	41	9.4		12.2								19.0	
6	315	66	5.2		6.4				9.9			18.3		
7	321	90	11.7	10.8	12.3				15.5		31.8			
8	325	90	11.8	12.8	12.7				15.8				35.3	
9	485	85	8.8		15.9					18.7			28.2	
10	424	92	11.5								28.5			

cent carbon dioxide. The curve for these data is shown in Figure 1. It is apparent from this curve that concentrations less than 40 per cent of carbon dioxide increase the rate of oxygen uptake slowly while higher concentrations increase the rate very rapidly.

Experiments 2, 3, and 4 inclusive were analyzed at various time intervals; thus by this procedure data were obtained on the rate of oxygen uptake by the tubers during a period of 45, 69, 92, and 140 hours from the start of experiment 2. As the storage period was extended under these conditions the rate decreased to some extent. This was found to be the case with the controls as well as with the treated tubers. This retardation in the rate of the oxygen uptake by the tubers may have been influenced by the oxygen supply. However, this factor should not have played a very important part in the results obtained since in no experiment did the oxygen supply to the control fall below 14 per cent. Because of the change in the rate of the respiration of the tubers with time it was impossible to compare any number of experiments with each other. However, control lots of tissue run in duplicate, Table I and experiments 7 and 8, Table II, show fairly good agreement for duplicate determinations of the oxygen uptake by the potatoes.

TABLE III

OXYGEN UPTAKE BY SPROUTING POTATO TUBERS (VARIETY GREEN MOUNTAIN) AT 25° C. IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour					Degree of sprouting
			Per cent of carbon dioxide within the container					
			0	18	35	40	60	
11	245	133	9.4		15.9			Sprouts just breaking through at the beginning of the experiment.
12	540	48	9.7		12.3			
13	540	140	10.7		12.1			Sprouts 6 mm. long. High CO_2 caused injury to tips of sprouts and prevented growth.
14	315	114	11.6	9.7	13.0		23.5	Sprouts 2 cm. long. High CO_2 caused injury to tips of sprouts.
15	300	90	11.4	12.5		17.5	27.0	Sprouted. Control grew to 1 cm. during treatment, in 18% CO_2 2 mm., and others just started to break through with tips blackened.

Upon holding the tubers at 25° C. for some time sprouting took place and the rate of oxygen uptake was determined as with non-dormant tubers. As shown in Table III the rate was increased from 30 to 120 per cent by

concentrations of carbon dioxide ranging from 35 to 60 per cent. This increase was observed with tubers just starting to sprout as well as with tubers having sprouts 2 cm. long. As found in previous work the higher concentrations of carbon dioxide did retard the growth and caused some blackening of the tips of the sprouts. This blackening or injury to the growing tip developed during the long periods of storage and appeared to be only a local effect. Upon removing the potatoes from the carbon dioxide other buds on the sprout developed as rapidly as those on the control tubers and produced healthy plants that gave no indication of having been injured by the previous treatment.

The results with freshly-harvested tubers are shown in Table IV. At carbon dioxide concentrations of about 20 per cent or less the rate of oxygen uptake was slightly decreased, but at carbon dioxide concentrations of 27 to 72 per cent the rate was increased, the percentage gains of the treated

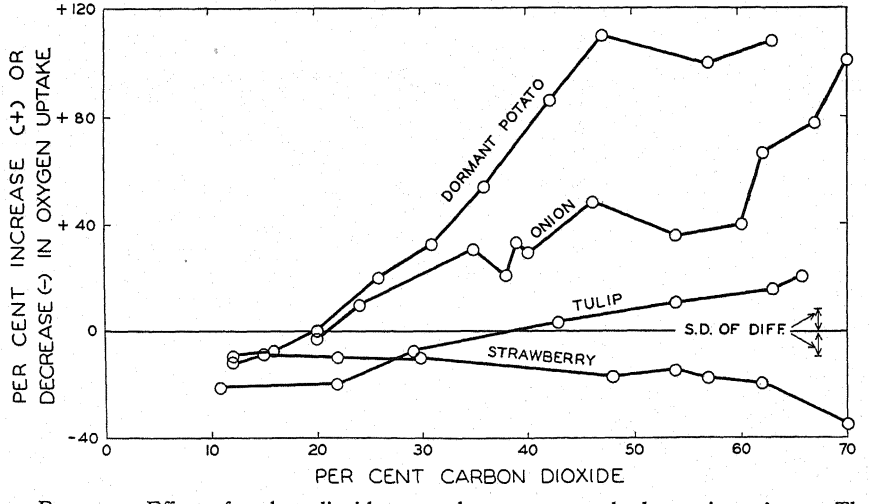


FIGURE 2. Effect of carbon dioxide upon the oxygen uptake by various tissues. The controls are plotted at zero. The curves show percentage increases or decreases in oxygen uptake by tissues treated with carbon dioxide at 25° C.

over control rising with increasing concentrations of carbon dioxide until at approximately 50 per cent carbon dioxide the maximum effect was obtained. At this point the gain in oxygen uptake due to the presence of carbon dioxide was about 100 per cent. The curve for these data is shown in Figure 2. An examination of this curve and that in Figure 1 for the non-dormant tubers indicates that the carbon dioxide has a greater effect upon the rate of the oxygen uptake by the older tissues.

Prolonged holding of the tubers within the closed container, even with replacing the gas mixtures, reduces the rate of oxygen uptake as shown by

TABLE IV

OXYGEN UPTAKE BY DORMANT POTATOES (VARIETY IRISH COBBLER) AT 25° C. IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour											
			Per cent of carbon dioxide within the container											
			0	8	13	15	21	27	30	37	42	47	57	60	72
16	866	141	15.2		14.1				21.6			36.8	35.6		
17	261	44	23.2	17.9			22.9				37.3			31.0	
18	267	42	22.6	21.0			19.5				45.8			43.5	41.9
19	970	67	16.2			14.7			23.4					30.0	
20	982	72	14.9		14.6			19.0						31.1	
21*		72	8.8			8.3				11.8					28.1

* Exp. No. 21 is a continuation of Exp. No. 20. After the first 72 hours the gas was completely removed and a fresh supply placed in the container and the analyses made as usual.

experiments 20 and 21 in Table IV. The tubers for the first period of 72 hours had a rate of 14.9 mg. of oxygen uptake as compared with 8.8 mg. for the second period of 72 hours. At the end of the first period the gas mixture was replaced by an atmosphere of approximately the original composition. By analyses it was found that this atmosphere contained an increased amount of carbon dioxide with 20 per cent oxygen in each test. However this did not alter the trend of the oxygen uptake since there was found in all tests a lower rate during the second period of treatment. The effectiveness of the carbon dioxide gas in increasing the oxygen uptake was, however, twice as great as in the previous experiment.

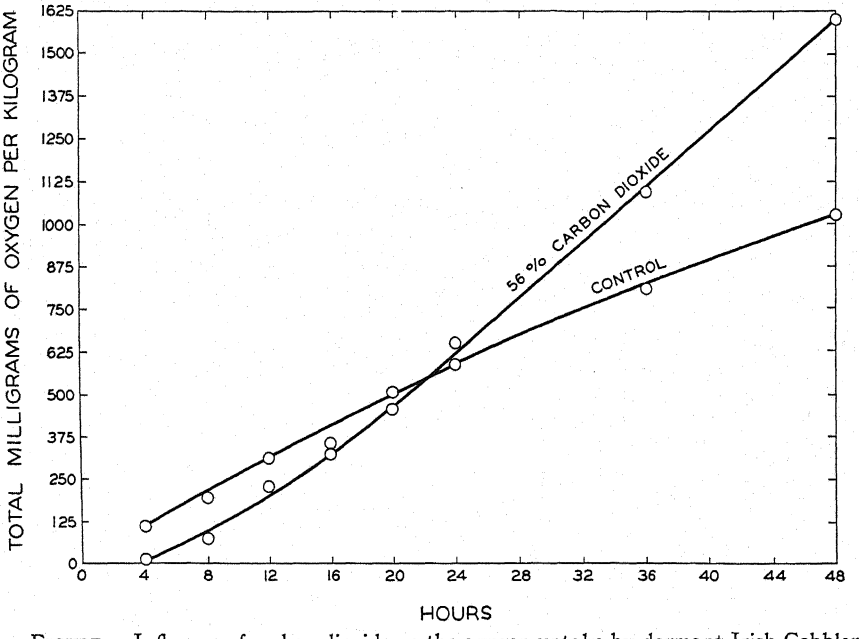


FIGURE 3. Influence of carbon dioxide on the oxygen uptake by dormant Irish Cobbler potatoes at 25° C.

The results shown in Tables II, III, and IV were obtained from experiments which extended over periods of 42 hours or more. Experiments were undertaken to determine the effect of carbon dioxide during shorter periods of exposure. The results are shown in Figure 3. It is evident that the oxygen uptake was reduced by carbon dioxide for all intervals up to about 20 to 24 hours, after which time the tubers treated with 56 per cent carbon dioxide gave increases. After eight hours the *decrease* due to carbon dioxide was about 66 per cent, but after 48 hours the *increase* due to carbon dioxide was about 77 per cent. The points on this graph were calculated from the rate

of the oxygen uptake, but a curve of the same form was obtained by plotting the amount of carbon dioxide produced.

With the knowledge that the source of carbon dioxide was unimportant a study was made upon the effect of the concentration of oxygen upon the rate of the oxygen uptake. The data presented in Table V show that reducing the oxygen concentration retards the rate of oxygen uptake. But it also shows that the rate of oxygen uptake by the potatoes treated with carbon dioxide was retarded more than that of the controls. Consequently, differences between the treated and control lots became smaller as the oxygen concentration was reduced. Maintaining the controls at 14 per cent oxygen or above, and allowing the carbon dioxide-treated lots to go below 14 per cent gave an estimate of the differences between the treated and the corresponding controls which was less than would have occurred if the oxygen supply had been equal. It may be concluded from these data that the effect of the reduction in oxygen supply was not a factor which would lessen the significance of the differences in oxygen uptake found between carbon dioxide-treated and control tissues.

TABLE V

EFFECT OF CARBON DIOXIDE ON THE OXYGEN UPTAKE BY POTATOES AT 25° C. IN LOW, NORMAL, AND HIGH OXYGEN ATMOSPHERES

Grams tissue used	Hrs. exp. ran	Per cent of carbon dioxide within the container	Milligrams of oxygen per kilogram per hour		
			Per cent of oxygen within the container		
			10	20	40
485	85*	0	7.3	8.8	14.0
		40	10.8	18.6	22.3
496	81**	0	10.7	11.9	14.0
		40	11.6	16.0	22.6

* Green Mountain variety (non-dormant).
** Irish Cobbler variety (dormant).

In view of the fact that these results were obtained with storage at 25° C. it was considered desirable to determine whether similar results could be obtained at a temperature of 15° C. Comparative experiments were set up using Maine potatoes, variety Green Mountain, of the 1932 crop. With storage for 96 hours at 25° C. it was determined that the controls absorbed oxygen at a rate of 5.1 mg. of oxygen per kilogram of tissue per hour as compared with 17.3 mg. for the tubers treated with 57 per cent of carbon dioxide. At 15° C. the controls absorbed oxygen at a rate of 2.5 mg. as compared with 5.8 mg. for the tubers treated with 57 per cent of carbon dioxide. These data show an increase in rate of oxygen uptake over

TABLE VI

OXYGEN UPTAKE BY ONION BULBS AT 25° C. IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour													
			Per cent of carbon dioxide within the container													
			0	17	18	33	35	37	39	40	41	57	60	63	67	70	71
22	308	91	21.9						20.3				25.3				
23	337	89	16.5		17.7						23.9			25.8		20.6	
24	254	92	14.9		18.3			23.6									
25	412	89	12.9							20.2							21.7
26	302	90	12.3 11.4	8.7	9.7	14.0	13.4								29.0		31.2
27	338	92	22.1 22.7	17.5					26.6			30.9	32.0				

TABLE VII

OXYGEN UPTAKE BY TULIP BULBS (PRIDE OF HAARLEM) AT 25° C. IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour									
			Per cent of carbon dioxide within the container									
			0	8	16	17	34	38	57	60	70	72
28	96	68	28.0	24.6		21.9		30.0		33.2		38.2
29	95	69	29.1	19.7						31.3	34.0	
30	118	66	28.0 28.1	26.3	18.8		26.7		30.3		34.7	

TABLE VIII

OXYGEN UPTAKE BY BEET ROOTS (GARDEN) AT 25° C. IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour											
			Per cent of carbon dioxide within the container											
			0	15	18	34	37	38	52	57	60	68	72	74
31	453	22	43.0 38.4		43.9		52.9			43.4			36.5	
32	545	21	38.1	29.6				40.7	41.9				44.9	
33*		23	29.3	25.9		32.5					47.7			48.6
34	383	18	33.1 38.3		48.0		57.4			46.0		65.5		
35**		23	27.3 28.5		33.7	44.5				64.2			74.5	

* Exp. No. 33 is continuation of Exp. No. 32 after the beets had been in the gas 42 hours. The gas mixtures were replaced twice then data of No. 33 was obtained.

** Exp. No. 35 is continuation of Exp. No. 34 after the beets had been in the gas 96 hours. The gas was replaced every 24 hours.

the control of 239 per cent at 25° C. and 132 per cent at 15° C. Temperature plays an important part in the rate of respiration of the tubers, but carbon dioxide even at the lower temperature increases the rate of oxygen uptake.

ONION

A study was made of the influence of carbon dioxide on the onion using methods similar to those used with the potato. Two varieties of the onion were studied, the Yellow Bermuda, in experiments 22 and 23, in Table VI, and the Southport White Globe, in experiments 24 to 27. Selected onions were used which weighed from 23 to 28 grams each except those taken for experiment 25 which averaged only 14 grams each. The rate of the respiration of the onions in experiments 22 and 27 was found to be high. The probable reason for this was that these onions had been harvested recently. The onions used in the other experiments had been in dry storage for some length of time.

The results given in Table VI show the effect of various concentrations of carbon dioxide upon the rate of the oxygen uptake of the onion. With 33 to 71 per cent of carbon dioxide the average increase in the rate of the oxygen uptake amounted to from 14 to 146 per cent. In experiment 26 a maximum increase of 162 per cent was obtained by storage of the onions in 71 per cent of carbon dioxide for 90 hours. With 17 to 18 per cent of carbon dioxide there was a slight reduction in the rate of the oxygen uptake of the onions. This reduction amounted to from 4 to 24 per cent and was especially noticeable with the freshly-harvested onions. The curve for these data is shown in Figure 2. Although there is some variation in the position of the points the trend shows a definite increase in oxygen uptake. These results with the onion are similar to those obtained with the dormant potatoes in which a small amount of carbon dioxide retarded and a larger amount greatly increased the rate of respiration as measured by oxygen uptake.

TULIP

Tulip bulbs in the dormant stage, weighing from 8 to 10 grams each, were stored in various concentrations of carbon dioxide. The experiments were conducted for only 66 to 69 hours because of the rapid depletion of the oxygen supply. As shown in Table VII, 38 to 72 per cent of carbon dioxide increased the rate of oxygen uptake of the tulips from 7 to 36 per cent. This increase was progressive with increase in the concentration of the gas in the storage atmosphere. However, 8 to 34 per cent of carbon dioxide brought about a reduction in the oxygen uptake of the tulip bulbs. This reduction was found greatest with the lower concentrations of carbon dioxide. The curve of these data in Figure 2 shows a very interesting response of the tulip to the carbon dioxide treatment. Here is observed a progression of

TABLE IX

OXYGEN UPTAKE BY CARROTS AT 25° C. IN VARYING CONCENTRATIONS OF CARBON DIOXIDE

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour											
			Per cent of carbon dioxide within the container											
			0	8	12	17	27	33	37	42	54	58	60	72
36*	270	25	41.8					39.4						
		66	36.2					39.1						
		113	30.9					34.7						
37	306	70	29.1 29.2			27.4			28.6				34.3	
38	305	91	29.2								33.9			
39	510	93	20.4								22.4			
40	303	91	32.7			27.8			31.0				41.0	
41	306	115	30.6 30.7			24.0				37.6	36.0			
42*	265	99	41.7	33.9		34.8			40.6		45.1			47.5
43	267	114	31.5	34.4		33.6			33.8			48.1		48.6

* Freshly-harvested.

TABLE IX (*continued*)

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour											
			Per cent of carbon dioxide within the container											
			0	8	12	17	27	33	37	42	54	58	60	72
44*	468	24	40.3	31.5		34.4			36.9			37.4		37.7
45*	385	24	41.8	38.7		37.2			37.2			35.3		36.0
46	454	5	36.2 38.3		55.1		43.0				—			
		18	36.2 37.0		51.5		39.4				43.1			
		26	37.9 34.9		40.4		40.9				44.4			
		44	36.4 37.2		38.4		38.0				43.3			
		67	35.3 36.0		37.4		36.6				44.0			
		92	34.4 35.1		28.0		36.9				40.0			
47	540	21	33.9				30.8				40.9			

* Freshly-harvested.

TABLE X

OXYGEN UPTAKE BY ASPARAGUS SHOOTS AT 25° C. IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour													
			Per cent of carbon dioxide within the container													
			0	3	6	9	14	16	23	28	39	43	58	60	70	74
48	315	20	194						141		134			127		120
49	601	18	133										111			
50	935	16	149 146			142	129			119		104	106			
51	969	24	155							120	114					
52	189	18	207	194	158	152	177	162				138		119	119	
53	201	18	198 187		159	170	154	149			156			143		131
54	142	22	182	180	183	154	140		132		139					
55	142	22	182	167	152	147	139		130		136					
56	251	18	209	214	198	188	177				160					

three stages in the oxygen uptake as affected by the treatment: a decrease, no effect, and an increase. It will be of interest to study this tissue further in both low and high concentrations of carbon dioxide.

BEET

The rate of oxygen uptake by garden beets was determined in the presence of many concentrations of carbon dioxide. In all the experiments the tops were removed from the roots which weighed from 57 to 68 grams each. The results, given in Table VIII, show that the treatment was effective in increasing the oxygen uptake of the beet roots. With periods of storage of over 40 hours the increase in the rate obtained was as much as 165 per cent with storage in 72 per cent of carbon dioxide. The shorter periods of storage gave somewhat variable results, but in two cases out of three there was obtained a maximum increase of 85 per cent. The lowest concentration of carbon dioxide used, 15 per cent, brought about a decrease in the oxygen uptake by the beet tissue. A curve for these data was found to conform approximately with that of the onion given in Figure 2.

CARROT

Freshly-harvested, as well as commercially-stored, carrots were used in these experiments. The tops, however, were removed in all cases from the freshly-harvested carrots before exposing to the carbon dioxide treatment. The roots were carefully selected and averaged in weight from 54 to 64 grams each. As shown in Table IX the rate of oxygen uptake by freshly-harvested carrots was rather high while the rate of the carrots obtained from the commercial storage was somewhat lower. Upon storage in carbon dioxide the rate of the oxygen uptake by the freshly-harvested carrots was depressed in three of the four experiments. With commercially-stored carrots, however, the rate was in general only slightly depressed with the low concentrations of carbon dioxide and slightly increased with the higher concentrations. Although there were a few cases of considerable increase in the oxygen uptake in the presence of the high concentration of carbon dioxide, the number is small in comparison with the total number of tests made. The curve for the carrot calculated from the data in Table IX is shown in Figure 1. It is to be seen that concentrations of carbon dioxide up to 40 per cent bring about no apparent change in the oxygen uptake. Even with higher concentrations of carbon dioxide it is questionable whether the curve shows a significant increase over that of the control. Should one require the increase to be greater than twice the standard deviation for significance, the present data for the carrot would show that there is but little definite effect of the carbon dioxide upon the rate of the oxygen uptake by this tissue.

ASPARAGUS

For these experiments turgid asparagus shoots 15 cm. in length from tip of the bud to the base, and weighing from 12 to 16 grams each, were se-

TABLE XI

OXYGEN UPTAKE BY ASPARAGUS SHOOTS AT 25° C. IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour								
			Per cent of carbon dioxide within the container								
			0	17	28	32	42	50	56	58	60
57	336	4	260 244						189	188	
		8	223 216						189	188	
		12	222 194						164	163	
		16	204 192						151	150	
		20	192 184						144	128	
		24	192 191						132	125	
58	415	4	277 313		217	238		255		261	
		8	277 313		186	238		248		203	
		22	232 252		167	181		180		141	
59	405	4	235		212	189					
		8	235		212	189					
		12	235		212	189					
		16	223		197	181					
		20	222		188	176					
		24	214		182	168					
60	163	8	270	228			205	166			
61	160	12	245	192			164				134
62	155	16	244	200			154				142
63	171	24	205	186			163				148

lected. In the experiments numbered 50 and 51 in Table X, however, the average weight per shoot was 25 grams. As was expected, the respiration rate of the asparagus shoots was found to be exceedingly high and therefore necessitated experiments of short duration. The data in Table X show that carbon dioxide exerts only a retarding action upon the rate of the oxygen uptake of asparagus. This retardation in the rate became noticeable with the addition of as small an amount as 3 per cent of carbon dioxide to the storage atmosphere. With increasing concentrations of carbon dioxide the average percentage decrease in the rate of oxygen uptake increased until a maximum of about 35 to 43 per cent was obtained with 60 to 70 per cent of carbon dioxide. The trend of the data in Table X has been calculated and is shown in Figure 1. From this curve it is seen that any concentration of carbon dioxide above 10 per cent produces a significant decrease in the rate of oxygen uptake by the asparagus tissue.

Experiments 57, 58, and 59 in Table XI were set up as one experiment in each case and the gas analyses were made at different intervals of time. This procedure permitted a determination of the effect of the carbon dioxide upon the rate of oxygen uptake by the asparagus at intervals of four hours from the start of the experiment. The results obtained again showed that the carbon dioxide had a definite retarding effect upon the rate of oxygen uptake by the asparagus. With accumulation of carbon dioxide

TABLE XII

OXYGEN UPTAKE BY BUSH LIMA BEAN AT 25° C. IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour									Remarks
			Percent of carbon dioxide within the container									
			0	9	20	27	29	42	55	58	60	
64	345	24	207	204	205					118		In shell
65	305	17	255			198	195			172	171	Shelled
66	482	19	171	158	157			156			156	In shell
67	98	22	468	405		405			309			Shelled

from respiration there occurred a retardation in the rate of the oxygen uptake of the control as shown in experiment 57. Thus with the accumulation of 2 per cent of carbon dioxide in 4 hours the rate was reduced from 260 mg. to 223 mg.; and further with the accumulation of 6 per cent carbon dioxide in 16 hours to 192 mg. In the same experiment the rate was reduced from 260 mg. of oxygen uptake for the control to 188 mg. by storage in 58 per cent of carbon dioxide for 4 hours. These results are further supported by the data given in experiments 60 to 63 inclusive. These four experiments were started at one time with asparagus from the same source, but the atmosphere of each was analyzed at different times as shown in

TABLE XIII

OXYGEN UPTAKE BY BANANAS AT 25° C. IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE*

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour													
			Per cent of carbon dioxide within the container													
			0	3	6	12	18	23	26	28	34	36	42	52	54	56	75
68	1171	6	54					35							41		
		18	41					32							39		
		26	42					48							42		
		42	61					75							59		
69	1199	39	67 69		82	30			37				45	48			
70	770	20	154 144		144	154					124		118			107	
71	902	6	180						121	148				134	134		
		12	176						130	136				132	131		
		24	159						137	155				128	121		

* Exp. Nos. 68 to 75—Variety, Gros Michel; No. 76—Lady Finger; No. 68—Green, very hard, 6 fingers per test, 2 from each of 3 middle hands, No. 69—Green, hard, 7 fingers, 1 from each of 7 hands of middle of bunch; No. 70—Removed from 15° to 25° C. for 16 hours then placed in carbon dioxide test. Six fingers, 2 from each of 3 hands from center of bunch; No. 71—Handled as Exp. No. 70. Six fingers, 3 from each of 2 hands; No. 72—Yellow ripe, few brown spots. In 15° C. 4 days then in 25° C. 2 days, then cut up in 2 hands, 4 fingers from each hand; No. 73—2 fingers each test from same hand from 25° C. room. Light green color, no yellow present; No. 74—Green, hard, 1 finger from each of 5 middle hands; No. 75—Green, hard at start, 1 outer, 1 inner finger from each of 3 hands; No. 75—17 hrs., bananas set up as Exp. No. 75—6 hrs., then at end of 43 hours the gas mixture was replaced as originally, 20 per cent O_2 with or without CO_2 and held 24 hours then replaced again and the 17 hour test run. The bananas were yellow with green tint and spots; No. 76—Lady Finger bananas, 4 per test, turning light green, some yellow showing.

TABLE XIII (*Continued*)

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour														
			Per cent of carbon dioxide within the container														
			0	3	6	12	18	23	26	28	34	36	42	52	54	56	75
72	591	4	106 118				101					106				104	123
73	249	18	46 47			55	37				37	46			42		
74	667	21	58 47	57	61	45			63				32	47			
		37	85 71	83	87	61			88				37	58			
75	805	6	52 51						50					37			
		19	48 66						50					45			
		43	65 101						86					63			
		17	121 104						128					114			
76	283	16	119** 115 111**			111 106				106 108						107 106	

** Exp. No. 76—From same hand and rest from 2 hands one above and one below the former.

Table XI. One observes that the rate of oxygen uptake by the controls decreased with time due to the accumulation of carbon dioxide from the respiration and that any additional amount of carbon dioxide greatly reduced the rate of oxygen uptake by the asparagus. The data in Table XI give percentage decreases in the rate of the oxygen uptake by the asparagus ranging from 16 to 38 per cent with concentrations of carbon dioxide ranging from 17 to 60 per cent. A curve was calculated from these data and its form was approximately the same as that given in Figure 1.

LIMA BEAN

Lima beans in pods were separated into groups depending upon the number of beans in each. By this procedure it was thought possible to select for the experiment fairly uniform material when using equal numbers of pods containing in each either one, two, three, or four beans. In view of the fact that the respiration rate of this material depends upon many uncontrollable factors critical selection of the beans as to size and age was carried out as far as possible. The data in Table XII show that carbon dioxide retarded only slightly the oxygen uptake by the beans within the shell. If, however, the beans were removed from the shell the retarding action of 9 to 60 per cent carbon dioxide on the rate of the oxygen uptake was found to range from 13 to 37 per cent. These results are in accordance with those of previous workers who used seed in making the respiration studies. The retardation of the rate of respiration of the shelled beans was comparable with that found with asparagus.

BANANA

Care was used in selecting uniformly well developed bananas from the center of a bunch. Paired inner and outer fingers were cut from each hand and distributed among the storage containers after a record of the weight

TABLE XIV

VARIATION IN OXYGEN UPTAKE OF INDIVIDUAL BANANAS AT 25°C. IN AIR

Milligrams of oxygen per kilogram per hour		
Light green color. Top hand of hanging bunch	Yellow color firm. No. 3 hand	Yellow color firm. 1 each of 8 hands. Top to bottom of bunch
100	102	108
101	106	145
143	115	125
158	119	102
162	122	107
177	140	108
		103
		101

had been made. Approximately the same weight of bananas was used in each concentration of carbon dioxide studied in any one experiment. A study of the rate of the oxygen uptake of the control bananas as given in Table XIII shows that the green fruit has the lowest rate of respiration and that this rate increases with ripening. A treatment of the green or

TABLE XV

OXYGEN UPTAKE BY STRAWBERRIES AT 25° C. IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE

Exp. No.	Grams tissue used	Hrs. exp. ran	Milligrams of oxygen per kilogram per hour											
			Per cent of carbon dioxide within the container											
			0	8	10	16	20	34	40	53	58	66	73	76
77*	439	4	141 174							115	124			
		8	106 139							96	124			
		12	106 116							102	110			
		16	106 113							96	103			
		20	106 111							92	99			
		24	106 110							96	103			
78**	106	37	73 77		72	78	75		79		67		45	50
79*	201	19	135		130		109		114			84		
80*	164	41	105 111	105		105		102				91		
81*	130	18	95 97							56				
		42	107 110							83				
82*	113	38	132	85		93		78			96		73	

* Variety Chesapeake.
** Steven's Late Champion.

ripe bananas with 30 per cent or more of carbon dioxide reduced the rate of respiration approximately 20 per cent. At the midway stage of ripening there was found in these experiments only a slight reduction in the oxygen uptake by storage in the carbon dioxide. The data in Table XIII gave a curve that was similar to the strawberry curve in Figure 2. In experiments

68, 71, 74, and 75 the gas analyses were made at frequent intervals but this gave little additional information on the effect of the carbon dioxide on the rate of oxygen uptake by the fruit. The variability in the rate of respiration of the fruit, especially when extended periods of storage were used, became very pronounced in these experiments. A storage period of six hours was found desirable for this work since less variation was encountered in the retarding action of the carbon dioxide in depressing the oxygen uptake by the fruit. The results of the longer periods show, however, that even in the presence of considerable carbon dioxide there is an increase in the rate of respiration accompanying the ripening of the fruit. This increase, however, is somewhat lower than that found with the control. With the precautions used in selecting the banana samples there was found considerable variation in the rate of oxygen uptake as given in Table XIII. In an attempt to account for this variation the rate of oxygen uptake by individual fingers from a hand or a bunch of bananas was determined and the results are given in Table XIV. Bananas selected by appearance to be alike may vary considerably as shown by these data when the maximum difference was 77 mg. of oxygen uptake. These results show that considerable variation in the rates from a sample of two to eight bananas such as used in the experiments of Table XIII could be expected.

STRAWBERRY

Strawberry fruits in a firm ripe condition and weighing from three to five grams each, except in experiment 77, Table XV, where the fruit weighed nine grams each, were carefully selected for these experiments. The data in Table XV show that the oxygen uptake by strawberries is somewhat retarded by storage in carbon dioxide. However, the rate is not greatly altered until a concentration of more than 50 per cent of the gas is employed. This observation is further shown by the curve in Figure 2. Although the trend of the curve is definitely downward it is of interest to find that the rate of oxygen uptake by the active fruit is not greatly altered by the carbon dioxide in concentrations from 10 to 40 per cent.

DISCUSSION

In these experiments it has been found that with the controls the oxygen uptake or carbon dioxide output gave the same results in volume of gas exchange. However, with storage in carbon dioxide the carbon dioxide output by the tissue was not equal to, but less than, the oxygen uptake. This difference between the two became greater, reaching a maximum of approximately 40 per cent, with increased amounts of carbon dioxide in the storage atmosphere. It may be that in these cases the course of respiration is altered so that less carbon dioxide is produced. An alternative suggestion is that the carbon dioxide produced in respiration is not com-

pletely eliminated. In this event there is the possibility of its combination with other substances in the tissue as well as the solution in the cell sap. Analyses only of the carbon dioxide in the atmosphere external to the tissue would not, in these cases, furnish a suitable measure of the amount produced by the tissue. This result has been shown before in the data of Kidd (6) with seeds, and has been discussed by Bennett and Bartholomew (2) in connection with potato respiration studies. Spoehr and McGee (11) in their work with leaves have considered this reduction of carbon dioxide output in the presence of carbon dioxide as due to an equilibrium between the leaf tissue and external concentration of carbon dioxide.

It seems that, at least in experiments in which high amounts of carbon dioxide are used, the oxygen uptake would be a better measure of respiration than the carbon dioxide production. However, it is not of critical importance in the present experiments whether oxygen uptake or carbon dioxide output is used as a measure of the effect of carbon dioxide in the storage atmosphere, since the same general conclusions are reached from considering the data upon either basis. In all of the tests where the data show an increase in the oxygen uptake there is also an increase in the carbon dioxide output over that of the control. Likewise the tests that show decreases in oxygen uptake also show decreases in the carbon dioxide production.

The variation in production of carbon dioxide by tissues after exposure to accumulating carbon dioxide during storage has been shown and discussed by Willaman and Beaumont (14, p. 52). They found that the evolution of carbon dioxide by apple twigs, potato tubers, and wheat seed during the first periods of aeration, following the accumulation period, was considerably higher than that previous to the accumulation. Furthermore, the production of carbon dioxide decreased with each successive period. They have suggested two theories to account for the decided increase in carbon dioxide produced under these conditions: (a) on the basis of the findings of Spoehr and McGee which they considered of minor importance and least possible, since they could not correlate the amount of carbon dioxide formed with the small amount the tissue would hold in a gaseous or dissolved state; and (b) "That the accumulation of CO_2 in the tissues increases the hydrogen-ion concentration in the latter; that this brings the proteins of the protoplasm nearer to their isoelectric point, and hence increases its permeability, which is responsible (perhaps through increased enzyme activity) for an actual increased rate of CO_2 production." These authors studied the effect of hydrochloric acid fumes upon the rate of the carbon dioxide production and found the same results as with the accumulation of carbon dioxide in the atmosphere. From this they conclude that the hydrogen ion factor is the important one. They did not, however, consider that hydrochloric acid is toxic to the wheat seed and may therefore

have altered the respiration. At a subsequent date Willaman and Brown (15) determined the amount of dissolved carbon dioxide in plant sap. In discussing their findings they referred to the results of Willaman and Beaumont (14) and suggested that the temporary increase in production of carbon dioxide was a result of the effect of higher acidity of the sap upon the respiration, augmented in part by previously formed carbon dioxide that was held in solution during the accumulation period. However, as will be shown in a paper to follow, carbon dioxide does not *increase*, but *decreases* the hydrogen ion content of the plant tissues. The theory that carbon dioxide affects the respiration of the tissue through altering the hydrogen ion concentration cannot be applied in this case. The proof of this is shown by the fact that carbon dioxide increases the respiration of the potato tuber and decreases the respiration of the asparagus, but in both cases it decreases the hydrogen ion concentration of the tissue.

In the light of the present researches the data of Willaman and Beaumont (14) may be interpreted further. In the case of the apple twigs and wheat seed the carbon dioxide output during the aeration periods, after reaching a maximum, decreased rapidly if the accumulation period had been of a long duration. With potatoes, however, following a prolonged accumulation period the carbon dioxide output during the subsequent aeration periods was maintained at a high rate, decreasing slowly, but never approaching the former rate during the experiment, as observed with other tissues. This would seem to indicate that the respiration of the potato tubers had been increased to some extent by the treatment.

Visible injury to the tissue as a result of carbon dioxide treatments has not been observed either during the treatment or after removal to air except in the case of the potatoes treated after sprouts had developed. The injury to the potato sprouts appeared to be a local effect on the growing bud, but these or other sprouts from the tuber grew well after removal to normal atmosphere. In the previous work (13) injury to plant tissue occurred because of the replacement of the normal atmosphere with various concentrations of carbon dioxide. In such experiments the initial concentration of oxygen was lowered considerably and only a short period of storage was necessary to deplete the oxygen supply. The experiments reported in this paper were carried out with the normal content of oxygen in the atmosphere at the beginning of the storage period and the treatment period was not long enough to deplete this supply.

SUMMARY

1. The oxygen uptake by plant tissue held at 25° C. in various concentrations of carbon dioxide with 20 per cent of oxygen was determined by gas analyses of the storage atmosphere at the beginning and end of the experiment as well as at various intervals during storage. These measure-

ments were made over a wide range of storage periods with plant tissue in various stages of development.

2. The rate of oxygen uptake by potatoes was increased 100 to 200 per cent by storage in concentrations of carbon dioxide ranging from 45 to 60 per cent. Similar results were obtained at 15° C.

3. If the periods of storage of potatoes in carbon dioxide were greater than 20 to 24 hours increases were found, but if the storage periods were less than this decreases in the rate of oxygen uptake resulted.

4. The rate of oxygen uptake by onion bulbs was increased from 20 to approximately 100 per cent by 30 to 70 per cent of carbon dioxide. The rate was slightly decreased with lower amounts of carbon dioxide.

5. The oxygen uptake by tulip bulbs was decreased with 10 per cent and increased with 65 per cent of carbon dioxide.

6. The oxygen uptake by beet roots was increased approximately 60 per cent by high concentrations of carbon dioxide, and was decreased slightly with 15 per cent of carbon dioxide.

7. The rate of oxygen uptake by carrot was not significantly altered by storage in carbon dioxide.

8. The oxygen uptake by asparagus shoots and shelled lima beans was reduced as much as 35 per cent by storage in carbon dioxide.

9. The oxygen uptake by banana and strawberry fruits was reduced by carbon dioxide, but the reduction was not marked until 30 per cent or more of carbon dioxide was employed.

10. Measurements of the carbon dioxide output gave results that substantiated the conclusions which were derived from the measurements of oxygen uptake.

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CARBON DIOXIDE STORAGE. IV. THE INFLUENCE OF CARBON DIOXIDE ON THE ACIDITY OF PLANT TISSUE[1]

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Since carbon dioxide has been found to have an effect on respiration as well as other metabolic processes it would be desirable to determine whether the effects were due specifically to carbon dioxide or merely to its effect on the acidity of the living cells. As carbon dioxide has acidic properties it might be expected that in the presence of a sufficient concentration of carbon dioxide plant tissue would become more acid. This is the prevailing conception of the problem as is expressed by the statement of Willaman and Beaumont (11, p. 52) "That the acidity of the tissue fluids is increased by the accumulation of carbon dioxide is well known, and does not need a specific illustration." Such a conclusion has been drawn from the fact that carbon dioxide has an acidic property and that it increases to a considerable extent the acidity of extracted plant juice (Small, 9) and of water and bacteriological media (Platz, 6). However, where living, intact plant tissues and not the extracted juices alone are concerned fundamental differences are obtained in the acidity of the tissues which have been stored in atmospheres enriched with carbon dioxide.

Scarth (8) has suggested the acidifying power of H_2CO_3 as an explanation of another physiological response. He correlated the pH value of the contents of the living guard cells with stomatal movement. The pH reaction with the open stomates was alkaline and the acid reaction with the closed stomates was considered to be due to the accumulation of carbon dioxide from respiration. With its disappearance, due to photosynthesis, the hydrogen ion concentration of the guard cells is reduced and the stomates open.

Magness and Diehl (4), while studying the effect of 0 to 100 per cent of carbon dioxide for ten days on the ripening of apples at 22° C., observed that in some cases the acidity of the fruit was decreased. With the Winesap apple the titratable acidity expressed as cc. N/10 acid per 10 g. wet weight of tissue decreased from 6.55 to 6.14 with treatment of 100 per cent of carbon dioxide. The acid changes in the Delicious apple was found to be less marked.

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Brooks and his coworkers (1) have reported that the flavor of peaches exposed to various concentrations of carbon dioxide was slightly less acidic than that of the controls.

The present results show that carbon dioxide in the presence of oxygen does not increase the hydrogen ion concentration of any plant tissue, so far investigated, but instead decreases the hydrogen ion concentration to a very marked degree. These studies were made with tissues ranging in pH from 3.3 to 6.4. This acidity change takes place rapidly with some tissues and slowly with others. An increase occurs even in tissues exposed to low concentrations of carbon dioxide accumulating from respiration of the tissue. The total change in the pH is dependent upon the kind of tissue, its initial pH, the concentration of carbon dioxide, and the length of the storage period. This change is a fundamental physiological process since potatoes in 100 per cent of carbon dioxide will become more acid, and in carbon dioxide with oxygen, more alkaline than the controls. The effect is not permanent since the tissue after removal from the treatment will resume approximately the original pH value.

MATERIAL AND METHODS

The tissues used were as follows: potato (*Solanum tuberosum* L.) tubers; onion (*Allium cepa* L.) bulbs; tulip (*Tulipa gesneriana* L.) bulbs; beet (*Beta vulgaris* L.) roots; carrot (*Daucus carota* L.) roots; asparagus (*Asparagus officinalis* L.) shoots; bush lima bean (*Phaseolus lunatus* L.); banana (*Musa paradisiaca* L. var. *sapientum* Kuntze) fruit; strawberry (*Fragaria chiloensis* Duchesne) fruit; apple (*Pyrus malus* L.) fruit; peach (*Prunus persica* (L.) Stokes) fruit; orange (*Citrus sinensis* Osbeck) fruit; tobacco (*Nicotiana tabacum* L.) plant; tomato (*Lycopersicon esculentum* Mill.) plant. The fruits and vegetables were obtained from a local wholesale dealer. The tobacco and tomato plants were taken from the greenhouses immediately before exposing to the carbon dioxide treatment.

The apparatus used for the production and maintenance of the gas mixtures and the methods employed in the treatment of the plant tissue were the same in this work as already discussed in a previous report (10). The gas mixtures were made up with 20 per cent of oxygen with varying percentages of carbon dioxide and nitrogen to make 100 per cent unless otherwise mentioned in the results. Gas analyses to determine the carbon dioxide and oxygen content of the atmospheres were made with an Orsat apparatus at the beginning and end of each experiment. The carbon dioxide used was the product of fermentation in a process connected with the refinement of cane sugar and manufacture of ethyl alcohol. Comparative tests made on tissue treated with carbon dioxide from the cylinder and from the action of sulphuric acid upon pure sodium carbonate showed

that the gas from the two sources had the same effect on the acidity of the plant tissue.

In a majority of the experiments the plant tissues were held at 25° C. not only during the tests, but also during the preliminary storage period for the temperature adjustment. The extraction of the juice and pH determinations were carried out at room temperatures of 22° to 25° C.

From 300 to 1000 g. of tissue were used in each concentration of carbon dioxide tested.

The tissues when removed from the carbon dioxide treatments were washed, peeled, or brushed and the excess moisture removed with cheesecloth. The tissue was ground through a butternut cutter of a food chopper, and then squeezed by hand through a double thickness of cheesecloth. The food chopper was rinsed with distilled water and dried after grinding each sample of tissue. The pH was determined upon most of the tissue extracts without diluting. With the banana fruit and green lima bean, since very little juice was obtained, a weighed sample of tissue was mixed and ground with a definite quantity of distilled water, then extracted as was done with the other tissues.

The pH determinations were made with the quinhydrone electrode, using a saturated calomel half cell as reference electrode. This apparatus was frequently checked against M/20 potassium acid phthalate buffer. Various indicators were used directly upon the cut surfaces of the tissues under examination. For further comparison the Youden and Dobroscky (12) glass electrode was used, especially in the tests with asparagus and banana. The glass electrode was depended upon entirely for the measurements of pH change in the onion. The quinhydrone electrode measurements of the onion juice were not dependable apparently because of some peculiar property of the juice. During measurements of the banana and asparagus it was necessary to clean the platinum electrode, coming in contact with the juices, after every few tests. This precaution was found necessary since a deposit was formed on the electrode which interfered with the measurements.

In all experiments a study was made of the change in pH of the tissue in air during the period of each test. For this purpose there were two room air controls, the pH of one determined at the beginning and the other at the end of the experiment. In the tables of data only one of these controls was used since there was no significant (less than 0.05 of a pH unit) change in pH of the tissue during the experiment. For a third control the tissue was placed in the sealed container with 20 per cent oxygen and 80 per cent nitrogen; any change in the pH of the tissue under this condition has been attributed to the effect of the carbon dioxide produced by the respiration of the tissue.

TABLE I

INCREASE IN pH OF PLANT TISSUE RESULTING FROM STORAGE IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE, WITH 20 PER CENT OF OXYGEN, AT 25° C.

Tissue used	Hrs. of test	pH of the tissue juice upon extracting														
		Control in		Per cent of carbon dioxide within the container at the beginning												
		Room	Container	8	14	18	22	28	35	40	47	52	57	60	72	76
Potato	168	6.34	6.34				6.41				6.59				6.78	
	144	6.36	6.39						6.73				6.90			
	144	6.20	6.19						6.51						6.85	
	144	6.19	6.29		6.29		6.44								6.80	
	144	6.12	6.17						6.37				6.47			
	144	6.03	6.22		6.34			6.53						6.61		
	141	6.03	6.32		6.36			6.58			6.70		6.75			
	140	6.10	6.17						6.56							
	133	6.10	6.17						6.58							
	120	6.36	6.36				6.41		6.61					6.71		
	114	6.10	6.25			6.36			6.49					6.61		
	96	6.27	6.30						6.54				6.70		6.85	
	90	6.19	6.25	6.31		6.31			6.41				6.81			
	90	6.19	6.24	6.29		6.29			6.36				6.80			
	90	6.10	6.17			6.25				6.34				6.47		
	72	6.03	6.27		6.32				6.61					6.63		
	67	6.05	6.10						6.27							
	67	6.03	6.27		6.32			6.61						6.63		
	66	6.10	6.17			6.24			6.26				6.37			
	44	6.03	6.27	6.36			6.53			6.68				6.68		
	42	6.03	6.05	6.08			6.27			6.37				6.53	6.79	
	41	6.00	6.05			6.05			6.31				6.41			

TABLE I (*Continued*)

Tissue used	Hrs. of test	pH of the tissue juice upon extracting														
		Control in		Per cent of carbon dioxide within the container at the beginning												
		Room	Container	8	14	18	22	28	35	40	47	52	57	60	72	76
Asparagus	24	6.34	6.36					6.61	6.70							
	24	6.25	6.25					6.63		6.70						
	20	6.39	6.63				6.81			6.95				7.13		7.10
	19	6.40	6.47										7.08			
	18	6.19	6.22	6.25	6.27									6.58		6.61
	18	6.39	6.47											7.08		
	24	6.34	6.46			6.59				6.90				7.07		
	16	6.34	6.48			6.63				6.85				6.90		
	12	6.34	6.39			6.60				6.90				6.95		
	8	6.34	6.36			6.59				6.71		6.78				
Lima bean	17	6.37	6.42					6.63						6.70		
	17	6.37	6.42					6.63						6.70		
	22	6.36	6.44	6.52				6.65						6.74		
Carrot	115	6.19	6.31			6.49				6.93			7.12			
	114	6.20	6.24	6.44		6.54				6.85				6.88	6.93	
	113	6.15	6.15						6.76							
	99	6.19	6.20	6.49		6.56				6.86		6.88			6.93	
	93	6.10	6.19									6.80				
	91	6.10	6.13									6.78				
	91	6.17	6.27			6.64				6.80				7.08		
	90	6.15	6.20			6.53			6.71				6.95			
	70	6.10	6.15			6.53			6.68					6.95		
	24	6.19	6.32	6.36		6.61			6.59				6.63		6.71	
Beet	96	5.85	5.93			6.36			6.66				6.78		6.83	
	64	6.09	6.10			6.36				6.73		6.91			7.02	
	22	6.09	6.12			6.31			6.44				6.46		6.51	

TABLE I (*Continued*)

INCREASE IN pH OF PLANT TISSUE RESULTING FROM STORAGE IN VARIOUS CONCENTRATIONS OF CARBON DIOXIDE, WITH 20 PER CENT OF OXYGEN, AT 25° C

Tissue used	Hrs. of test	pH of the tissue juice upon extracting														
		Control in		Per cent of carbon dioxide within the container at the beginning												
		Room	Container	8	14	18	22	28	35	40	47	52	57	60	72	76
Tulip	69	5.56	5.61	5.68										5.80	5.87	
	68	5.56	5.65	5.68										5.80	5.85	
	66	5.56	5.59												5.85	
Onion	116	5.30	5.47			5.65				5.64			5.89	5.89		
	92	5.35	5.38			5.43			5.55				5.94			5.96
Banana	20	5.31													5.92	
	20	5.31													5.92	
	6	5.00													5.21	
	2	5.00													5.09	
	2	4.99													5.09	
Strawberry	41	3.40	3.40	3.40		3.42			3.48		3.50				3.60	
	37	3.36	3.36	3.36	3.36	3.36	3.38			3.46			3.53	3.53		3.53
	24	3.33	3.41												3.51	
	20	3.44	3.48							3.51						
Tobacco plant growing in soil	2	5.85	5.93				6.36			6.47					6.60	
	2	5.93	6.02				6.49			6.63					6.73	
	4*	5.90	5.92				6.36			6.36					6.53	
	4*	5.92	5.91				6.32			6.34					6.44	
Tomato plant growing in soil	5	5.53	5.68				6.09			6.27					6.34	
	5	5.53	5.65				6.07			6.20					6.27	
	5	5.54	5.66				6.02			6.17					6.27	
	4.5	5.59	5.68				6.09			6.17					6.19	
	4.5	5.59	5.68				6.05			6.12					6.10	

* Plants had only three hours of dim light during 20 hours preceding the experiment.

RESULTS

pH OF JUICE FROM TREATED AND CONTROL TISSUE

The change in pH of the tissue resulting from treatment with carbon dioxide is shown in Table I. In all cases an increase in the pH of the plant tissue is observed with increasing concentrations of carbon dioxide and lengthening of the storage period. This increase amounts to as much as 0.9 of a pH unit with some tissues having an original pH value greater than 5.0. With the tissues having an initial pH value less than 4.0 there was found a small, but consistent, difference between the control tissue and that treated. with carbon dioxide. In these cases as in all others no tissue has been observed that became more acid during treatment with carbon dioxide in the presence of oxygen.

An increase in the pH of plant tissue treated with carbon dioxide is obtained even with plants growing in soil. Tobacco and tomato plants, 6 to 8 inches high, removed from the greenhouse at 10 o'clock in the morning and placed in carbon dioxide for various lengths of time, gave decided increases in pH as shown in Table I. The maximum increase of 0.8 of a pH unit was obtained by the treatment of the tomato with 72 per cent of carbon dioxide for five hours. With the tobacco plant treated for two hours the increase amounted to approximately 0.7 of a pH. These pH determinations made by the quinhydrone electrode were duplicated with the glass electrode. The pH determinations on this very active tissue must be taken immediately upon removal from the carbon dioxide; otherwise the fullest extent of the change is not obtained because the tissue, upon standing in air, gradually becomes more acid and approaches its original pH value. In no case with these periods of treatment or concentrations of carbon dioxide was injury to the tissues observed.

The accumulation of carbon dioxide by respiration of the tissues, as little as 2 per cent with potato and as much as 12 per cent with asparagus and lima bean, brought about a change in the pH of the tissues. Although some of the differences in pH values obtained under these conditions are not significant, the consistency of the results warrants consideration.

A few tests have been made upon the McIntosh apple having a pH value of 3.4. The results to date indicate that an increase of 0.2 to 0.3 of a pH unit may be obtained with treatment of freshly-harvested apples with 70 per cent of carbon dioxide for 120 hours. However, the apples treated with carbon dioxide 30 or more days after harvesting showed an increase of approximately 0.1 of a pH unit. Further tests on the apple at various stages of maturity would be of interest.

A maximum increase of 0.15 of a pH unit was obtained with the Elberta peach held in as much as 70 per cent of carbon dioxide for periods up to 48 hours. Lower concentrations of carbon dioxide produced a consistent increase in the pH of the peach.

Oranges, having a pH value of 3.5, were held in various concentrations of carbon dioxide up to 70 per cent for 26 to 120 hours. After treatment the pH was determined upon the pulp and skin ground together and upon the pulp and skin separately. The extract of pulp and skin together showed a maximum increase of 0.15 of a pH unit with the treatment. With the juice alone there was no significant change in the pH. However, with the peel there occurred an increase of approximately 0.4 to 0.5 of a pH unit. These results indicate the importance of active cells in the production of the change in pH value of the tissue with carbon dioxide treatment.

RAPIDITY OF THE PH CHANGE

Length of treatment. The extent of the pH change of the tissue is influenced to a considerable degree by the length of the storage period in carbon dioxide. The rapidity of the pH change, however, is apparently regulated by the activity of the tissue. With active tissue such as asparagus shoots the increase in pH occurs soon after placing in the gas, but with a less active tissue such as potato tuber the pH change occurs only after many hours of the treatment. Experiments with asparagus in 50 to 70 per cent of carbon dioxide with 20 per cent of oxygen for 15 minutes showed an increase of 0.1 of a pH unit over the control; for 30 minutes an increase of 0.24 of a pH unit; and for one hour an increase of approximately 0.4 of a pH unit. The less active beet root tissue required a longer period of time, approximately 4 to 6 hours, before there was any indication of a change in pH with the treatment. With carrot roots an increase in pH occurred after 6 to 8 hours of the treatment. Tests with the potato indicated that 12 or more hours of treatment are necessary before an increase in the pH of the tubers occurs.

pH change after removal from the treatment. Upon removal of the tissue from the carbon dioxide treatment to room air a gradual reduction in the pH of the sap occurs. It is apparent that this lowering of the pH to the original value is influenced by the activity of the tissue. Tests made with asparagus shoots and potato tubers in 50 per cent of carbon dioxide with 20 per cent oxygen gave the following results: asparagus treated 24 hours increased 0.5 of a pH unit, and potato tubers treated 48 and 96 hours increased 0.3 and 0.4 of a pH unit, respectively. Upon removal to air at 25° C. the asparagus resumed its original pH value in approximately 20 to 24 hours as compared with 48 to 72 hours for the potato. These results are the average of a number of experiments and again show the influence of the activity of the tissues upon the pH change induced by the carbon dixoide treatment.

SPECIAL TESTS

Treatment of cut pieces of potato tubers separately. In each experiment six potato tubers were used. The individual tubers were cut into three

pieces each of which represented about one-third of the total volume of the tuber. Upon treating each part separately with various concentrations of carbon dioxide there resulted an increase in pH of the tissue juice. The

TABLE II

EFFECT OF CARBON DIOXIDE UPON PIECES OF CUT POTATO TUBERS TREATED FOR 95 HOURS AT 25° C.

Treatment	pH of extracted juice	
	Dormant	Non-dormant
Control, in room	6.09	6.19
Control, in container	6.15	6.27
33% CO_2	6.44	6.70

data in Table II show this change obtained with both dormant and non-dormant potatoes. It is apparent that the effect of the carbon dioxide is independent of whether the potatoes are whole or cut into pieces when treated. Upon removal to air the pH of the treated pieces dropped slowly to approximately the same value as the controls.

pH of juice from different parts of the potato tubers. Determinations of the pH of various parts of the potato tuber have been made according to the method employed by Miller (5, p. 326–327). The pH data for various layers of three tubers in each treatment are given in Table III. As Miller

TABLE III

pH OF EXTRACTED JUICE FROM THE OUTER, MIDDLE, AND INNER PORTIONS OF AN IRISH COBBLER POTATO TUBER AFTER TREATMENT IN A WHOLE CONDITION FOR 144 HOURS AT 25° C.

Treatment	pH of juice from layers		
	Outer	Middle	Inner
Control, in room	5.58	5.71	5.80
Control, in container	5.85	6.02	6.09
30% CO_2	6.42	6.53	6.53
60% CO_2	6.98	6.90	6.70

observed, the outermost layer of the potato tuber has a low pH value which increases as one approaches the center of the tuber. Storing the tubers in 30 per cent of carbon dioxide increases the pH of the entire tuber. However, 60 per cent of carbon dioxide increased the pH to a greater extent in the layer next to the skin than in the center of the tuber. The total increase in pH of the layers upon treatment with 60 per cent of carbon dioxide becomes respectively: inner 0.9, middle 1.19, and outer 1.30. This apparent gradient in pH change may possibly be correlated with the penetration of oxygen into the tissue. Parallel with the pH change the

course of activity of this tissue is being influenced by the presence of the carbon dioxide.

Direct effect of carbon dioxide upon the extracted juice. Small (9) has shown that the pH of extracted juice is depressed by storage in atmospheres enriched with carbon dioxide. This change is brought about by the direct acidic effect of the the carbon dioxide. The same results may be obtained by bubbling the gas through the extracted juice for various periods of time as is shown by the data in Table IV. With the potato and carrot

TABLE IV

PH OF EXTRACTED JUICE AS AFFECTED BY BUBBLING CARBON DIOXIDE THROUGH IT FOR VARIOUS LENGTHS OF TIME

Minutes of passing CO_2 into the juice	pH of extracted juice		
	Green Mt. potato	Carrot	McIntosh apple*
0	6.02	6.14	4.07
10	5.85	5.93	4.07
20	5.85	5.93	4.07
60	5.51	5.81	4.07

* After storage at 2° C. for five months, same result obtained at harvest time except pH at about 3.4.

the pH of the juice was lowered considerably by the dissolving of the carbon dioxide in the juice. However, in the case of the apple juice the carbon dioxide did not change the pH even after one hour of exposure. Similar tests conducted on juice of freshly-harvested apples showed the same results.

Effect of dissolved carbon dioxide upon the pH of the extracted juice of treated whole tubers. Since carbon dioxide dissolving in the extracted juice of untreated potatoes tends to lower the pH, it was necessary to study the effect of the dissolved gas in the extracted juice of treated potatoes. The data in Table V show the effect of various treatments to rid the juice from the treated whole tubers of the dissolved carbon dioxide. The results show that centrifuging the juice after extracting alters the pH to only a slight

TABLE V

PH STUDIES OF THE EXTRACTED JUICE OF CARBON DIOXIDE-TREATED AND UNTREATED WHOLE POTATOES. THE TUBERS WERE TREATED 144 HOURS AT 25° C.

Treatment	pH of juice				
	Upon extracting	After centrifuging	After aerating		After boiling and filtering
			5 min.	10 min.	
Control, in room	5.80	5.87	5.98	6.05	6.03
Control, in container	6.17	6.22	6.37	6.42	6.44
30% CO_2	6.34	6.41	6.64	6.68	6.66
60% CO_2	6.80	6.80	7.18	7.44	7.59

extent. A more complete removal of carbon dioxide from the juice was obtained by aerating with air or nitrogen with the result that there was a decided increase in the pH. However, the fullest extent of the pH change was found by boiling and filtering the juice. After boiling and cooling the juices the difference between the treated and control was 1.56 of a pH unit. These same lots of juice showed a difference of 1.00 of a pH unit when tested before the carbon dioxide dissolved in the juice was expelled by boiling.

Titration of extracted juice. Titration curves for the extracted juices of treated and control Irish Cobbler potatoes show no significant differences in the buffer capacity of the tubers after the 40 to 70 per cent carbon

TABLE VI

CHANGE OF pH OF GREEN MOUNTAIN POTATOES WITH CHANGE IN TEMPERATURE AND THE EFFECT OF VARIOUS CONCENTRATIONS OF CARBON DIOXIDE ON THIS CHANGE DURING STORAGE OF 144 HOURS

Treatment	pH of extracted juice			
	2°C.	5°C.	15°C.	25°C.
Control, in room		5.76	6.09	6.14
Control, in container		5.80 5.82	6.12 6.12	6.20 6.20
60% CO_2		5.98 6.00	6.30 6.30	6.47 6.49
Control, in room Control, in container	5.53 5.52	5.51 5.51	5.75 5.75	5.81 5.80
30% CO_2	5.63 5.68	5.75 5.73	6.05 6.09	6.36 6.34
60% CO_2	5.68 5.70	5.85 5.88	6.17 6.19	6.36 6.37

dioxide treatment. Titrating the juice with N/10 acid and alkali through a pH range of 3.0 to 8.4 the curves were found to be nearly parallel in most cases. The position of the curves is altered somewhat, but this is expected since the treated tissue has a higher pH value than the control. Any deviation from the usual position of the titration curves for the treated and control juices was shown by their being a little closer in the acid range than in the alkaline. If, however, unaerated or unboiled juice is used for the titrations the curve for the treated juice does not remain as smooth as the control, due to the presence of a large amount of dissolved carbon dioxide. The curve for the treated juice is altered on the acid side of the observed pH of the extracted juice. The addition of from 0.01 cc. to as

much as 0.2 cc. of N/10 acid may not alter the pH or in some cases may increase slightly the pH of the juice. The addition of more acid, however, will decrease the pH of the juice, giving a curve nearly parallel with the curve for the control. The presence of dissolved carbon dioxide brings about this change in the titration curve since after its removal by stirring, aerating with air or nitrogen, or boiling, the titration curve for the same juice becomes smooth over the pH range studied.

pH change with indicators. The differences in pH of treated and control potatoes could be observed by direct application of bromthymol blue and phenol red to the cut surfaces of the tubers. Similar results were obtained with bananas by placing methyl red on the cut surface of the pulp.

Temperature of storage and pH change. The temperature of storage of the potato tuber has a marked effect upon the pH of the juice extracted from the tissue as shown by the data in Table VI. With the Green Mountain variety of potatoes used in these experiments a difference of approximately 0.3 of a pH unit was observed between storage temperatures of 2° and 25° C. Treatment of the potato tubers with various concentrations of carbon dioxide in 20 per cent of oxygen resulted in an increase in pH of the extracted juice in all cases. The results show the greatest increase in pH when the treatment was at a storage temperature of 25° C. and least with a temperature of 2° C. These results show further that the magnitude of the pH change with the treatment is dependent upon the activity of the tissue.

CONCENTRATION OF OXYGEN IN THE STORAGE ATMOSPHERE AND THE PH CHANGES OF POTATO TISSUE AS AFFECTED BY CARBON DIOXIDE

It was observed that, after storage periods of more than 96 hours with carbon dioxide, the tissue in the containers having the highest concentration of oxygen always gave the greatest increase in pH over the control. Likewise the tissue in the lowest concentration of oxygen at the end of the test gave the smallest increase in pH. This observation led to the question of what would happen in respect to the pH change of the tissue if there was little or no oxygen present with the carbon dioxide at the beginning of the storage period. Tests of this nature were made and the results given in Table VII show that *potato tubers in the presence of carbon dioxide without oxygen become more acid than the controls during the same period of time that tubers in carbon dioxide with oxygen become more alkaline than the controls.*

The results given in Table VII show the effect of various lengths of storage upon the pH change of the tissues held in the different gases. It appears that from 96 to 168 hours of storage are necessary to bring about the maximum pH change. The results for periods of storage less than 96 hours are probably affected by the oxygen present within the tissue, while

periods longer than 168 hours result in too great a depletion of the oxygen supply. The effect of depleting the oxygen supply may be observed with the experiment in Table VII carried on for 192 hours. The tissue placed in 5, 10, and 20 per cent of oxygen used up this supply before the end of the test, with the result that the tissue became more acid in the case with 5 and 10 per cent oxygen and was approaching its original pH in the case with 20 per cent oxygen. However, the tissue placed in 40 per cent of

TABLE VII

EFFECT OF CARBON DIOXIDE, WITH AND WITHOUT OXYGEN, ON THE pH OF THE JUICE OF POTATO TUBERS TREATED IN THE WHOLE CONDITION. TUBERS HELD AT 25° C. PRECEDING AND DURING THE EXPERIMENTS

Treatment	pH of extracted juice							
	Hours of storage							
	48	96		144			168	192
		Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 3		
Control, in room	6.02	6.02	5.95	6.19	6.20	6.10	6.19	6.19
Control, in container		6.02			6.20		6.19	
100% O_2				6.19	6.22	6.09	6.24	6.17
100% N_2				6.19	6.05	6.09	6.05	6.14
100% CO_2		5.61		5.89	5.70	5.61	5.87	5.53
80% CO_2 20% N_2	6.03		5.85				5.76	
80% CO_2 5% O_2 15% N_2				6.42		5.90		5.87
80% CO_2 10% O_2 10% N_2	6.10		6.09	6.47		6.27	6.30	5.93
80% CO_2 15% O_2 5% N_2				6.51		6.40		
80% CO_2 20% O_2	6.20	6.42	6.27	6.68	6.54	6.49	6.46	6.25
60% CO_2 40% O_2								6.70

oxygen at the start did not deplete this supply before the end of the test; therefore, the tissue was still more alkaline than the control. From these results it appears that the decrease in pH of the potato tuber produced by carbon dioxide without oxygen is approximately equal to the increase in pH produced by carbon dioxide with 20 per cent of oxygen.

The pH of the potato tissue in pure oxygen changed very little, while that in pure nitrogen became more acid with the depletion of the oxygen within the tissue and production of carbon dioxide by respiration.

Upon boiling the extracted juices of the potatoes receiving these varied treatments it was found that the pH increased with the elimination of the dissolved carbon dioxide. The total amount of the pH change, either on the acid or alkaline side of the control, was not greatly altered since the increase in all cases was approximately the same. This result indicates that the acidifying effect of this treatment of potatoes with carbon dioxide in the absence of oxygen involves a far more complicated process than that by which carbon dioxide merely becomes dissolved in the tissue sap.

DISCUSSION

The relation of pH of the tissue to its respiration has been investigated by many research workers. Gustafson (2) has reported that in the case of *Penicillium chrysogenum* a variation in pH of the medium between 4.0 and 8.0 did not affect the respiration, but that a more acid medium increased and a more alkaline medium decreased the respiration. At a later date Gustafson (3) studied the respiration of maturing tomato fruits having a pH value between 4.0 and 5.0. From these studies he suggested that at the point of minimum respiration the maximum hydrogen ion concentration was reached and with increasing respiration there occurred a reduction in the hydrogen ion concentration of the cell sap. Although the plants used by Gustafson show a rise and fall of the rate of respiration with the pH change, it cannot be concluded that this is the general reaction for all plant tissue, a fact that is evident from the results of these and of previous experiments (10). Potato tubers that showed an increase in the rate of respiration as a result of treatment with carbon dioxide at the same time showed a decrease in the hydrogen ion concentration. On the other hand carbon dioxide decreased both respiration and hydrogen ion concentration of asparagus shoots. With the tulip bulbs low concentrations of carbon dioxide reduced and high concentrations increased respiration, but all concentrations of carbon dioxide decreased the hydrogen ion concentration. A further and still more striking example was in the tests with the carrot; the rate of respiration was not significantly altered by carbon dioxide, but the tissue sap was among those that showed the greatest depression of the hydrogen ion concentration of any tissue studied.

The changes in pH of the tissue upon storage in atmospheres enriched with carbon dioxide brings up the question of the direct and indirect effect of the gas upon living processes. The direct action of carbon dioxide upon the extracted juice of plant tissue is in effect to make it more acid, that is, to increase the hydrogen ion concentration. This fact is well established and has been used to predict the effect of carbon dioxide upon the sap in

the living plant tissue. However, when living tissue is exposed to carbon dioxide there results an indirect effect upon the tissue whereby the sap becomes more alkaline. These results demonstrate the fact that predictions of the effects of chemicals upon living tissue based entirely upon their chemical properties without due consideration of the response of the living tissue may lead to false conclusions.

A further indication that this change depends upon a living process is shown by the fact that oxygen is necessary for the development of alkalinity in the presence of carbon dioxide. With the removal of oxygen from the storage atmosphere enriched with carbon dioxide there results some change in the metabolism of the potato tuber whereby the sap becomes more acid. A review of the data of Sando (7), who stored tomatoes during ripening periods of 11 days in an unventilated box and wrapped in paper, suggests a similar result to that found with the potato. He reports that in both cases there was an increase in the acidity of the fruit; more so in the fruit within the box than that wrapped in paper. He found that the air within the unventilated box at the end of the test would not support combustion.

SUMMARY

1. The treatment of various types of plant tissues such as fruits, roots, stems, tubers, bulbs, and even of entire plants growing in pots with carbon dioxide in the presence of oxygen at 25° C. for various periods of time resulted in a decided *decrease* in the hydrogen ion concentration of the sap extracted from that tissue. The pH difference between treated and control tissue sap was determined with the quinhydrone and glass electrodes and with indicators.

2. The approximate changes in pH of the extracted juice of the tissues stored for various periods of time in 50 to 70 per cent of carbon dioxide was as follows: potato tubers 0.53 during 41 to 168 hours; asparagus shoots 0.59 during 8 to 24 hours; green lima beans 0.35 during 17 to 22 hours; carrot roots 0.72 during 24 to 115 hours; beet roots 0.74 during 22 to 96 hours; tulip bulbs 0.27 during 66 to 69 hours; onion bulbs 0.59 during 92 to 116 hours; strawberry fruits 0.18 during 37 to 41 hours; apple fruits 0.25 during 120 hours; peach fruits 0.15 during 48 hours; oranges 0.15 during 26 to 120 hours; tobacco plants 0.77 during 2 hours; and tomato plants 0.76 during 5 hours.

3. A significant change in pH of the asparagus shoots became evident after 15 minutes of the treatment. With the potato tubers the change in pH is not noticeable until after 12 or more hours of the treatment.

4. Storage of the tissues in air after the treatment results in the restoration of the pH value at the same level as that of the control tissue. This process requires from 20 to 24 hours with asparagus shoots and from 48 to 72 hours with the potato tubers.

5. Cut pieces of potato tubers show the change in pH with the treatment as well as the whole tubers.

6. The control potato tubers have a low pH value at the surface which increases toward the center. Treatment of the potato tuber with 60 per cent of carbon dioxide reverses this gradient whereby the outside has the highest pH value which decreases toward the center.

7. Titration curves of potato juice do not show differences in buffering capacity of the treated and control juices.

8. The pH of potato tubers is decreased with lowering of the storage temperature. Carbon dioxide increases the pH of potatoes at a temperature as low as 2° C., but not to as great an extent as at 25° C. during the same period of treatment.

9. The change of pH of the potato tissue sap in the alkaline direction in the presence of carbon dioxide is dependent upon the presence of oxygen. In the absence of oxygen the change in pH is not in the alkaline direction but in the direction of increased acidity.

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INTRACELLULAR BODIES ASSOCIATED WITH RING-SPOT[1]

MARK W. WOODS[2]

Intracellular bodies characteristic of numerous virus diseases of plants have been studied by a number of workers. Iwanowski (7) described such bodies in tobacco mosaic. Kunkel (10) described and illustrated intracellular inclusions characteristic of corn mosaic, and later (11, 12, 13, 14), similar intracellular bodies in *Hippeastrum* mosaic, sugar cane mosaic, and mosaic of Chinese cabbage and tobacco. Goldstein (1, 2) studied the bodies associated with mosaic of tobacco, and also of dahlia (3). Rawlins and Johnson (15) described the intracellular bodies in tobacco mosaic. Hoggan (4, p. 668) reports that "up to the present, they (intracellular bodies) have been reported in connection with 10 different plant diseases, namely, mosaic of tobacco, corn, sugar cane, Fiji disease of sugar cane, mosaic of wheat, *Hippeastrum equestre*, *H. johnsonii*, Chinese cabbage, potato, and dahlia." She presents evidence in her paper (4, p. 668) of "eight virus diseases of solanaceous plants with which such inclusions definitely do not occur, at least under greenhouse conditions." (4, p. 668) "These are: Cucumber mosaic, speckled tobacco mosaic, mild tobacco mosaic, spot necrosis, ring spot, bleaching mosaic, tomato stem necrosis, and petunia mosaic." She also reports that intracellular bodies were not observed in association with leaf roll, streak, or calico of potato. All of these viruses she classified according to the scheme of Johnson (8, 9). Holmes (5) made a careful cytological study of the intracellular body characteristic of *Hippeastrum* mosaic. He gave special attention to the possibility of the presence of nuclear material and chondriosomes in the body. Goldstein (2) and Hoggan (4) have given very thorough reviews of the literature dealing with intracellular bodies.

This paper reports observations on the occurrence of intracellular bodies in ring-spot.

MATERIALS AND METHODS

The virus used in these studies of ring-spot was obtained from W. C. Price then at the Boyce Thompson Institute. It originally was obtained

[1] By permission of the Boyce Thompson Institute for Plant Research, Yonkers, New York, an investigation on ring-spot, which was begun there in the summer of 1931, has been carried on in the Department of Botany, University of Maryland, College Park, Maryland, in partial fulfillment of the requirements for the degree of Master of Science. This paper represents a part of the thesis requirement.

[2] The writer wishes to state that he is greatly indebted to Professor Charles E. Temple of the Department of Botany at the University of Maryland for many helpful suggestions and criticisms. He also wishes to thank other members of the Department of Botany at the University of Maryland, and members of the Boyce Thompson Institute for many facilities and kindnesses extended to him.

from S. A. Wingard of the Virginia Agricultural Experiment Station, where it was secured from a diseased plant in a commercial tobacco planting in Virginia.

All plants used in these experiments were grown from seed in sterile soil, and at a suitable age were transplanted to two and one-half or four-inch pots, the latter being more generally used.

Plants were inoculated by rubbing whole leaves or portions of leaves with cheesecloth swabs saturated with juice extracted from diseased Turkish tobacco plants. In nearly every experiment the leaves were rinsed with tap water immediately after inoculation to remove any adhering substances that might injure the leaf tissues.

Lesions were cut out of the leaf with a razor blade and fixed in either Flemming's weaker solution or formol-acetic-alcohol (90 cc. 50 per cent alcohol, 5 cc. formalin, 5 cc. glacial acetic acid). As a general rule the latter fixative gave much better results, which is in accord with the findings of Rawlins and Johnson (15) and Hoggan (4). Material was generally fixed from 24 to 48 hours. Each lesion was cut so as to include several millimeters of normal-appearing tissue outside of the visibly lesioned area. In practically every case other apparently non-lesioned areas of the same leaf were removed, near the lesion concerned, or from the opposite half of the lamina. In some instances only one-half of the leaf was inoculated. In all cases visibly lesioned areas were compared with apparently non-lesioned areas of the same leaf. It was concluded that this method gave a more accurate means of comparing lesioned with non-lesioned tissues than the use of samples from leaves other than those in which the lesions occurred.

A large number of sections were carefully observed, cell by cell, before any conclusions were drawn. Certain tests were performed at different times with *Nicotiana tabacum* L. var. Turkish and *N. glutinosa* L. to check the purity of the ring-spot virus used. These tests will be reviewed under another section of this paper. The results obtained at all times indicate that a pure virus was used in every series of inoculations.

The intracellular bodies associated with ring-spot are described as they were observed in the different species or varieties of *Nicotiana* and in *Petunia*.

EXPERIMENTAL RESULTS

NICOTIANA TABACUM L. VAR. TURKISH

Primary lesions. Intracellular bodies were observed in both primary and systemic lesions of ring-spot in Turkish tobacco. The bodies were not found in all primary lesions examined. In lesions of the type where zones of normal-appearing tissue alternated with zones that were necrosed or chlorosed they were found in two cases only four days after inoculation. In this instance three primary lesions on one leaf and one lesion on another

leaf of one plant contained typical intracellular inclusions. The bodies were confined to the visibly lesioned areas included within from one to two necrotic rings. Normal-appearing tissues surrounding the outermost rings and in the non-lesioned half of the leaves were apparently entirely free of the bodies. In primary lesions of this type, formed on old basal leaves 15 to 30 days after inoculation, the bodies appeared to occur much more frequently. In every case the bodies were confined to cells actually included by the visibly lesioned area, being most numerous in the cells of the central portions of the lesions. The cells in close proximity to the necrotic areas contained more bodies than those at some distance from them.

Figure 1 A illustrates a leaf with a primary lesion as it appeared 15 days after inoculation. This was one of the basal leaves of the plant, and was inoculated in late June at College Park, Maryland. A careful cell-by-cell study was made of a large number of sections of this lesion cut 20 microns in thickness. This was a very typical "summer" lesion of ring-spot and the distribution of intracellular bodies within the lesion likewise appeared to be typical as judged by comparison with other primary lesions of this type. About 5 per cent of the cells of the mesophyll included by the initial necrotic ring contained typical intracellular bodies. In the band of green tissue just outside of the initial necrotic ring, and included by the secondary necrotic ring, the bodies occurred with about the same frequency. A few bodies were observed in cells immediately outside of the latter necrotic area. Though a careful study was made of a large number of sections, intracellular bodies were not observed in the areas lesioned more recently than the ones just described. A large number of sections cut from a non-lesioned region of the lamina directly across the midrib from this lesion, were apparently free of intracellular bodies.

It is interesting to note the cytological and histological pictures presented in the different regions of this lesion. They appeared to be quite typical of those observed in similar areas of other lesions of this size and age. In the central island of green tissue, many of the cells of the palisade and spongy parenchyma were obviously affected, containing smaller and fewer than normal plastids. The reduction in number seemed to be due to disintegration of some of the chloroplasts which was evidenced by the presence of a certain type of granular material in the cells. The nuclei of these cells appeared to be unaltered. Starch grains were present in the normal-appearing as well as the much disintegrated plastids. The amount of cellular disintegration was greatest in close proximity to the necrotic regions. The palisade cells were the most severely affected as a rule. The debris in the cells in many instances seemed to have accumulated about the nucleus. In cells of the second band of green tissue the situation was in general similar to that in the center of the lesion, but with less severe cellular modification. Necrotic cells occurred more or less scattered in this re-

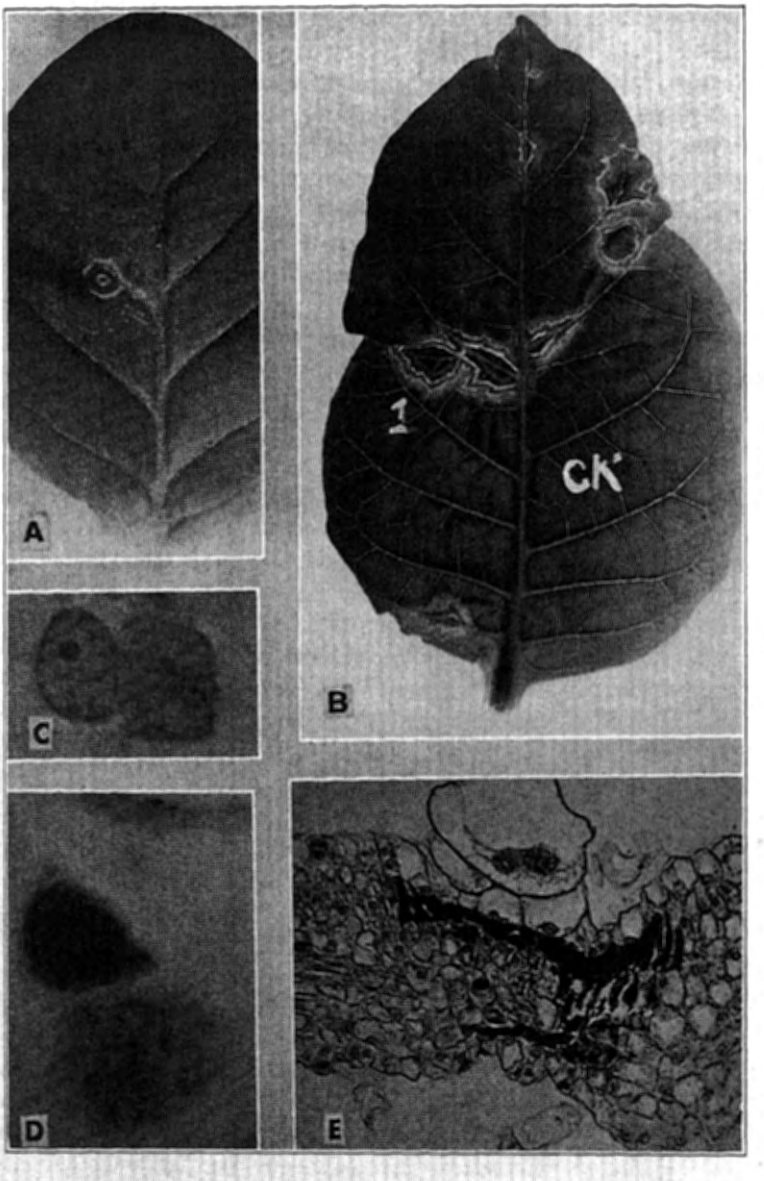


FIGURE 1. Ring-spot on Turkish tobacco. A. Primary lesion 15 days after inoculation. B. Systemic lesions 15 days after inoculation. C, D. Intracellular bodies in primary lesions 28 and 4 days after inoculation respectively. E. Intracellular body in trichome over necrotic area of systemic lesion 15 days after inoculation.

gion. In lesioned areas where no intracellular bodies were observed, most of the cells appeared to be normal or nearly so. Scattered necrotic areas a few cells in width were common throughout the lesioned area, however.

Intracellular bodies in the two regions described above occurred in some instances in cells that showed very little or apparently no plastid disintegration, and in others in connection with considerable plastid breakdown. In the latter case the debris formed had accumulated about the body. Cells containing bodies or much granular material often displayed what appeared to be an abnormal thickening of the cytoplasmic strands and of the peripheral cytoplasm. This may have been due, however, to the accumulation of by-products of plastid disintegration. In this lesion a few bodies were observed in the epidermal cells overlying the central regions. Most of the trichomes had been destroyed and no bodies were observed in those remaining.

The cells that contained bodies did not necessarily show visible signs of degeneration, but they apparently were always located adjacent or very close to regions in which there was quite obvious cellular degeneration.

Primary lesions formed in winter on Turkish tobacco are generally more completely necrotic than those formed in summer. "Winter" lesions four days old were observed. No intracellular bodies were observed in these lesions, but this may have been due to the short time elapsed following inoculation or to the extreme necrosis.

In primary lesions in Turkish tobacco, intracellular bodies have been observed in cells of the epidermis and mesophyll. In lesions on old leaves, where the cells were very large, the bodies were commonly found in either the palisade or spongy parenchyma and were easily discerned. They were also found in the ordinary epidermal cells and in the trichomes.

In Turkish tobacco the conditions under which intracellular bodies form seem to be limited. The fact that only a few bodies are found in some lesions, and that the age of leaf, time after inoculation, and environmental conditions all seem to play an important part in stimulating or making possible their formation, tend to explain their absence from some lesions and their presence in others. If intracellular bodies occur in one primary lesion on a leaf they are likely to be found in other similar lesions on the same leaf, but never in the non-lesioned area.

Typical intracellular bodies from primary lesions are illustrated in Figure 1 C and D. Figure 2 I shows a drawing of a large body in contact with the nucleus of a cell of the spongy parenchyma adjacent to a necrotic ring in the primary lesion illustrated in Figure 1 A. Considerable plastid distingegration has taken place, but the cytoplasm still appears to be intact. The large nucleus is slightly indented where the body lies in contact with it. The nucleus is in contact with the peripheral cytoplasm, the body extends into the vacuole and is partly surrounded by cytoplasm. The body

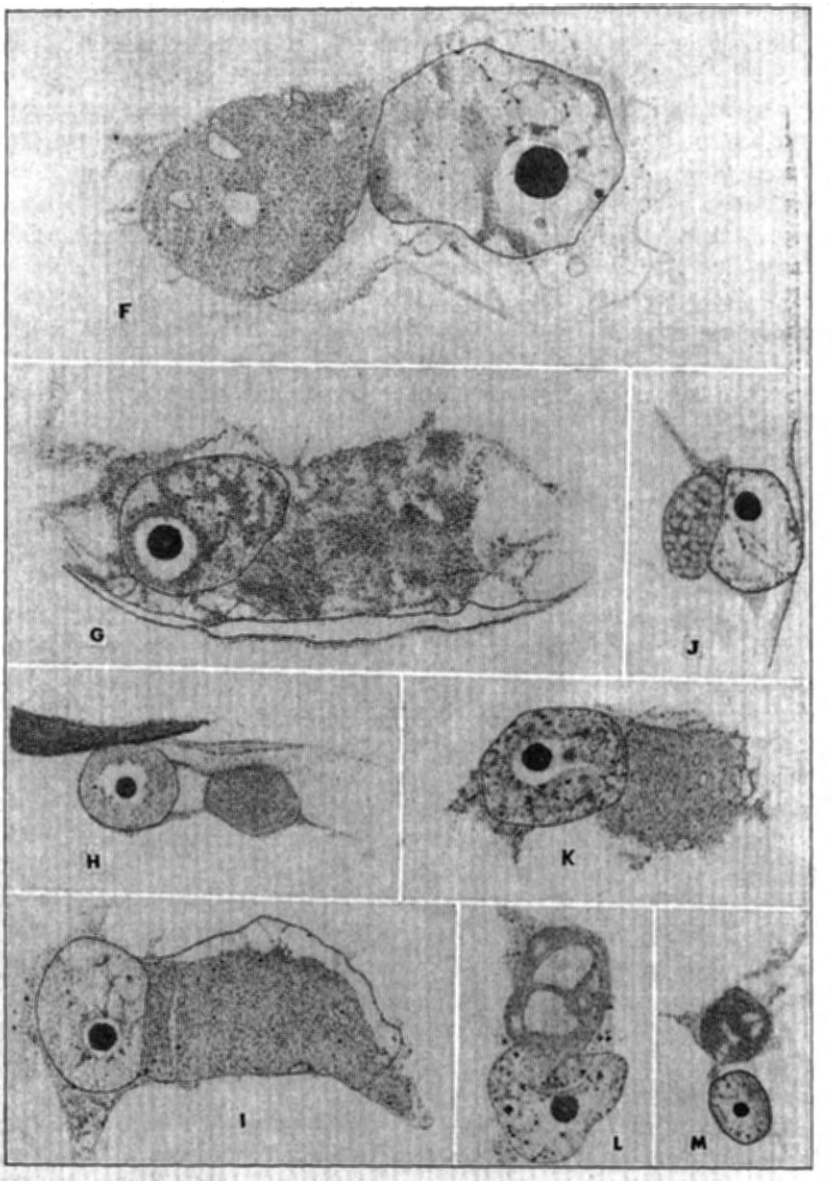


FIGURE 2. Intracellular bodies in ring-spot. F, G. In systemic lesions 15 days after inoculation of Turkish tobacco. H, I. In primary lesions on Turkish tobacco 15 days after inoculation. J. In primary lesion on *Nicotiana glutinosa* 13 days after inoculation. K. In primary lesion on Havana Seed-leaf tobacco 5 days after inoculation. L. In primary lesion on *N. rustica* 8 days after inoculation. M. In primary lesion on petunia 8 days after inoculation. (Camera lucida drawings magnification ×1800.)

is elongate, being somewhat kidney-shaped, and tapering slightly toward the end opposite the nucleus. The main mass of the body is densely granular, containing several small vacuoles. The surface of the body also appears to be vacuolate. The vacuolation is such that on one side the body appears to consist of a central compact mass of granular material stained with orange "G" containing some small vacuoles, surrounded by a hyaline membrane-like portion. Some loosely aggregated granular material is irregularly distributed between the outer "membrane" and the inner compact granular mass. As cell cytoplasm containing bubble-like vacuolate structures very similar in appearance to the peripheral vacuolation described here has been observed, it may be that this vacuolation is not in the body itself but in the enveloping cytoplasm. This may account for the "foam-like" appearance of bodies occasionally observed after fixation with Flemming's weaker or formol-acetic-alcohol solution. The inner mass of the body has for the most part a definite uniform structure but does not appear to be limited by a distinct membrane. One end of the body, as shown in Figure 2 I, has either been ruptured in sectioning or is loosely aggregated. The cytoplasm of the cell contains considerable abnormal granular material, and envelops at least partly the intracellular body and the nucleus.

Cuboidal bodies. There are present, rather uniformly distributed throughout the body shown in Figure 2 I, numerous very small, densely staining (with safranin) cuboidal bodies, apparently crystalline in nature. In sections stained in 0.2 per cent solution of acid fuchsin in camphor water these cuboidal structures are an intense red in contrast to pink on the cytoplasm, and deep brownish-red on the nucleus. From their appearance and staining reactions it seems possible that these cuboidal bodies are protein crystalloids. They occur in the cytoplasm of the cell as well as in the intracellular body. As similar inclusions have been observed in several other intracellular bodies and frequently in the cytoplasm or nuclei of diseased and healthy cells, they will be discussed at this point. These cuboidal bodies seem to be identical with those described by Rawlins and Johnson (15) and Goldstein (2) in healthy and mosaic diseased tobacco. In the writer's preparations they varied in size from less than 0.2 micron to approximately 0.8 micron square. Similar crystals were observed in the peripheral and transvacuolar cytoplasm of diseased and healthy cells. Per unit volume these cuboidal bodies probably occurred with about the same frequency in the intracellular body as in the ordinary cytoplasm of the cell containing the body. They apparently occur normally in the tissues of the tobacco plant. Rawlins and Johnson (15) state that they stain black with Heidenhain's haematoxylin, and bright red after Flemming's triple stain. Goldstein (2) found cuboidal bodies present in the cytoplasm of the cell, but not free in the cell vacuole.

In studies on ring-spot the writer observed them commonly in the cells of chlorotic regions where they occurred along with the debris accompanying plastid degeneration. It was observed that they occurred most abundantly in some lots of material in close proximity to the vascular tissues or actually in them. In such cases they were observed most frequently in cells of the parenchyma of secondary veins or midrib, in the phloem cells, or in cells of the mesophyll close to the veins. They were also observed in the trichomes and epidermis. The relation of the vascular elements to the occurrence of these cuboidal bodies was in certain instances very striking and in other cases they appeared more uniformly distributed throughout the leaf. While they were sometimes found very abundantly in diseased cells it cannot be said that they were observed more frequently in diseased than in healthy cells; the proximity of the cells containing the cuboidal bodies to the vascular elements seemed more often to be a determining factor. Observations on these cuboidal bodies were made in tissue fixed in formol-acetic-alcohol and stained with Flemming's triple combination.

Figure 2 H illustrates a rather small more or less oval body in a palisade cell from the lesioned area shown in Figure 1 A. A portion of an adjacent, completely necrotic cell is shown. This body is densely granular and apparently contains no visible vacuoles.

Intracellular bodies were observed in which radiating strands of cytoplasm extended from the peripheral cytoplasm to the body and connected with pointed protuberances at the surface of the latter. It appeared as though a state of tension had existed between the body and the strand of cytoplasm. Some bodies were observed that appeared much flattened against the peripheral cytoplasm and the cell wall.

Figure 1 C shows a photomicrograph of a body in a cell of the palisade parenchyma in a degenerating area of a primary lesion 28 days after inoculation. The body is roughly spherical in form and typical in all respects. The cell adjacent contained a considerably vacuolate, kidney-shaped body a little larger than this one. Considerable plastid degeneration was apparent in both cells.

Systemic lesions. Intracellular bodies were observed in numerous, but not all, systemic lesions of ring-spot in Turkish tobacco. Here, as in the primary lesions, certain limiting factors for their formation seemed to exist.

The bodies occurred most commonly in the large trichomes and ordinary epidermal cells, overlying lesioned areas. A cell-by-cell study of several hundred sections resulted in the following observations.

Intracellular bodies in systemic lesions, as in primary lesions, were observed only in visibly lesioned leaves. In the lesions studied they were almost always observed in trichomes overlying visibly affected mesophyll, the latter being completely necrosed or apparently with only plastid disintegration as in some chlorotic areas. Bodies were sometimes observed in

ordinary epidermal cells over such areas. They were not found over normal-appearing mesophyll outside of the visibly lesioned portions of the same sections.

Figure 1 B illustrates a leaf with several systemic lesions of ring-spot 15 days after inoculation of the plant. Lesion 1 was very carefully studied section by section and cell by cell, as was an area of approximately the same size at the place marked CK.

In the lesioned area 212 sections, each 20 microns in thickness, were studied. Each section included the visibly lesioned area and a portion of the normal-appearing tissue surrounding the outermost chlorotic halo. At least 25 intracellular bodies were observed all occurring in cells of trichomes or the epidermis included by the visibly lesioned area. They were most common over the chlorosed or necrosed areas. No bodies whatever were observed outside of the lesioned area. Necrotic or otherwise visibly degenerate cells in this region were confined in nearly all sections to the mesophyll, particularly the palisade. It was frequently observed that trichomes remained apparently unaltered in appearance even after the underlying epidermis and mesophyll had become completely necrotic. Such trichomes apparently must obtain water for a short time by imbibition from the underlying dead cells, before the latter completely dry out. Though they are usually the last cells to die in a lesioned area, they are apparently the most likely ones to contain bodies. One hundred eighty-one sections from the region marked CK were similarly studied, and no intracellular bodies or similar appearing materials of any kind were observed in any of the cells.

Another lesion of this leaf and similar lesions on leaves of another plant were observed. The above observations appeared to be substantiated in every case. If bodies had occurred in cells of the mesophyll of these lesions it would have been difficult to distinguish them, due to the small size of the cells and to the by-products of degeneration contained therein. In some cells of the mesophyll in the lesioned area, masses of material resembling intracellular bodies were observed.

The general appearance of the bodies in systemic lesions seems to be the same as in primary lesions of Turkish tobacco. In the large trichomes, however, the bodies are often very large.

Figure 2 G shows a body from one of the systemic lesions just described. The body is very large and is cut into two sections, thus affording a good view into its interior. This body is typical of the more loosely aggregated type sometimes observed. The mass of the body is finely granular, forming a somewhat reticulated structure, and contains dense and irregular lumps. These lumps are surrounded by the more loosely aggregated material. Both lumpy and loosely aggregated materials seem to be formed of the same substance, differing only in their state of aggregation. The mass is

finely vacuolate in part. The periphery of the body is not limited by a membrane and at places the body substance is almost indistinguishable from the cytoplasmic strands with which it is connected. The nucleus was apparently not deformed.

Figure 1 E is a photomicrograph of a very large intracellular body in the basal cell of a trichome over a necrotic area in a typical systemic lesion 15 days after inoculation. The epidermis in this area is necrotic, but the palisade and much of the spongy parenchyma is completely necrosed. Structures resembling small intracellular bodies are present in two of the underlying ordinary epidermal cells.

Figure 2 F is a camera lucida drawing of this intracellular body as it appeared with an initial magnification of 1800 diameters. The body is roughly spherical, densely granular, and contains several distinct vacuoles that extend throughout the body. The body is touching the large nucleus, but the latter does not seem to be deformed as a result of this. The body and the nucleus are enveloped in cytoplasm. Distributed throughout the body and cell cytoplasm of this and other cells of the same section are structures resembling the minute cuboidal bodies previously described under primary lesions. Comparing the body with the peripheral cytoplasm of the cell, these cuboidal structures apparently occur with about the same frequency per unit volume in the body substance as they do in the cytoplasm. This intracellular body, while easily distinguishable from the surrounding cytoplasm, does not seem to be limited by a definite membrane.

In general it may be said that the appearance of the intracellular bodies, and their location within the cells in which they occur in the systemic lesions, is essentially the same as in the primary lesions.

Terminal growing regions of a systemically infected plant, in which numerous bodies were found in the primary and systemic lesions of the older leaves, apparently did not contain any intracellular bodies in the cells in and beneath the growing point, or young leaves surrounding, even though two of the latter were partly necrotic.

NICOTIANA TABACUM L. VAR. HAVANA SEED-LEAF

The primary symptoms of ring-spot in Havana Seed-leaf tobacco were very similar to those in Turkish tobacco. Primary lesions formed in the month of December were observed in this study. Some of these were of the typical winter or almost completely necrotic type and some consisted of alternate zones of normal-appearing and necrosed tissues. Typical intracellular bodies were observed in both types, more abundantly in the latter. The bodies were always confined to the visibly lesioned areas, and were not observed in apparently non-lesioned regions of the same leaf. Typical

bodies were observed in primary lesions 5 and 13 days after inoculation. The bodies occurred most commonly in the latter.

The appearance, distribution in the cells, etc., of these bodies was apparently the same as in Turkish tobacco. It is interesting to note that while some of the bodies in the lesions 5 days after inoculation were small, some were fully as large as any observed in lesions 13 days after inoculation.

Figure 2 K shows an approximately spherical body lying next to the nucleus of an epidermal cell from a primary lesion five days after inoculation. The body is stained orange, and is densely granular with numerous small vacuoles distributed throughout its mass. The periphery of the body is also partly vacuolate. The surface of the body apparently is not limited by a definite membrane. Though the body lies in contact with the nucleus, the latter does not seem to be deformed. Both nucleus and body are surrounded by cytoplasm.

NICOTIANA RUSTICA L.

In *Nicotiana rustica* typical intracellular bodies were observed in cells, included by lesioned areas, eight days after inoculation. These lesions consisted of faint narrow necrotic rings surrounding a central island in which some cells contained slightly disintegrated plastids. The bodies were observed in cells of the mesophyll and epidermis, but more commonly in the latter. The cytological and histological pictures in these lesions were very similar to those observed in similar lesions in Turkish tobacco. The plants were inoculated in late November, 1931.

The general appearance and location of the intracellular bodies in primary lesions on *N. rustica* were similar to those in Turkish tobacco. The bodies tended to be smaller, but the ratio of size of nuclei and cells to the bodies apparently was about the same in either plant. In *N. rustica* the bodies were possibly a little more definite in form than in Turkish tobacco, particularly in regard to their peripheral structure. They were granular in structure but tended to be more highly vacuolate than in Turkish tobacco.

Figure 2 L illustrates a highly vacuolate body next to the nucleus of an upper epidermal cell. The nucleus appears to be indented opposite the point of contact of the body. The body is densely granular and contains two large central vacuoles. The red, cuboidal, and apparently crystalline structures observed in Turkish tobacco are present in the cytoplasm of the cell. Larger and more distinct cuboidal bodies of the same type are present in the nucleus. The surface of the body appears very much as though limited by a definite membrane.

NICOTIANA GLUTINOSA L.

Only a small amount of material was observed in *Nicotiana glutinosa*. Primary lesions 13 days after inoculation were examined. These appeared as rather circular areas, consisting of a central very slightly chlorotic island of tissue surrounded by from one to several alternating bands of green tissue and very fine slightly necrotic rings.

Though some workers have experienced trouble in infecting *N. glutinosa* with ring-spot, the writer has nearly always succeeded in readily infecting this species, and in securing very typical ring-spot symptoms. Vigorous young plants were inoculated by lightly rubbing the upper surfaces of the leaves with a cheesecloth-covered spatula dipped in virus-containing juice. The plants were rinsed with tap water immediately following inoculation. Primary symptoms of ring-spot in *N. glutinosa* were often slow in appearing, but after 8 to 13 days were usually visible. Both primary and systemic lesions tended to be very slightly necrotic, consisting of alternate bands of green tissue, and very fine necrotic or chlorotic rings.

Three lesions from the same leaf were examined, one contained two very definite and typical intracellular bodies in the central faintly chlorotic region, and two lesions contained structures that may have been bodies appearing as irregular masses of orange-stained granular material aggregated about or near the nucleus.

Figure 2 J illustrates an intracellular body in contact with the nucleus of an upper epidermal cell over the central faintly chlorotic area of one of the lesions. The body is oval in shape, nearly as large as the nucleus, stained orange, and is granular in structure, containing a large number of small vacuoles. The body lies in contact with the nucleus which is flattened along the region of contact. Both body and nucleus are partly surrounded by cytoplasm. The surface of the body appears to be membrane-like in nature. The cuboidal crystal-like structures referred to before were not definitely observed.

NICOTIANA GLAUCA GRAHAM

Several primary and systemic ring-spot lesions in *Nicotiana glauca* were observed, but no intracellular bodies were located in them. All of the material studied was fixed in Flemming's weaker fluid and was poorly preserved.

PETUNIA SP.

In petunia numerous typical intracellular bodies were observed in primary lesions of ring-spot of the zonate chlorotic type six and eight days after inoculation. These lesions were found in collections of July, 1931. The bodies were observed only in the epidermal cells overlying the lesioned area. They were not observed in non-lesioned areas of the same leaves in

which the bodies were found. Primary lesions of the zonate, partially necrotic type did not contain bodies three days after inoculation.

The intracellular bodies in petunia appeared to be of essentially the same nature in regard to position in the cell, structure, staining reactions, etc., as similar inclusions observed in the cells of other suscepts to ring-spot.

Figure 2 M illustrates a dense, rounded body almost touching the nucleus of an epidermal cell in a primary lesion eight days after inoculation. The body is vacuolate, compactly granular, but, apparently, not limited by a definite membrane-like peripheral region.

Numerous other intracellular bodies were observed. The amount of vacuolation in the bodies varied. The red cuboidal bodies, described elsewhere, were not observed.

DISCUSSION

The purity of the virus used in the experiments, and the elimination of chance contamination of the plants by mosaic or other virus diseases, are important factors to be considered in a study of this kind.

The plants used in this investigation were frequently observed in order to detect any visible signs of contamination. The plants were often allowed to grow for a considerable time after complete recovery from ring-spot, but symptoms of mosaic or other virus diseases were not observed. *Nicotiana glutinosa* plants were frequently inoculated with the virus stock and never developed any virus disease symptoms other than those of ring-spot. If mosaic had been present necrotic spots should have developed on the inoculated leaves. The following more specific tests were performed in order to demonstrate the purity of the virus in plants containing intracellular bodies.

A series of 14 Turkish tobacco plants, 12 young and vigorous and two old and stunted, were inoculated with undiluted juice from two Turkish tobacco plants in which typical intracellular bodies were found in primary lesions 28 days after inoculation. Typical symptoms of ring-spot appeared on all of the plants. Seven vigorous *Nicotiana glutinosa* plants, also inoculated, developed typical primary lesions of ring-spot. No necrotic spots developed on the *N. glutinosa* plants though they were abundantly lesioned with typical chlorotic and faint ring-necrotic lesions of ring-spot. All of the plants were held for 25 days following inoculation and no symptoms of a virus disease other than ring-spot were observed. The plants from which the inoculum was taken and a number of other plants inoculated at the same time recovered completely without developing symptoms of mosaic or other virus diseases. Apparently the only virus present was that of ring-spot.

The fact that typical intracellular bodies have been observed in

primary lesions of ring-spot in *Nicotiana glutinosa* and *N. rustica* 13 and 8 days respectively after inoculation further attest the freedom from tobacco mosaic contamination. The latter virus produces completely necrotic spots on the inoculated leaves of these species as described by Holmes (6).

The intracellular bodies observed in ring-spot seem to be similar in most respects to those described for tobacco mosaic by different workers. In regard to occasional peripheral vacuolation they resemble the bodies in cells of mosaic-diseased dahlia plants as described by Goldstein (3). She states that such vacuolation of the bodies was never observed during her work with tobacco mosaic. The types of striate material described by different workers in cells diseased with tobacco mosaic virus were never observed, either after fixation in Flemming's weaker fluid or in formol-acetic-alcohol.

The only type of inclusion observed within the intracellular bodies associated with ring-spot, with the exception of the vacuoles, were the red-staining cuboidal bodies. As the cuboidal structures sometimes occurred with about the same frequency per unit volume in cell cytoplasm and body substance, it may be that they indicate a similarity of composition or content existing between the two. These crystal-like bodies do not occur free in the vacuole. It is suggested that they are protein crystalloids.

Because of both the loose and compact states of aggregation of the substances composing the bodies, it seems probable that they represent the formation and accumulation of certain materials in the cytoplasm of the diseased cell, which generally congregate near the nucleus. The fact that bodies were observed with and without apparently membrane-like peripheries in young and old lesions, seems to indicate that such structures form only under certain conditions. Bodies in primary lesions on *Nicotiana rustica* appeared to be more often limited by a membrane-like layer than in Turkish tobacco. In regard to appearance and staining reactions, the intracellular bodies in ring-spot bear a strong resemblance to masses of young cytoplasm.

From a study of the material collected in this investigation it seems quite probable that the metabolic condition of the cell at the time of inoculation is a much more important factor in the formation of intracellular bodies than the lapse of time following inoculation. This is evidenced by the fact that large and typical bodies may occur in primary lesions only a few days after inoculation. Some of these appear to be as large as any of the bodies formed in lesions three times as old on similar plants inoculated at the same time as the ones from which the younger lesions were collected. As a general rule the oldest and largest cells are the most likely to contain intracellular bodies. The bodies apparently may form rapidly,

but the time required for the conditions in the suscept cell to become suitable for their formation usually seems to be considerably longer than that required for visible lesions to develop.

Goldstein (2) has shown in tobacco mosaic that intracellular bodies may be present in cells of the growing point of the stem. In the writer's study, only two terminal buds from plants systemically infected with ring-spot were sectioned. The cells of the growing point, maturing stem, and the very young leaves surrounding the growing point were apparently free of intracellular bodies, even though obvious cellular degeneration occurred in some of these very young leaves of one of the plants. A primary lesion and several systemic lesions on older leaves of this same plant contained numerous intracellular bodies.

The distribution of the bodies within the lesions studied indicates that there is a close relationship existing between cellular degeneration occurring in connection with the formation of necrotic or chlorotic areas in the leaf, and the development of intracellular bodies in that leaf. In systemic lesions in Turkish tobacco, cells of the trichomes or epidermis, lying over or near degenerating mesophyll, are very likely to contain intracellular bodies even though these cells are very resistant to breakdown, living after all the underlying mesophyll has been killed.

While intracellular bodies were not observed in all lesions of ring-spot, it seems safe to conclude, from the studies herein reported, that when they do occur they bear a definite relationship to the ring-spot virus in the diseased areas.

SUMMARY

1. Intracellular bodies, very similar to those associated with several plant virus diseases, are described as occurring in connection with the primary and systemic lesions of ring-spot in *Nicotiana tabacum* var. Turkish and primary lesions in *N. tabacum* var. Havana Seed-leaf, *N. rustica*, *N. glutinosa*, and *Petunia* sp.

2. The intracellular bodies are described with reference to structure, inclusions, position in the cells, and distribution within the diseased leaves.

3. Evidence is presented to demonstrate the purity of the ring-spot virus used, and the freedom from contamination in the experimental plants.

4. The development of intracellular bodies seems to bear a direct relationship to the formation of visibly lesioned areas in the leaf by the virus.

5. The rapidity with which intracellular bodies develop after inoculation seems to depend more upon the physiological condition of the suscept cells at the time of inoculation than upon the length of time that the virus remains in the cells.

6. The intracellular bodies observed in ring-spot bear resemblance to masses of cytoplasm.

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EFFECT OF ETHYLENE CHLORHYDRIN VAPORS UPON THE CHEMICAL COMPOSITION OF GLADIOLUS CORMS[1]

F. E. DENNY

INTRODUCTION

Previous papers (2, 4) have dealt with the chemical changes that occur in dormant potato tubers and lilac twigs in the interval between the time of treatment and the time at which growth of buds could be observed. The present paper relates to similar measurements with dormant gladiolus corms.

At intervals after the treatment with vapors of ethylene chlorhydrin samples of the tissue were taken for chemical analysis and the values so obtained were compared with those from corms that had been subjected to the same procedure except that they had not been treated with vapors.

The most pronounced changes found in the chemical composition were decreases in reducing sugars and increases in sucrose and in forms of nitrogen soluble in 70 per cent alcohol. These changes were found to occur within two to three days from the end of the period of treatment with vapors.

METHODS

Corms. Corms of *Gladiolus* L., varieties Alice Tiplady, Souvenir, and Halley, were harvested September 23, 1931, from plants which had produced good blooms and which had reached maturity as shown by the yellowing of the tops. The corms were allowed to dry indoors in a shallow layer on the floor. The outer brown husks were removed before treatment.

Treatment. The corms were placed in glass or stoneware jars of about one gallon capacity. Pieces of cheesecloth containing the required amount of 40 per cent ethylene chlorhydrin were placed loosely at the top of the jar which was then closed with a clamp top to the margin of which modelling clay was applied in order to perfect the seal. In the case of the treatments on September 30, October 2, and October 3, the amounts of ethylene chlorhydrin used were 3 cc. per liter of volume of the container, and the treatment lasted 3 days. For the treatments on October 13, 14, and 15, the amount used was 2 cc. per liter, and the period of the treatment was 2 days. The check lots were placed in similar containers for the same lengths of time but were not exposed to vapors of the chemical. At the end of the treatment the treated and check bulbs were planted in soil in flats, were stored at room temperature, and the soil was kept moist.

Analyses. At the intervals shown in column 3 of the tables, 10 to 15 bulbs were taken from each lot, were cut into small cubes with a knife,

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 67.

and samples of the well mixed tissue were dropped into an evaporating dish containing boiling 95 per cent alcohol. Subsequently, the alcohol was evaporated slowly on the steam bath, and the removal of the moisture was continued at room temperature by means of a current of air from an electric fan. The air dry tissue was reduced to a fine powder by means of a food grinder and by a power driven mill operating on the mortar and pestle principle. The powder was passed through a 60-mesh sieve and dried to constant weight in a vacuum oven regulated at 70° C. Portions of this powder were weighed out for the chemical analyses. The methods of analysis were the same as those described in a previous article (3). The sucrose was inverted by HCl in the cold (1, p. 95) and the values for sucrose shown in the tables include all constituents which are hydrolyzable by dilute acid at room temperature to produce substances which reduce Fehling's solution. Polysaccharides were estimated by hydrolyzing of the alcohol-insoluble residue with acid (1, p. 95), and they represent, therefore, all constituents from which reducing substances can be formed under such conditions of hydrolysis.

EXPERIMENTAL RESULTS

CALCULATIONS ON DRY WEIGHT BASIS

The results of the analyses of the corms are shown in Table I, in which the amounts of constituents are computed upon the dry weight basis.

Reducing sugar. The outstanding difference in the composition of the treated and check lots is with respect to the percentage of reducing sugar, column 8. The values for the treated lots are consistently low, being in fact about one-fourth or one-fifth of the corresponding check values. In many cases the reducing sugar was brought to a very low concentration by the treatment of the corms with vapors of ethylene chlorhydrin.

Sucrose. The sucrose values, on the other hand, were higher in the treated than in the check lots, column 9, samples at two to three days after treatment showing increases of about 15 per cent in the amount of sucrose, and those at later periods showing increases of about 30 per cent.

Nitrogen. Larger amounts of the nitrogenous substances which are soluble in 70 per cent alcohol were found in the treated lots (column 5). The increase was about 10 per cent for the samples taken at the end of two to three days after treatment, and about 15 per cent for the later samples. The nitrogen insoluble in 70 per cent alcohol was in most cases somewhat lower in the treated than in the check lots.

Polysaccharides. Starch is the principal constituent in the gladiolus tissue included under this heading, and, since it is the main storage product from which sugars could be obtained it might be expected that the treatments would bring about decreases in this constituent. Table I, column 7, however, shows that only in the case of Halley were there consistently large decreases in polysaccharides. With the other two varieties the polysac-

TABLE I

EFFECT OF ETHYLENE CHLORHYDRIN UPON CHEMICAL COMPOSITION OF GLADIOLUS

Variety	Date treated	Days after treatment	Tr. or ck.	Per cent of dry weight					
				Nitrogen		Poly-sacc.	Sugar		Resid. dry wt.
				Sol.	Insol.		Reducing	Sucrose	
Alice Tiplady	Sept. 30	3	Tr.	0.77	1.00	56.9	0.05	2.90	40.15
			Ck.	0.67	0.87	54.5	0.47	2.64	42.39
		7	Tr.	0.73	0.85	52.3	0.05	3.78	43.87
			Ck.	0.70	0.94	53.9	0.62	3.11	42.37
		12	Tr.	0.82	0.84	53.4	0.06	3.70	42.84
			Ck.	0.74	0.96	54.8	0.16	3.32	41.72
	Oct. 15	2	Tr.	0.90	0.88	53.7	0.07	3.25	42.98
			Ck.	0.79	0.89	53.5	0.47	2.93	43.10
		6	Tr.	0.74	0.88	53.3	0.03	3.51	43.16
			Ck.	0.68	0.98	52.7	0.10	3.38	43.82
		10	Tr.	0.81	0.93	52.7	0.05	3.58	43.67
			Ck.	0.69	1.01	53.5	0.09	3.51	42.90
Souvenir	Oct. 2	2	Tr.	0.60	0.84	55.0	0.32	2.71	41.97
			Ck.	0.49	0.88	54.2	1.43	2.46	41.91
		8	Tr.	0.66	0.79	46.8	0.24	4.52	48.44
			Ck.	0.54	0.79	51.0	1.19	2.55	45.26
		12	Tr.	0.63	0.82	50.0	0.10	4.18	45.72
			Ck.	0.56	0.99	51.8	0.95	2.59	44.66
	Oct. 13	3	Tr.	0.78	0.88	53.4	0.79	3.22	42.59
			Ck.	0.74	0.90	53.9	1.28	2.42	42.40
		6	Tr.	0.82	0.84	51.7	0.20	3.56	44.54
			Ck.	0.71	0.88	55.0	1.26	2.53	41.21
		12	Tr.	1.02	0.89	52.6	0.09	3.46	43.85
			Ck.	0.75	1.00	53.8	0.86	2.43	42.91
Halley	Oct. 3	3	Tr.	0.78	1.18	48.5	0.29	3.72	47.49
			Ck.	0.74	1.22	49.5	1.46	4.15	44.89
		8	Tr.	0.82	1.11	44.4	0.45	4.07	51.08
			Ck.	0.71	1.18	49.6	1.27	3.86	45.27
		13	Tr.	1.02	1.20	43.5	0.29	3.51	52.70
			Ck.	0.75	1.44	49.1	1.20	3.52	46.18
	Oct. 14	3	Tr.	0.79	1.10	49.1	0.13	4.01	46.76
			Ck.	0.83	1.19	51.5	0.62	3.63	44.25
		7	Tr.	0.84	1.15	46.5	0.10	4.45	48.95
			Ck.	0.64	1.34	51.3	0.74	3.28	44.68
		11	Tr.	0.79	1.31	48.3	0.08	4.60	47.02
			Ck.	0.73	1.43	51.1	0.65	3.25	45.00

TABLE II

ANALYSES RECALCULATED ON RESIDUAL DRY WEIGHT BASIS

Variety	Date treated	Days after treatment	Tr. or ck.	Per cent of residual dry weight				
				Nitrogen		Poly-sacc.	Sugar	
				Sol.	Insol.		Reducing	Sucrose
Alice Tiplady	Sept. 30	3	Tr.	1.91	2.49	142	0.12	7.22
			Ck.	1.58	2.05	129	1.10	6.22
		7	Tr.	1.66	1.94	119	0.11	8.61
			Ck.	1.65	2.21	127	1.46	7.34
		12	Tr.	1.91	1.96	125	0.14	8.63
			Ck.	1.77	2.30	131	0.38	7.95
	Oct. 15	2	Tr.	2.09	2.05	125	0.16	7.56
			Ck.	1.83	2.06	124	1.09	6.79
		6	Tr.	1.71	2.04	123	0.07	8.13
			Ck.	1.55	2.24	120	0.23	7.71
		10	Tr.	1.85	2.13	121	0.11	8.19
			Ck.	1.60	2.35	125	0.21	8.18
Souvenir	Oct. 2	2	Tr.	1.42	2.00	131	0.76	6.45
			Ck.	1.16	2.10	129	3.41	5.86
		8	Tr.	1.36	1.63	97	0.50	9.33
			Ck.	1.19	1.75	113	2.62	5.63
		12	Tr.	1.37	1.79	109	0.22	9.14
			Ck.	1.25	2.22	116	2.12	5.79
	Oct. 13	3	Tr.	1.83	2.07	125	1.85	7.56
			Ck.	1.74	2.12	127	3.01	5.70
		6	Tr.	1.84	1.89	116	0.45	7.99
			Ck.	1.72	2.14	133	3.05	6.13
		12	Tr.	2.32	2.03	120	0.21	7.89
			Ck.	1.74	2.33	125	2.00	5.66
Halley	Oct. 3	3	Tr.	1.64	2.48	102	0.61	7.83
			Ck.	1.64	2.72	110	3.25	9.24
		8	Tr.	1.60	2.17	87	0.88	7.96
			Ck.	1.56	2.61	110	2.80	8.52
		13	Tr.	1.93	2.28	83	0.55	6.66
			Ck.	1.62	3.12	106	2.59	7.62
	Oct. 14	3	Tr.	1.68	2.35	105	0.28	8.57
			Ck.	1.87	2.69	116	1.40	8.20
		7	Tr.	1.71	2.35	95	0.20	9.09
			Ck.	1.43	3.00	115	1.65	7.34
		11	Tr.	1.68	2.79	103	0.17	9.78
			Ck.	1.62	3.18	114	1.44	7.22

charide values were lower for the treated lots in the 7, 8, 10, and 12 day samples but samples taken at periods earlier than this did not show decreases in the treated lots.

CALCULATIONS ON THE RESIDUAL DRY WEIGHT BASIS

Since Table I shows evidence of changes in the constituents on the dry weight basis, it is necessary to consider the question whether these changes were due to changes in the constituents themselves, or to changes in the dry weight which was used as a basis for computing them.

Another basis for comparison can be obtained by determining the residual dry weight of each sample and by expressing each constituent as a percentage of this value. The residual dry weight was obtained by subtracting the sum of the percentages of polysaccharide and the sugar from 100. For example, in Table I, top line, in 100 grams of dry weight were found 56.9 g. of polysaccharide, 0.05 g. of reducing sugar, and 2.90 g. of sucrose; therefore, the residual dry weight (non-carbohydrate material) was $100-(56.9+0.05+2.90)=40.15$ g. It is in this way that the residual dry weight values in Tables I, column 10, were obtained. These values allow us to find the amounts of each constituent associated with 100 grams of residual dry weight. This was done by finding the ratio which each constituent in Table I has to the residual dry weight value in Table I, column 10, and the values so obtained are given in Table II.

This method of calculation required a modification of the conclusions that were derived from the dry weight percentages only with respect to the data for Halley. So far as the Alice Tiplady and Souvenir results are concerned the differences are about the same by the calculations on the two bases. But with the Halley data the differences in soluble nitrogen are small, on the residual dry weight basis, and in sucrose it cannot be shown that any differences exist. This contrast between Halley and the other two varieties is related to differences in the behavior with respect to residual dry weight. Column 10 in Table I shows that for Alice Tiplady and Souvenir there were no consistent differences between the pairs of treated and check residual dry weight values, but in the case of Halley the residual dry weights of the treated lots, in every pair of comparable samples, are higher in the treated than in the check lots.

DISCUSSION

A comparison of these results for gladiolus corms with those previously obtained with potato tubers and with lilac twigs shows certain contrasts in the responses of the three species toward vapors of ethylene chlorhydrin. The sucrose was increased by the treatment in the cases of potato and gladiolus but was decreased in the case of lilac. The reducing sugar was decreased in the cases of lilac and gladiolus but was not consistently influenced in the case of potato. The soluble nitrogen was definitely increased in the cases of lilac and gladiolus, but the effect in the case of potato was doubtful.

Since the effect produced by the vapors of ethylene chlorhydrin upon the growth of the dormant buds was the same in the three species, either the initiation of growth was not the result of the attainment of a particular chemical composition in the tissue, at least so far as these constituents are concerned, or else the requirements in this respect are different in the various species.

Although these measurements fail, therefore, to establish a simple biochemical basis from which can be developed a theory to account for the growth response of the three species to vapors of ethylene chlorhydrin, they are, nevertheless, of interest in showing how sensitive the living cells are to small amounts of the chemical, and how pronounced, in certain respects, is the effect of the chemical vapors upon the metabolism of the plants.

SUMMARY

1. *Gladiolus* corms of varieties Alice Tiplady, Souvenir, and Halley were harvested on September 23, and were treated at intervals between September 30 and October 15 with vapors of ethylene chlorhydrin. These corms were planted in soil, as were also control bulbs which were not treated.

2. At intervals of 2 to 12 days after planting, in all cases before visible sprouting occurred, samples of bulbs were taken for chemical analysis. Reducing sugar, sucrose, polysaccharides (alcohol-insoluble substances hydrolyzable by dilute acids), and soluble and insoluble forms of nitrogen (70 per cent alcohol being the solvent) were determined.

3. The most pronounced effect of the vapors upon the chemical composition was with respect to the reducing sugar, the treatments in some cases causing a decrease in this constituent almost to zero. Sucrose, however, was higher in the treated than in the check lots.

4. The treatment caused increases in the soluble and decreases in the insoluble nitrogen.

5. The changes in sucrose and in nitrogen were more pronounced with Alice Tiplady and Souvenir than with Halley. But the reducing sugar changes were as large with Halley as with the other two varieties.

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EFFECT OF POTASSIUM THIOCYANATE AND ETHYLENE CHLORHYDRIN UPON AMYLASE ACTIVITY[1]

F. E. DENNY

INTRODUCTION

As an explanation of the effectiveness of small amounts of chemicals, such as ethylene chlorhydrin and sodium thiocyanate, in inducing growth in dormant potato tubers, the suggestion has been made that the cause may be found in the action of these chemicals in activating amylase.

Johnson and Wormall (5) were the first to propose such an explanation as a possible factor, their suggestion regarding potato amylase being in the nature of a corollary to their main conclusion, which related to the effect of thiocyanates upon salivary amylase.

In a more recent paper, Clark, Fowler, and Black (2) present further evidence regarding the favorable effect, not only of thiocyanates, but also of ethylene chlorhydrin and of thiourea, upon malt amylase. Their experiments were initiated because of our previous reports regarding the effectiveness of these chemicals in breaking dormancy, and the object was to throw some light on the mechanism by which the chemicals produce their effect.

In previous papers (3, 4, 6) we have presented evidence for the view that, so far as potatoes are concerned, the chemicals do not act in a direct way by increasing the activity of the amylase already present in the tissue, and there are no data in the paper by Clark, Fowler, and Black which require a modification of this conclusion.

But apart from the question of the effect of the chemicals upon potato amylase, the reported activating action upon malt amylase is so strikingly high as to challenge attention. The curves in the paper cited (2) show that an amylase preparation which required more than three weeks for the achromic stage in the absence of thiocyanate required only about one day in the presence of it, an increase in activity approximately twenty-fold.

Since these chemicals, especially thiocyanate and ethylene chlorhydrin, have been used so extensively in this laboratory for several years, experimental work regarding this phase of their relation to amylase activity seemed almost obligatory.

METHODS

In the preliminary experiments an attempt was made to use the Wohlgemuth (7) method which measures amylase activity by the rate at which starch is hydrolyzed to produce substances which no longer give a blue

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 68.

color with iodine. In using this method care was taken to avoid an error which apparently was made by Clark, Fowler, and Black. Since iodine reacts with thiocyanate it can be preferentially absorbed, and if iodine is not present in excess, the mixture can fail to show blue even when rather large amounts of starch are present. To avoid this possibility of error, the control lots which do not receive KSCN at the start of the test should receive an equivalent amount of KSCN at the end of the test before the iodine is added. In making the test, the solution is acidified, and iodine is added drop by drop until all of the thiocyanate has reacted, after which time starch, if present, will react to give a blue color. Clark, Fowler, and Black do not mention the necessity of precautions in this respect. They added a definite quantity of iodine which was the same for all of the tubes. And if some of the tubes contained KSCN, while others did not, an inadequate starch test might result, since tubes containing considerable quantities of starch can show a negative starch test by this method of testing.

The difficulty is even greater in the case of thiourea, because, although alkalinity favors the union of iodine with KSCN, the absorption of iodine by thiourea takes place under both acid and alkaline conditions.

A more complete discussion of the difficulties encountered in applying the iodine test for starch in experiments of this kind is given by Miller (6).

A number of preliminary tests with the Wohlgemuth method showed that it was difficult to demonstrate any influence either of KSCN or of ethylene chlorhydrin upon the activity of malt diastase. Consequently, use was made of the method which measures amylase activity by determining the amount of copper-reducing substances formed from soluble starch.

The reaction-mixture consisted of 50 cc. of 6 per cent soluble starch +10 cc. of buffer solution +10 cc. of enzyme solution +10 cc. of H_2O or solution of the chemical to be tested. Toluene was added as a preservative. This mixture was placed in 200 cc. Erlenmeyer flasks, and was allowed to stand at room temperature for 18 hours. Then the flasks were placed in boiling water for a few minutes and subsequently were cooled in running water. The reaction-mixture was neutralized, made up to 250 cc., and aliquots were taken for the sugar determination. The Munson and Walker procedure (1, p. 78) was used, and the cuprous oxide was titrated with 0.05 N potassium permanganate. From the permanganate value was deducted the value of a blank determination obtained by the same procedure except that no enzyme solution was added. The difference between these two $KMnO_4$ values was regarded as a measure of amylase activity, and the values plotted in Figures 1, 2, and 3 are those given by the aliquot which was taken from the reaction-mixture for the sugar determination.

Enzymes. Commercial preparations of malt diastase U.S.P. IX, pancreatin U.S.P., and pangestin (all three obtained from Eimer and Amend), and takadiastase (obtained from Parke, Davis and Co.) were used, and,

in addition, an extract of barley malt prepared from germinated barley seeds. The amount of each enzyme preparation used was adjusted to give a convenient reading in the sugar determination which was made at the end of the experimental period. For the malt diastase, 0.2 g. was dissolved in 2000 cc. of water, and, after filtration, 10 cc. of this solution were used for each 80 cc. of reaction-mixture consisting of starch, buffers, chemical, and enzyme; for pancreatin, 0.1 g. was dissolved in 2000 cc. of water, and 10 cc. of this were taken; for pangestin, 0.1 g. was dissolved in 6000 cc. of water and 10 cc. of this were taken; for takadiastase, 0.2 g. was dissolved in 1000 cc. of H_2O, and of this 10 cc. were taken. In preparing the malt extract from barley, hereafter called barley extract to distinguish it from the malt diastase U.S.P., the barley seeds were allowed to germinate until the sprouts were about one-half inch long; the sprouted grains were dried at room temperature in a current of air, and the tissue was pulverized until it would pass through a 60-mesh sieve; to 50 grams of this powder 125 cc. of H_2O were added, and the mixture was stirred with a mechanical stirrer for two hours, after which it was placed in cheesecloth and squeezed with a hand-press; this suspension was centrifuged, and the liquid which was decanted was filtered and used in the experiments; 2 cc. were diluted to 2000 cc. with H_2O, and 10 cc. of this liquid were taken for the enzyme tests.

Reagents. The ethylene chlorhydrin was obtained from the Eastman Kodak Company and was their 40 per cent solution, catalog No. T 131. It was redistilled. All of the other chemicals were recrystallized from C. P. salts, except in the case of the acetic acid which was reagent quality glacial acetic. The starch was soluble starch purchased from Eimer and Amend. It was extracted with cold 95 per cent ethyl alcohol, and was then dried at room temperature.

Buffers. In the range from pH 3.5 to 5.5, mixtures of 0.2 M acetic acid and sodium acetate were used, and in that from pH 5.5 to 8.0, mixtures of 0.1 M sodium dihydrogen phosphate and mono-potassium phosphate were used. Hydrogen ion determinations were made by the quinhydrone method.

RESULTS

WITH NON-DIALYZED ENZYMES

Sodium Chloride Not Added

Figure 1 shows the effect of ethylene chlorhydrin and potassium thiocyanate upon the amylase activity of takadiastase, pancreatin, malt diastase, and barley extract. The amounts of chemicals added per 100 cc. of reaction-mixture were 2.5 cc. of 40 per cent ethylene chlorhydrin and 2.4 g. of KSCN. These amounts were chosen because they gave large increases in amylase activity in the experiments of Clark, Fowler, and Black (2). It is seen that the pH is a controlling factor in the effect of the

added chemical. In the acid range of each enzyme KSCN retarded the activity, and, in fact, practically inhibited it. Note, for example, the KSCN

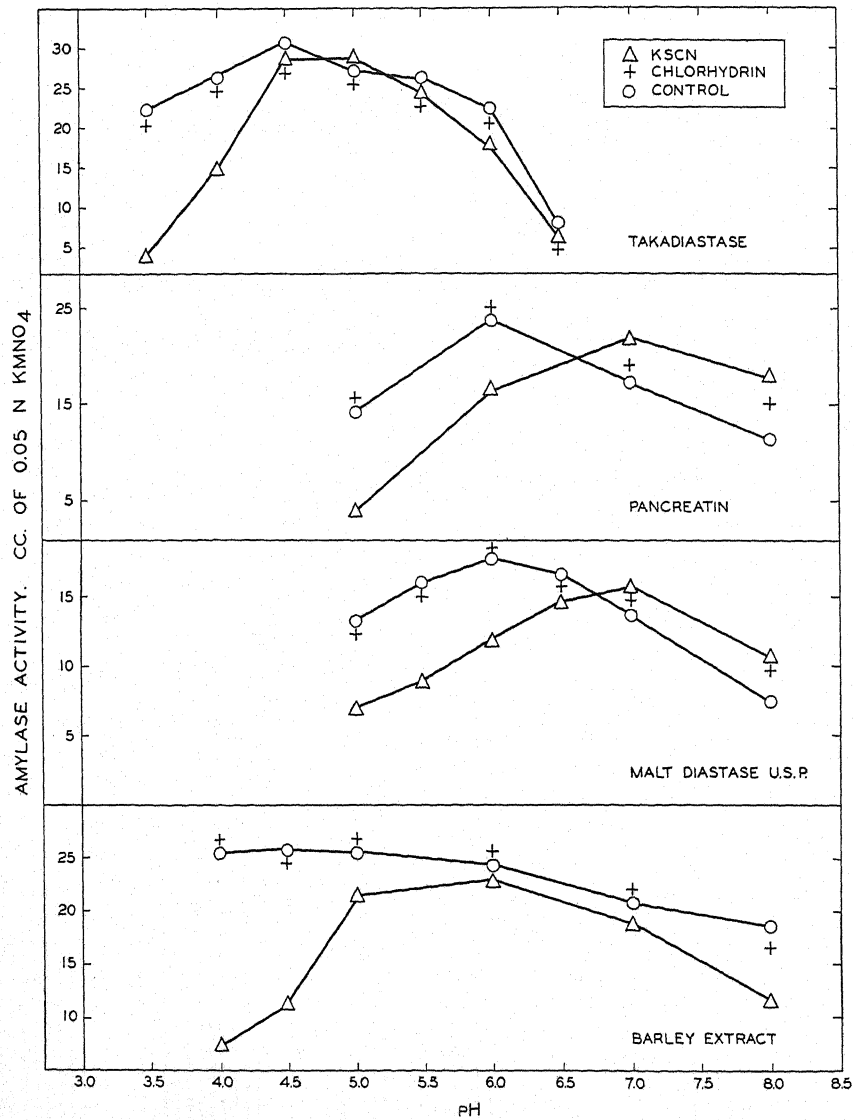


FIGURE 1. Effect of ethylene chlorhydrin and potassium thiocyanate upon amylase activity at different pH values.

values for takadiastase at pH 3.5, for pancreatin and malt diastase at pH 5.0, and for barley extract at pH 4.0. In the less acid ranges of these en-

zymes the response was different for the different enzymes. Takadiastase showed no effect of the added chemicals in the range from pH 4.5 to 6.5, and barley extract showed little effect from pH 6.0 to 8.0. However, pancreatin and malt diastase showed increases in activity as a result of adding KSCN at pH 8.0. The curves for KSCN and the control cross at about pH 6.8, at which point the addition of KSCN had no effect.

The experiments with pangestin showed results that were similar to those described for pancreatin.

Figure 1 also shows that the addition of ethylene chlorhydrin had very little effect upon the activity of any of the enzymes. At pH 8.0 there may have been a small increase in the case of pancreatin, and possibly also for malt diastase U.S.P.

Sodium Chloride Added

The experiments on the effect of the chemicals in the presence of NaCl were an outgrowth of an attempt to determine why Clark, Fowler, and Black (2) obtained activation of amylase with ethylene chlorhydrin, whereas in the present experiments this did not occur. Solutions of ethylene chlorhydrin always contain small amounts of chloride which cannot be removed, and in order to minimize the influence of these small amounts of chloride, relatively large amounts of NaCl were added, in which case the chloride accompanying the chlorhydrin should have little influence, and any effect under such conditions could be regarded as due mainly to the chlorhydrin and not to the accompanying chloride.

The result was to show that, although the addition of ethylene chlorhydrin under such conditions had little effect, the addition of KSCN had a pronounced effect especially with barley extract and with pancreatin.

Barley extract. Preliminary experiments showed that when about 0.1 g. of NaCl was added per 100 cc. of reaction-mixture in the absence of KSCN the amylase activity was retarded, especially at pH values higher than 5.0, and that, in fact, at pH 8.0 amylase action was nearly inhibited. However, when KSCN was added simultaneously with the NaCl, the unfavorable action of NaCl in the alkaline range was nullified, and amylase values as high as those without the addition of either NaCl or KSCN were obtained. The results of such an experiment are shown in Figure 2. The unfavorable effect of NaCl in the alkaline range was prevented by the addition of KSCN, using 2.4 g. per 100 cc. of reaction-mixture.

Pancreatin. The amount of NaCl which retarded the amylase of barley extract *increased* the amylase activity of the pancreatin, and the experiment which has just been described for malt extract was repeated with pancreatin. The results are shown in Figure 2.

Comparison of the results with the two enzymes shows certain contrasts in their relation to KSCN. In the alkaline range, pH 7.0 to 8.0, the

activity of pancreatin was increased by KSCN only in the absence of NaCl, while that of barley extract was increased only in the presence of it.

WITH DIALYZED ENZYMES

Dialysis was carried out in bags made from collodion prepared by dissolving 5 g. of Union Cotton Negative in a mixture of 50 cc. of ethyl ether and 50 cc. of ethyl alcohol. The bags containing the enzyme solutions

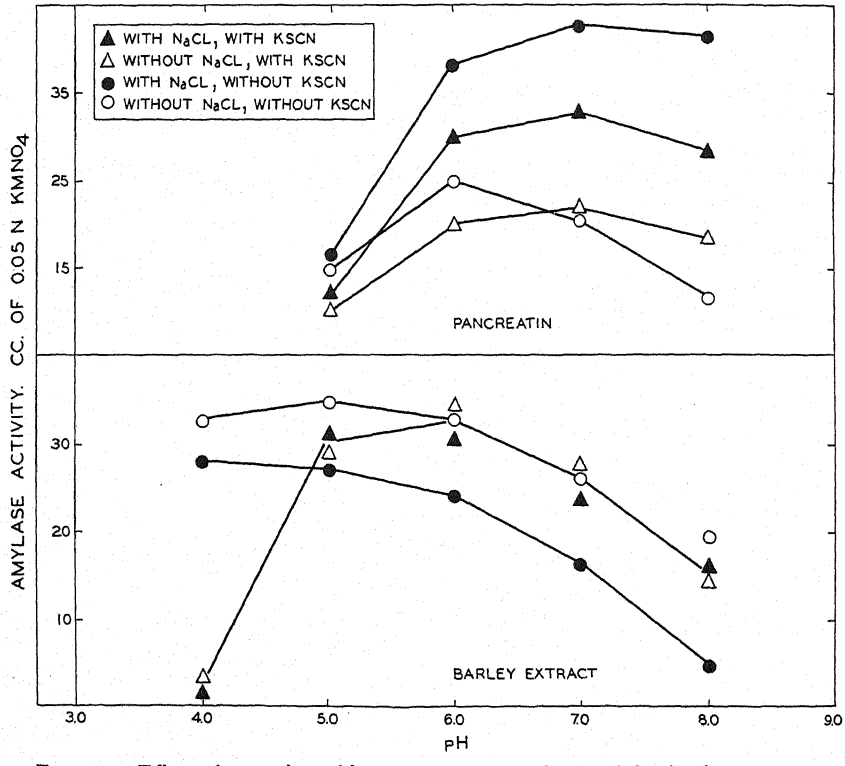


FIGURE 2. Effect of potassium thiocyanate upon amylase activity in the presence and in the absence of sodium chloride.

were suspended for 24 hours in running tap water, and then for 24 hours in distilled water which was continuously renewed by constant dropping from a large storage tank of distilled water. After removal from the collodion bags at the expiration of the dialysis period, the solutions were filtered, and preliminary tests were made to determine the dilution that was necessary to adjust the strength of the dialyzed enzymes to give amylase readings comparable to those obtained with the non-dialyzed enzymes. The barley extract decreased in activity only slightly as a result of dialysis,

but the takadiastase was much weaker, and the malt diastase U.S.P. lost most of its activity.

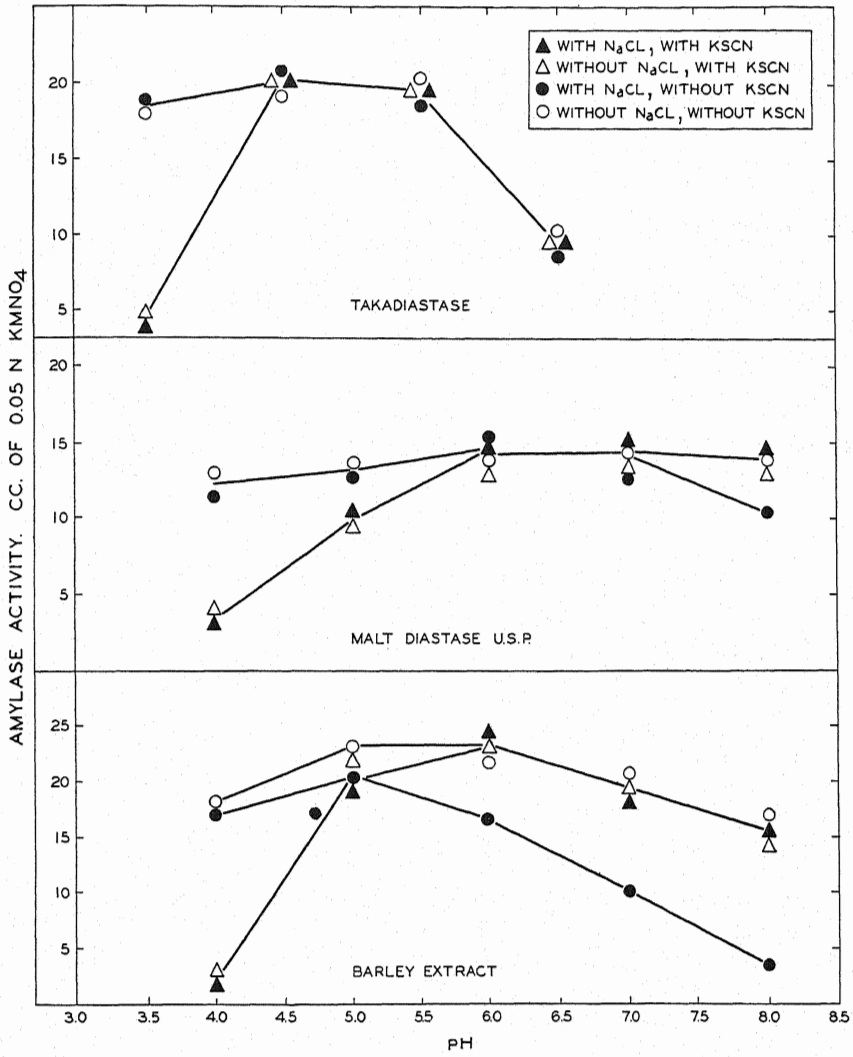


FIGURE 3. Effect of potassium thiocyanate upon the amylase activity of dialyzed enzyme preparations in the presence and absence of sodium chloride.

The results with the dialyzed enzymes are shown in Figure 3. Tests were made with and without NaCl, and with and without KSCN. The dialyzed barley extract and takadiastase gave results similar to those that were obtained with the non-dialyzed preparations. Here again, in the acid

range KSCN strongly retarded amylase activity for both enzymes. The retarding action of NaCl upon barley extract in the pH range 6.0 to 8.0, and the capacity of KSCN to prevent this retardation, were again evident. The dialyzed malt diastase preparation failed to show the increases due to addition of KSCN at pH 8.0, at least in the absence of NaCl, but the depression in the rate in the acid range was again very striking.

Both the retardation at pH 4.0 in the absence of NaCl, and the increase at pH 8.0 in the presence of NaCl, were brought about by the addition of 2.4 g. of KSCN per 100 cc. of reaction-mixture. A test was made with the dialyzed barley extract to determine the effect of smaller quantities of KSCN. It was found that at pH 4.0 definite retardation with about 1.0 g. of KSCN, and at pH 8.0 definite increases with about 0.3 g. of KSCN, were obtained.

The addition of 2.5 cc. of 40 per cent ethylene chlorhydrin per 100 cc. of reaction-mixture had no effect upon the amylase activity of these three dialyzed enzyme preparations, either in the presence or absence of NaCl. The tests with ethylene chlorhydrin were carried out, but in order to avoid confusion in the graphs, the values are not plotted in Figure 3.

DISCUSSION

These experiments emphasize how diverse are the results obtained by the use of KSCN with different amylases under different experimental conditions. The pH is an important factor; the presence or absence of NaCl is another; and two different preparations from the same species (malt diastase and barley extract in these experiments) may respond differently to KSCN, even when the pH and NaCl content are the same.

A general statement to the effect that KSCN activates amylase cannot be made. It may activate, retard, or have no effect; all three of these possibilities may be true of one enzyme preparation (e.g., barley extract), and only two of them of another (e.g., takadiastase). In the present experiments only one statement is true for all of the enzymes tested under all of the conditions of the experiments: in the acid range of each enzyme KSCN always retarded.

These results show how difficult it is to make use of the *in vitro* effect of a chemical upon the activity of an enzyme as the basis for an explanation of the physiological effect upon a plant which has been treated with this chemical. Even if *in vitro* experiments showed a simple relation between the activity of the enzyme and the chemical which is added, much difficulty would be encountered in applying this information directly to the problem that exists in the living cell. But when *in vitro* experiments show that the relation between the enzyme and the chemical is not simple but complex, the hypothesis of a direct enzyme effect does not offer a hopeful basis for a solution of the problem.

The hypothesis that activation of amylase is an important factor in the effect of the chemical treatments in inducing growth of dormant plants requires that the activating effect shall be brought about by all of the chemicals that break dormancy. This was one of the most hopeful phases of the report by Clark, Fowler, and Black, since the chemicals which hastened sprouting also hastened amylase action. But in the present experiments this correlation was not confirmed. Ethylene chlorhydrin and potassium thiocyanate have very similar effects upon the growth of dormant potatoes, but in their effect upon the amylases tested in the present experiments, differences rather than similarities should be emphasized.

SUMMARY

Because of suggestions from two sources that the effectiveness of such chemicals as ethylene chlorhydrin, potassium thiocyanate, and thiourea in inducing growth in dormant buds was related to the capacity of these chemicals to increase amylase activity, tests were made of the effect of ethylene chlorhydrin and potassium thiocyanate upon five different amylase preparations: takadiastase, malt diastase, pancreatin, pangestin, and an extract from germinated barley seeds.

The effect of 2.4 g. of KSCN per 100 cc. of reaction-mixture depended upon the pH. In the acid range of each amylase preparation KSCN strongly retarded; at intermediate values of the pH range it had no effect; in the alkaline range it increased the amylase activity of pancreatin, pangestin, and malt diastase, and had no effect upon takadiastase and barley extract.

Addition of 0.1 g. of NaCl per 100 cc. of reaction-mixture decreased the activity of the barley extract, but if KSCN was added simultaneously this retarding action of NaCl was nullified.

Ethylene chlorhydrin in amounts up to 2.5 cc. of the constant-boiling 40 per cent solution per 100 cc. of reaction-mixture had no important effect upon any of the amylases tested, at any pH value, whether NaCl was present or absent.

The failure to obtain a simple relation between these chemicals and amylase activity, and the non-agreement in the effect upon amylase of these two chemicals which produce nearly identical results in initiating sprouting of dormant potatoes, indicate that the basis for an explanation of the effectiveness of the chemicals is not to be found in the direct effect which they have upon amylase activity.

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SEEDLING PRODUCTION OF TREE PEONY

LELA V. BARTON

INTRODUCTION

No intensive scientific investigation has been made of the germination of the seeds of tree peony (*Paeonia suffruticosa* Andr.). The herbaceous peony has long been a favorite with flower growers but only recently has the tree peony become increasingly popular. Consequently, reports of problems on the germination of peony seeds are usually concerned with the herbaceous type.

Kinzel (4) was of the opinion that the germination of herbaceous peonies was inhibited by light in spite of their thick black coats. He cited the work of Vilmorin who found that it took two years for the germination of *Paeonia* seeds which were not "entirely fresh."

Wheeler (9) observed that the peony seeds should be gathered as soon as they are ripe before they have time to harden. He further stated that if they once get hard nothing will make them germinate before the second year. This same fact has been noted by a number of other authors (1; 2, p. 217; 3). Harrison (3) remarked that if the seeds were fully dried not one out of thirty pounds ever came up in less than two years.

Saunders (5) in 1918 stated that the seed germinates the second season after planting. The same year he reported (6) that treatment of tree peony seed with concentrated sulphuric acid for one or two hours before planting undoubtedly hastened germination. However, his data seem rather inadequate for this conclusion. He further observed that these first sprouts were roots, not leaves. He described the sequence of events with ordinary dry peony seed as follows (6, p. 19): "If sown in autumn of 1917, growth begins about August 1918 and consists in the sending down of a root to a depth of 2 or 3 inches and then nothing more that year. In the spring of 1919 leaf growth begins."

Weinard (8) in his discussion of the problems of peony growers does not mention any difficulty in connection with seed germination but he advises the use of lime soils.

Contrary to all of the reports mentioned above Seyler (7, p. 9), in speaking of the peonies, said that "they can be grown from seed without much trouble and, unfortunately, have been with the resulting confusion and bewilderment of innumerable seedling varieties."

The main problem in the production of tree peony seedlings has proved to be the finding of a method of forcing the dormant shoot bud after the root has already started to grow. The present paper deals mainly with this problem. Because of the difficulty of obtaining seed material in large

quantity the number of seedlings used for each test has been comparatively small. However, each test has been repeated a sufficient number of times to show certain definite trends in response to various treatments.

MATERIALS AND METHOD

Seed material used was obtained through the courtesy of Mrs. A. H. Scott, Mr. J. C. Wister, and Mr. A. P. Saunders, and from Herbst Bros., New York City, and the Aomori Horticultural Station, Kuroisi, Japan.

Tests were first conducted to determine the temperature favorable for germination. The seeds were mixed with moist granulated peat moss and placed in electrically controlled ovens at various constant and alternating temperatures.

As soon as the seeds germinated (hypocotyls 1 to 3 cm. long) they were removed from the granulated peat moss and planted in pots or flats containing a mixture of equal parts of granulated peat moss, sand, and wood soil. The flats or pots were then placed in a greenhouse (13° C. or 21° C.) immediately or after two weeks to four months in ovens at various temperatures (1°, 5°, 10°, 15°, 20° C., and refrigerator). The refrigerator referred to throughout the paper is comparable to an ordinary electric refrigerator. It does not keep a constant temperature but fluctuates between 3° and 15° C. with an average of about 8° C.

All transfers of treated seedlings to the greenhouse conditions were made in the late fall, winter, or early spring when the greenhouse temperatures were fairly well controlled.

Seeds were also planted directly in flats which were kept in the greenhouse or which were placed in cold frames in open, mulched, or board-covered conditions for the winter. Consecutive plantings made at intervals of one to three months throughout the year were placed in a cold frame.

EXPERIMENTAL DATA

OVEN TESTS

Experiments were begun in the fall of 1929 on new seeds furnished by Scott and on seeds of the 1926 crop furnished by Saunders. The latter had been stored at approximately 8° C. First it was desirable to determine the temperature at which these seeds would germinate. Samples were placed at 1°, 5°, 10°, 15°, and 20° C., as well as daily alternating temperatures of 5° to 10° C., 10° to 30° C., and 15° to 30° C., and weekly alternating temperatures of 5° to 10° C., and −15° to 5° C. Only 15 seeds were available for each sample and yet it was quite evident that the higher temperatures (20° C. and daily alternation of 15° to 30° C.) were effective in producing roots. The first seedlings appeared in about two months and the germination extended over a period of approximately four months.

Subsequent tests of different seed crops at these high temperatures

showed that the germination percentages varied greatly. As low as 4 per cent and not more than 53 per cent were obtained.

From these results it appeared that the problem was a very simple one since the seeds needed no pre-treatment of any kind for germination. However, these seedlings failed to produce shoots when they were placed in the greenhouse. We could assume then that the greenhouse temperature was not favorable to the development of the epicotyl or that the epicotyl was really dormant and hence would require a pre-treatment to break this dormancy.

In the fall of 1930 additional experiments were started using seeds obtained from Scott and Wister. These seeds were divided in three lots, mixed with granulated peat moss, and one lot each placed at 20° C. and daily alternating temperatures of 10° to 30° C., and 15° to 30° C.

Germination tests here on a somewhat larger scale (using a total of 1228 seeds) confirmed the results of the tests of the previous year. As soon as the hypocotyls appeared at one of these high temperatures, the seedlings were removed and planted in pots in a mixture of granulated peat moss, sand, and wood soil. These pots were then placed in low temperature ovens (1°, 5°, 10°, and 15° C.) for intervals of one to three months after which times the pots were transferred to a greenhouse. The first transfers were made to a greenhouse with a temperature of 21° C. It was noticed that even if the shoots were starting to break through the soil at the time they were transferred, they all died in the greenhouse. Consequently, later transfers were made to a cooler greenhouse (13° C.). Here the growth of the shoots was very satisfactory.

The results of this treatment are shown in Table I. It will be noted that in spite of the fact that the number of seedlings involved was very small due to the irregular germinations at high temperatures, good development of epicotyls in the cool greenhouse followed a treatment of two and one-half or three months at 1° or 10° C.

Again in 1931 seeds were obtained from Scott and from Japan. All of these were mixed with moist granulated peat moss and put at a daily alternating temperature of 15° to 30° C.

As rapidly as the hypocotyls started to grow, the seedlings were planted in pots in the mixture described above and placed at various low temperatures where they were allowed to remain from two weeks to four months. Twenty seedlings were used in each pot in most instances and never less than eleven seedlings per pot were used. An effort was made to get as many replicates of each condition as possible. As far as was possible, comparable seedlings were planted in pots of the same series.

In twenty-one cases, duplicate lots were transferred to the warm (21° C.) and the cool (13° C.) greenhouses, always with the result that seedlings in the cool greenhouse grew well if the epicotyl had been held for the

TABLE I

PRODUCTION OF SHOOTS FROM SEEDLINGS (ROOT 1 TO 3 CM. LONG) WHICH HAD BEEN EXPOSED TO VARIOUS LOW TEMPERATURES. NUMBERS IN PARENTHESES INDICATE NUMBER OF SEEDLINGS USED IN EACH TEST

Seed lot	Temperature ° C.	% shoot production in greenhouse after months at low temperatures								
		0	0.5	1	1.5	2	2.5	3	3.5	4
Scott 1930	1	—	—	0(12)	—	22(22)	76(33)	90(21)	—	—
	5	—	—	18(11)	—	45(11)	—	—	—	—
	10	—	—	—	22(9)	27(30)	44(34)	68(25)	—	—
	15	—	—	—	22(9)	33(10)	—	—	—	—
Wister 1930	1	—	—	—	—	50(6)	56(9)	—	—	—
	10	—	—	—	—	50(6)	88(8)	—	—	—
Scott 1931	1	—	5(21)	20(14)	45(11)	42(17)	70(20)	80(20)	—	—
	5	—	5(21)	30(14)	10(11)	71(17)	80(20)	100(20)	—	—
	10	—	19(21)	20(14)	18(11)	47(17)	70(20)	85(20)	—	—
	15	—	5(21)	10(14)	10(11)	35*(17)	20*(20)	80*(20)	—	—
	Refrig.	—	—	—	—	—	—	55(20)	—	—
	Control	7(69)	—	—	—	—	—	—	—	—
Japan 1931	1	—	0(20)	35(60)	—	49(51)	62(55)	20(15)	63(35)	65(40)
	5	—	20(20)	18(65)	21(33)	58(106)	73(70)	77(35)	89(35)	78(40)
	10	—	20(20)	22(60)	41(32)	48(50)	78(50)	63(35)	63(35)	73(40)
	15	—	—	—	—	50*(18)	—	75*(20)	—	—
	Refrig.	—	25(20)	40(40)	24(33)	41(51)	64(36)	70(20)	—	—
	Control	7(60)	—	—	—	—	—	—	—	—
Scott 1932	1	—	—	7(15)	29(15)	33(15)	73(15)	—	—	—
	5	—	—	7(15)	24(15)	53(15)	47(15)	—	—	—
	10	—	—	7(15)	21(15)	20(15)	60(15)	—	—	—
	15	—	—	0(15)	—	53(15)	93(15)	—	—	—
	Refrig.	—	—	0(15)	—	11(15)	13(15)	—	—	—
	Control	5(42)	—	—	—	—	—	—	—	—

* Seedlings above ground when pot was transferred to greenhouse but died later.

proper time at a favorable low temperature previous to transfer to the greenhouse. Those in the warm greenhouse either grew very slowly or died.

Good shoot production in the greenhouse followed the pre-treatment of the seedlings for at least two months at 1°, 5°, 10° C., and refrigerator. (See Table I and Figs. 1 and 2.)

Figure 1 shows pots of seedlings from Scott, 1931 crop, transferred to the greenhouse after two, two and one-half, and three months at various temperatures except the control pots which were put in the greenhouse at the same time that the others were placed in the ovens. It will be seen that after three weeks in the greenhouse (Fig. 1 A) a large percentage of the seedlings from 5° and 10° C. (for two and one-half and three months) have developed shoots above ground. Fifteen degrees C. appears here as a favorable temperature also, while 1° C. appears unsatisfactory. After seven weeks in the greenhouse, however, it is evident that the epicotyl has been after-ripened at 1° C. while most of the seedlings which had appeared at 15° C. have died (Fig. 1 B). There is an indication here that 5° C. may be better than 10° C. for overcoming dormancy of the epicotyl. A treatment for two months is also effective, especially at 5° and 10° C.

Seedlings allowed to remain at 15° C. for two months or longer and at 10° C. for over three months appeared above ground while the pots were still in the ovens. In spite of the fact that these seedlings had developed more rapidly, they did not grow well when transferred to the greenhouse. This was especially true if they were allowed to remain at 15° C. for two and one-half months or at 10° C. for four months.

Control lots of seedlings planted and put directly in the greenhouse rarely showed epicotyl growth (Figs. 1, 2, and 3). The roots lived for several months but eventually died if they did not receive low temperature treatment. Figure 3 shows that, even after a year when pots were left in the greenhouse all summer and placed in a board-covered cold frame over winter, no seedlings appeared from the control pot. In other cases, however, it will be noted that a few shoots appeared above ground in the pots of seedlings which were placed immediately in a greenhouse at 13° C. (Table I) but these seedlings showed poor growth.

The comparative effects of one, one and one-half, two, and three months at various low temperatures are shown in Figure 2 and Table I. In Figure 2 it will be seen that the refrigerator temperature is also effective. This will be of interest to the peony grower who has an electric refrigerator available. Even one month at low temperatures is effective in hastening the appearance of the epicotyl of the tree peony, but as the time is increased, the effectiveness increases proportionally up to two and one-half months of treatment, after which there is no additional advantage. A prolonged period at low temperatures becomes harmful since the seedlings appear above ground and suffer from lack of light.

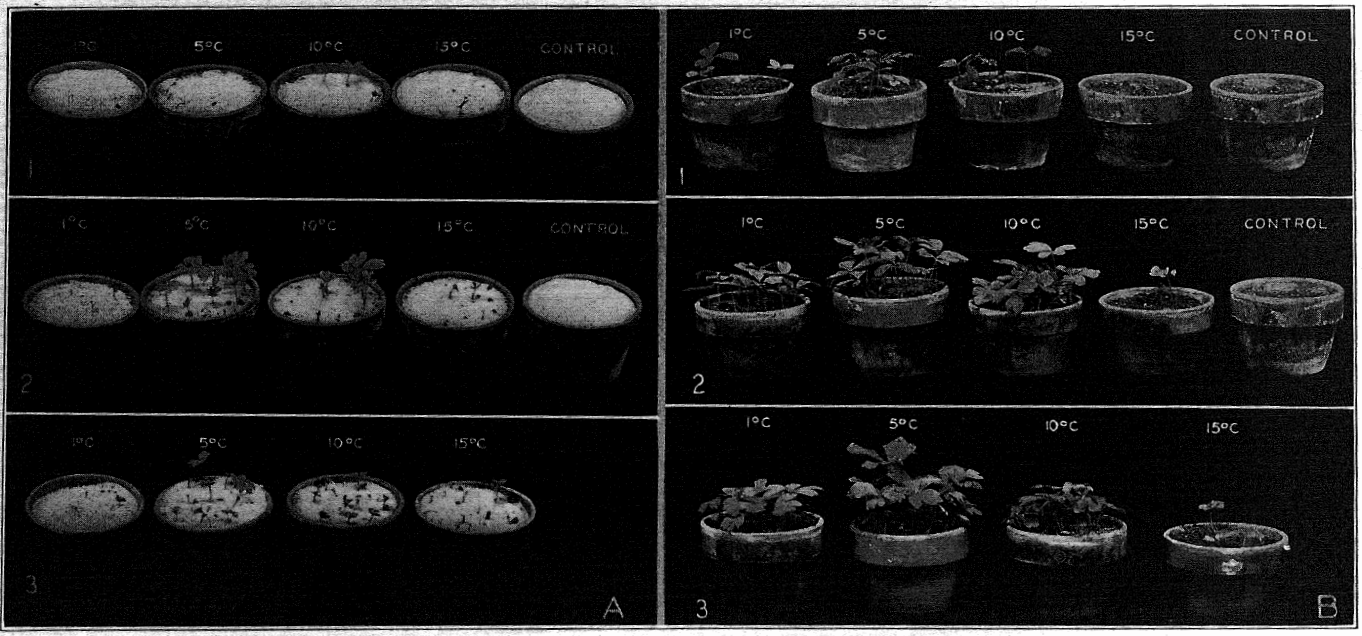


FIGURE 1. The effect of low temperature treatment for (1) two months, (2) two and one-half months, and (3) three months, on production of tree peony (Scott, crop 1931) shoots in a greenhouse (13° C.). Twenty seedlings (with hypocotyls 1 to 3 cm. long) planted in each pot. A. After three weeks in greenhouse; B. After seven weeks in greenhouse.

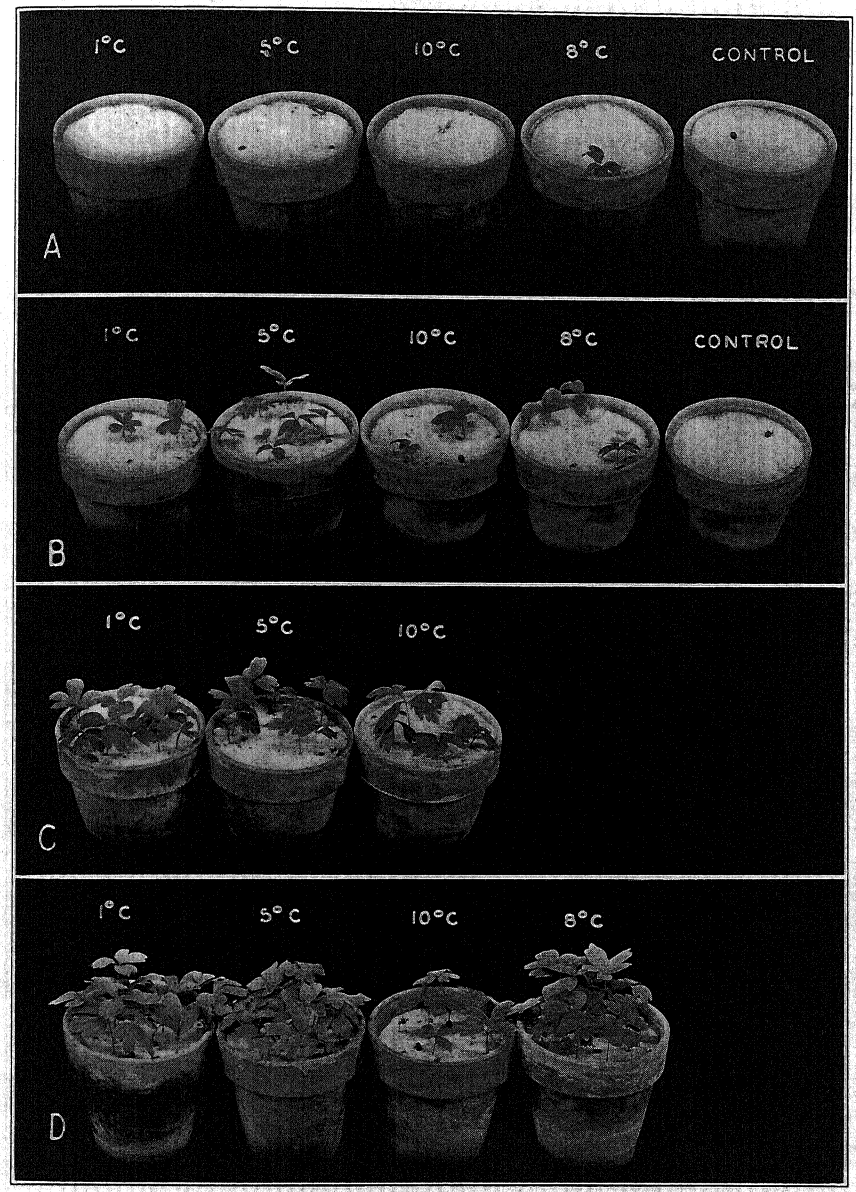


FIGURE 2. The effect of low temperature treatment for (A) two weeks, (B) one month, (C) two months, and (D) two and one-half months, on production of tree peony (Japan, crop 1931) shoots in a greenhouse (13° C.). Fifteen seedlings (with hypocotyls 1 to 3 cm. long) planted in each pot. Photographed after three months in greenhouse.

It is of interest to note the growth, at the end of a year, of the peony seedlings which had received two and one-half months' treatment (Fig. 3). This photograph was taken one year later than Fig. 1 B, and shows the same pots after a summer in the greenhouse followed by a winter in a board-covered frame. It appears that 5° and 10° C. are most favorable for good subsequent growth of seedlings.

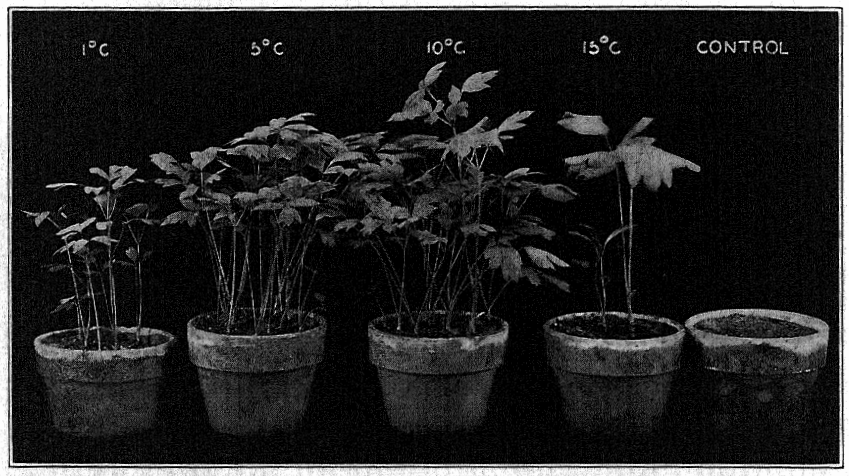


FIGURE 3. Tree peony (Scott, crop 1931) seedlings, one year and seven weeks after transfer to greenhouse (13° C.) following a pre-treatment of two and one-half months. Kept in board-covered frame over winter.

PLANTINGS IN FLATS

Plantings from a collection of seeds obtained from Herbst Bros. were made in flats in April, June, July, and September 1931. These flats were placed in cold frames immediately after planting and left over winter in open, mulched, and board-covered conditions. The seeds were poor in quality, a sample of 50 in moist granulated peat moss at a daily alternating temperature of 15° to 30° C. giving only 4 per cent germination.

No seedlings were obtained in either open or mulched conditions, but, in the summer of 1932, seedling production in the board-covered frame had reached up to 9 per cent (Table II). The September planting showed poorest seedling production (2 per cent). The ineffectiveness of mulch was probably due to the fact that the slightly higher temperature favored the early appearance of the seedlings which were then killed before the mulch was removed. In the case of the open frame, the freezing and thawing probably killed the young roots.

Again flat plantings were made in December 1931, and in February, March, May, and July 1932. Here the 1931 crop of seeds from Japan was

TABLE II

SEEDLING PRODUCTION IN FLATS PLANTED ON VARIOUS DATES AND KEPT IN A BOARD-COVERED COLD FRAME OVER WINTER; 100 SEEDS PLANTED IN EACH FLAT

Seed material	Date of planting	% seedling production Sept. 1932
Japan crop 1931*	Dec. 30, 1931	0
	Feb. 1, 1932	5
	Mar. 1, 1932	10
	May 2, 1932	25
	July 1, 1932	18
Herbst Bros. crop 1931**	April 9, 1931	6
	June 9, 1931	9
	July 9, 1931	9
	Sept. 10, 1931	2

* Gave 26% germination in oven test.
** Gave 4% germination in oven test.

used. From Table II and Figure 4, it will be seen that the May and July plantings were superior to the others. Again the board-covered condition proved best for cold frame treatment. No seedling production resulted from the open condition and from 0 to 12 per cent from the mulched condition. The May planting in the board-covered frame gave 25 per cent seedling production by April 1933 (Fig. 4 D). This germination can be regarded

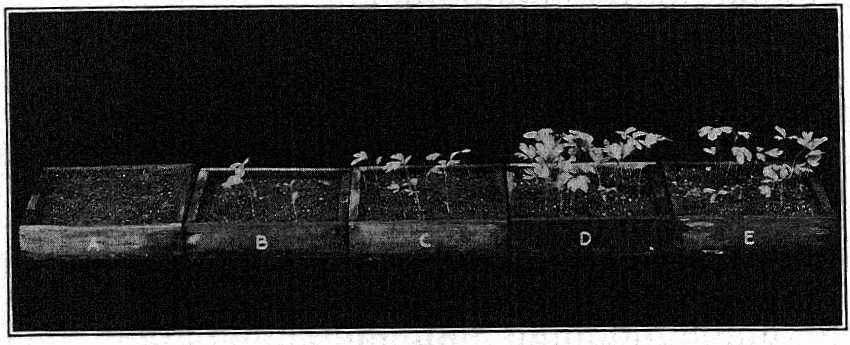


FIGURE 4. Seedling production in May 1933 from seeds planted in flats which were placed in a board-covered frame. Seeds planted: A. Dec. 1931; B. Feb. 1932; C. March 1932; D. May 1932; and E. July 1932.

as satisfactory since the oven test of these seeds showed 26 per cent germination. Seeds planted in December 1931 showed no seedling production in 1933 in spite of the fact that the seeds had the summer of 1932 for root development. The February and March 1932 plantings were better than the December 1931 planting but not so good as the May planting. Seeds planted in July gave about the same seedling production as those planted in March, although the former developed more quickly (Fig. 4 C and E).

Flats of seeds kept in the greenhouse showed no seedling production after two years.

SUMMARY

Tree peony seeds were germinated at a daily alternating temperature of 15° to 30° C., but if kept at this high temperature, the shoot failed to develop and the roots finally died.

When small seedlings (roots 1 to 3 cm. long) were planted in pots and kept at low temperatures (preferably 5° C., 10° C., or refrigerator) for two to three months, good shoot production followed their transfer to a cool greenhouse (about 13° C.).

When seeds were planted in flats in the fall or winter and kept in cold frames, the seedling production was very poor. Seeds planted in May, June, or July gave good seedling production the following spring. The roots appeared in the summer and then the cold winter period broke the dormancy of the epicotyl and the shoots appeared the following spring. Board-covered cold frames gave best results. If an earlier production of seedlings is desired, the seeds should be planted in a warm greenhouse immediately after harvest. Here they should be kept for about three months or until root production is complete. Then the flats should be transferred to a cellar or cold storage room with a temperature between 1° C. and 10° C. After two and one-half or three months, the flats may be transferred to a cool greenhouse where shoots should appear within a few days.

No attempt was made to treat the seeds in order to obtain a greater percentage of germination. It is possible that an effective pre-treatment may be found which will increase root production. The effect of soil acidity should also be determined. Only acid soils were used in the experiments reported here. These tests, together with others in which brown, undried seeds will be used, are now being planned.

The method here described insures good shoot production after the roots have once started to grow.

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GREENHOUSE FUMIGATIONS WITH NAPHTHALENE SOLUTIONS[1]

FRANK WILCOXON, ALBERT HARTZELL, AND W. J. YOUDEN

INTRODUCTION

In a previous publication (3) a method was described for controlling the concentration of naphthalene vapor during fumigation. The method consisted in passing air drawn from a source outside the greenhouse to be fumigated, and passing this air through a saturator containing shelves filled with naphthalene. The air carrying the desired percentage of naphthalene vapor was then discharged into the greenhouse.

Experience with this apparatus has shown that it is satisfactory except in one respect. The necessity of continually drawing air from an outside source is a decided disadvantage, particularly in winter when the outside temperature is low. If the greenhouse air is continually recirculated through the saturator instead of drawing fresh air from the outside, then the concentration of naphthalene vapor continually increases, reaching a value which will cause injury to sensitive plants. In order to avoid this difficulty, and still permit recirculation, use was made of the well-known fact that the vapor pressure of a substance is lowered when it is dissolved in a solvent. If the naphthalene were dissolved in a suitable solvent its vapor pressure could be lowered to any desired degree, and the concentration of naphthalene vapor in the greenhouse could never rise beyond a value determined by the concentration of naphthalene in the solution. The solution used might be a solid solution or a liquid solution, and in fact both kinds were successfully used in the experiments described below.

APPARATUS FOR USE WITH SOLID SOLUTIONS

For fumigating with a solid solution of naphthalene the saturator was used which has been described previously. Instead of pure naphthalene, however, cakes of a sulphur-naphthalene mixture consisting of a solid solution of naphthalene and sulphur were placed on the shelves, and the greenhouse air was recirculated through the apparatus instead of being drawn from an outside source.

The material was prepared by melting together naphthalene and sulphur in the desired proportions, which ranged from 20 to 90 per cent of naphthalene. The molten mixture was poured into shallow pans to cool, and the resulting cakes were broken up into small pieces and used in the saturator described in a previous paper.

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 70.

APPARATUS FOR USE WITH LIQUID SOLUTIONS

Figure 1 shows two views of the apparatus constructed for the recirculation of greenhouse air through a solution of naphthalene. The design of this saturator is such that it may readily be moved through the narrow aisles encountered in greenhouses and therefore serve a large number of

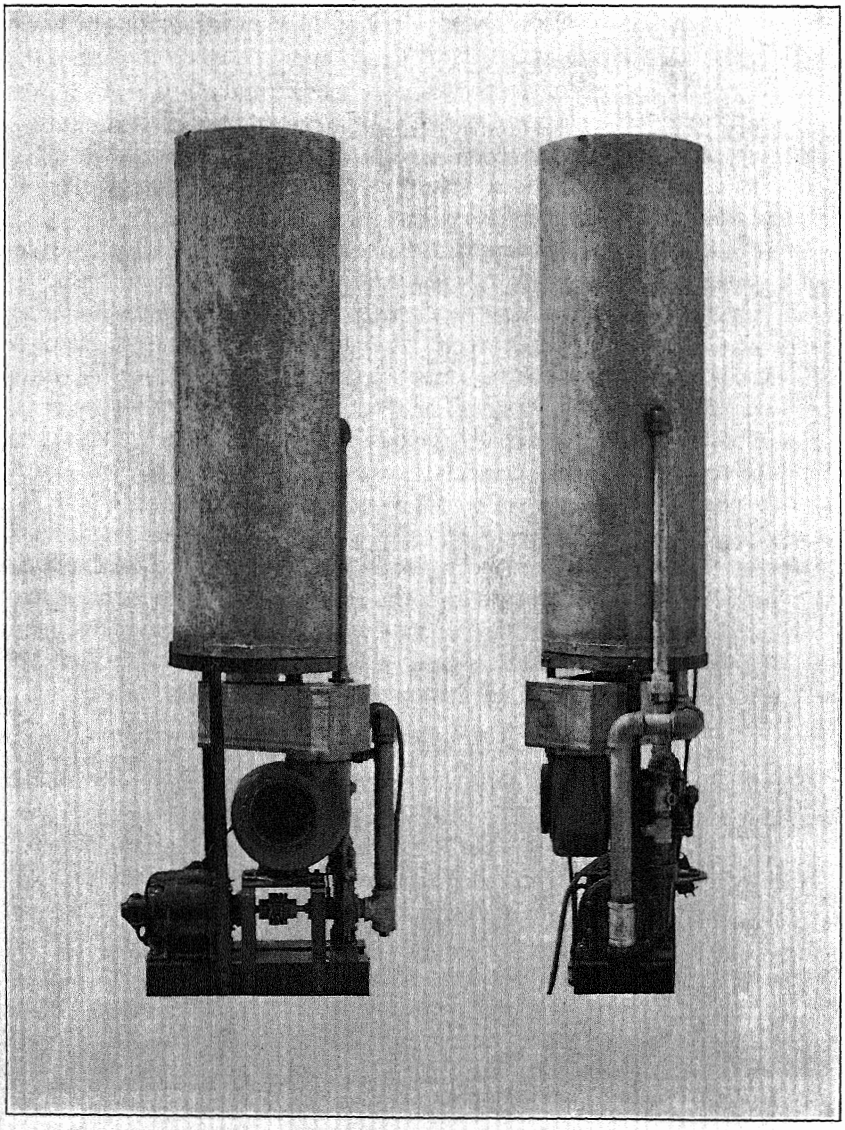


FIGURE 1. Two views of the oil-naphthalene apparatus.

houses. The arrangement is shown diagrammatically in Figure 2. The liquid, drawn from the bottom of the cylindrical container (A), is pumped over the top of a sheet metal cone (C) which surmounts a cylinder (B) which serves as the air inlet. A rectangular box connects a fan with this air inlet. The air drawn in by the fan (E) is deflected downward by the metal cone and passes through the rain of liquid pouring over the cone and then escapes through the top of the large cylinder. Four hoops (D) covered with cheesecloth serve to trap any droplets of liquid carried by

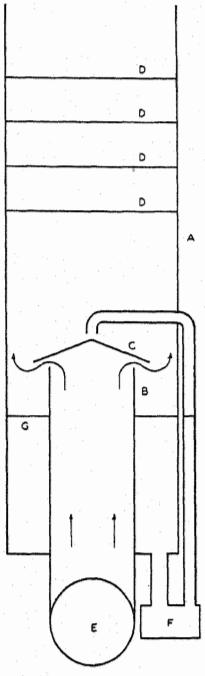


FIGURE 2. Diagrammatic sketch of the oil-naphthalene apparatus. A, outer cylinder; B, inner cylinder; C, sheet metal cone; D, cheesecloth filters; E, fan; F, pump; G, level of the oil-naphthalene solution. The arrows show the path of the air current.

the air. Important specifications for the construction of this piece of equipment are listed as follows:

Gauge of sheet metal No. 16
Diameter of outer cylinder 16 inches
Height of outer cylinder 50 inches
Diameter of inner cylinder 8 inches
Height of inner cylinder 17 inches
Overall height 80 inches
Pipe sizes 1 and 1.5 inches

Fan delivers 530 cubic feet per minute free delivery
Fan delivers 450 cubic feet per minute at one-quarter inch static pressure
Pump delivers 42 gallons per minute at 20 feet head
Pump delivers estimated 50 gallons per minute as installed
Fan motor $\frac{1}{8}$ H.P. 1725 R.P.M.
Pump motor $\frac{1}{4}$ H.P. 1725 R.P.M.

SELECTION OF SOLVENT

The choice of an appropriate solvent for the naphthalene necessitated a consideration of the requirements that such a solvent must meet. The vapor pressure of the solvent should be very low; otherwise the large volumes of air passed through the solution will remove the solvent along with the naphthalene. The solvent must not be toxic to plants in the concentration built up by its partial pressure. The solubility of naphthalene in the solvent ought to be considerable so that the removal of naphthalene during the fumigation will not greatly alter the original concentration. The solvent must not corrode the metal parts. It should be inexpensive and readily obtained in quantity on short notice. Hygroscopic qualities are undesirable since dilution might occur through the accumulation of water. Many of these qualities are found in light motor oils. The solubility of naphthalene in an oil with an S.A.E. rating of 20[2] was about ten per cent by weight.

LABORATORY EXPERIMENTS WITH OIL SOLUTION OF NAPHTHALENE

Some preliminary laboratory experiments were performed with oil solutions containing various percentages of naphthalene. Leaves infested with red spider mite (*Tetranychus telarius* L.) were confined in a shallow glass vessel in which was also placed an open dish of the oil solution. These experiments are listed in Table I and show that complete control was ob-

TABLE I

RESULTS OF LABORATORY TESTS ON RED SPIDER MITE EXPOSED TO NAPHTHALENE DISSOLVED IN OIL

Per cent naphthalene by wt.	Number alive	Number dead	Per cent kill
2.0	5	4	45
3.0	15	16	52
3.5	4	27	87
4.0	2	14	88
4.0	3	26	90
4.5	0	44	100
5.0	2	12	86
5.0	0	38	100
5.5	0	20	100
5.5	0	36	100
6.0	0	17	100
8.0	0	20	100

[2] The oil used was Mobiloil Arctic manufactured by the Vacuum Oil Company, New York, New York.

tained with solutions containing 5 or more per cent of naphthalene by weight. In some cases a desiccator, arranged so that both the oil and air were stirred, was used to insure equilibrium conditions.

The solutions prepared for these laboratory tests were utilized to establish a curve relating the composition of the solution to its specific gravity. The change in density of the oil is rather small and it is important to bring the solutions to a constant temperature before reading the hydrometer. A high grade hydrometer with a range from 0.880 to 0.940 and a long stem which made it possible to estimate the fourth decimal place was used. The relationship is practically a straight line and is shown in Figure 3.

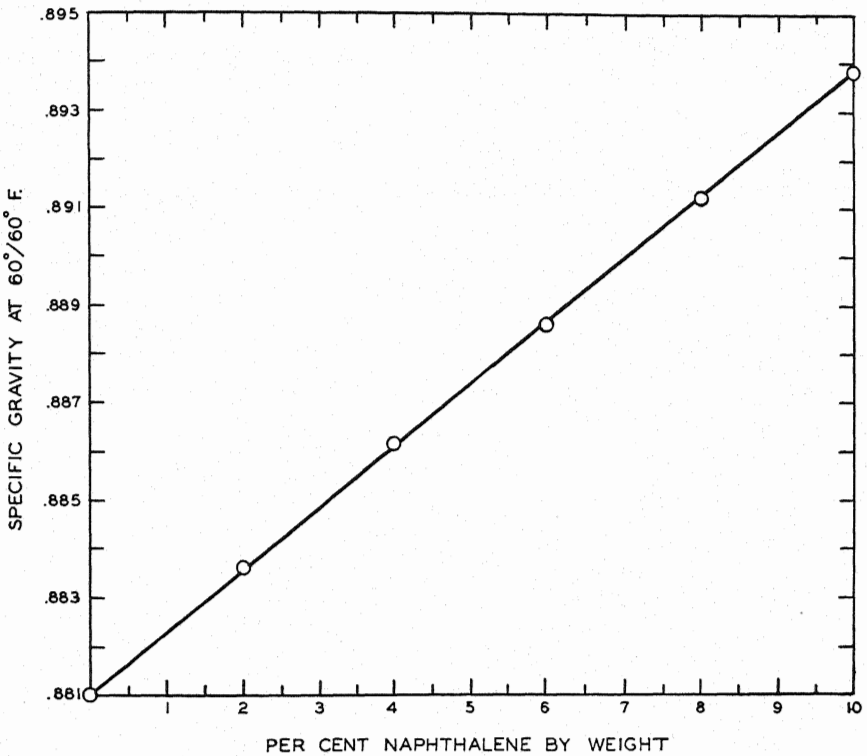


FIGURE 3. Curve showing the relation between density and concentration of naphthalene in oil solution.

The 5 per cent solution represents approximately a one-half saturated solution. If the similarity in chemical nature of the solute and solvent justify the assumption of a perfect solution, then the partial vapor pressure of naphthalene is one-half that of the vapor pressure of pure naphthalene, which at room temperature is approximately 0.1 mm. (International

Critical Tables). A greenhouse with a capacity of 6000 cubic feet requires then $\frac{6000 \times 28.3}{24.6} \times 128 \times \frac{.05}{760} = 58.2$ grams of naphthalene to build up a toxic concentration. The gram molecular volume at 30° C. is 24.6 liters and one cubic foot equals 28.3 liters. The molecular weight of naphthalene is 128.

In greenhouse practice a higher concentration than 5 per cent of naphthalene in oil will be required due to the inevitable leakage of air which constantly dilutes the equilibrium mixture. The fact that about 1.5 pounds of naphthalene are removed from the solution in the course of a fumigation indicates the extent of this loss. Consequently the initial concentration of naphthalene in the oil was made as high as 8 or 9 per cent in some instances. The concentration of the solution is readily adjusted by adding naphthalene flakes and running the pump for a few minutes to circulate the oil and bring about solution. The original solution was prepared by adding 7.5 gallons of oil and 4 pounds of naphthalene, resulting in a solution of 6.8 per cent naphthalene by weight. The specific gravity was determined at the start and conclusion of each run in order to establish the amount of naphthalene consumed.

GREENHOUSE FUMIGATIONS

Fumigations were begun either at 4 p.m. or at 5 p.m. and continued until 6 a.m., 8 a.m., or 9 a.m., depending on whether periods of 13, 14, 15, or 16 hours' duration were desired. Greenhouse sections of a capacity of 6000 cubic feet were fumigated. The plants were grown in pots or in flats and were watered before the fumigation was started as previous experience had shown that a high relative humidity is desirable to avoid injury to the foliage. The most favorable temperature range for fumigation was found to be between 68° F. and 80° F.

The difficulty of detecting analytically the concentration of naphthalene vapor in air led to the use of tomato plants of the variety Bonny Best and buckwheat as test plants which previous investigation (3) had shown would indicate the maximum and minimum concentrations respectively for satisfactory control of the red spider mite, and at the same time would be within the tolerance limit of other plants commonly grown in greenhouses. The foliage of sunflower, digitalis, and balsam was found to be as sensitive as that of buckwheat (Table II) to naphthalene fumigation and these plants should be added to the lists previously published (1, 2, 3). Since in a number of cases the degree of injury depends on the age of the plants, heights are indicated in Table II. Tests with solid solutions of naphthalene and sulphur, the content of which ranged from 20 to 90 per cent of naphthalene, indicated that a solid solution containing 90 per cent naphthalene and 10 per cent sulphur could be used without injury to the

TABLE II

SENSITIVITY OF HOST PLANTS TO NAPHTHALENE FUMIGATION AT CONTROLLED CONCENTRATIONS

Plant	Naphthalene-sulphur		Naphthalene-oil	
	No. plants	Plant height, inches	No. plants	Plant height, inches
Plants sensitive to naphthalene vapor				
Balsam (*Impatiens balsamina* L.)	116	6–14	30	20
Buckwheat (*Fagopyrum esculentum* Moench.)	10	6	10	6
Digitalis (*Digitalis purpurea* L.)	5	10	—	—
Sunflower (*Helianthus annuus* L.)	74	10–36	22	14
Plants not sensitive to naphthalene vapor				
African marigold (*Tagetes erecta* L.)	335	8–18	82	28–36
Begonia (*Begonia semperflorens* Link & Otto)	—	—	9	7–10
Broccoli (*Brassica oleracea* L. var. *italica* Plenck.)	—	—	40	8
Bryophyllum (*Bryophyllum* sp.)	10	8	—	—
Calendula (*Calendula officinalis* L.)	12	14	—	—
Chrysanthemum (*Chrysanthemum carinatum* L.)	12	16	—	—
Coleus (*Coleus blumei* Benth.)	—	—	31	8–18
Cosmos (*Cosmos bipinnatus* Cav.)	88	4–22	23	60
Cotton (*Gossypium hirsutum* L.)	1	12	—	—
Cyclamen (*Cyclamen indicum* L.)	5	6	—	—
Eggplant (*Solanum melongena* L. var. *esculentum* Nees.)	10	10	—	—
English ivy (*Hedera helix* L.)	—	—	1	14
Euonymus (*Euonymus japonica* L. f.)	16	14–22	—	—
Fuchsia (*Fuchsia hybrida* Voss.)	38	12–24	—	—
Holly (*Ilex opaca* Ait.)	—	—	1	14
Hydrangea (*Hydrangea paniculata* Sieb. var. *grandiflora* Sieb.)	50	12–16	—	—
Japanese maple (*Acer palmatum* Thunb.)	4	8	14	6
Jerusalem cherry (*Solanum pseudocapsicum* L.)	150	4	30	20
Mazzard cherry (*Prunus avium* L.)	—	—	12	4
Nasturtium (*Tropaeolum minus* L.)	5	6–10	—	—
Peach seedlings (*Prunus persica* (L.) Stokes.)	700	2–12	—	—
Pepper (*Capsicum frutescens* L.)	200	3	—	—
Popcorn (*Zea mays* L. var. *everta* Bailey)	—	—	48	40
Privet (*Ligustrum vulgare* L.)	—	—	39	8–10
Rose (*Rosa* sp. var. Briarcliff)	8	30–36	16	24
Rose (*Rosa* sp. var. Madame Butterfly)	15	30–36	—	—
Rose seedlings (*Rosa* sp.)	—	—	15	16
Sensitive plant (*Mimosa pudica* L.)	4	8	—	—
Silver bell (*Halesia carolina* L.)	—	—	14	7
Strawberry (*Fragaria chiloensis* Duchesne var. *ananassa* Bailey)	5	4	—	—
Sweet potato (*Ipomoea batatas* Lam.)	6	12	1	9
Tobacco (*Nicotiana tabacum* L. var. Turkish)	—	—	11	36
Tomato (*Lycopersicon esculentum* Mill. var. Bonny Best)	232	3–6	113	8–14
Willow (*Salix* sp.)	—	—	30	24

foliage of the most sensitive plants tested. No foliage injury was observed on plants fumigated by means of the naphthalene-oil method at concentrations reported in Table III. Marigold, calendula, cosmos, carnation, and hydrangea were fumigated while in bloom without injury to the flowers.

The red spider mite was selected as a suitable test species for the efficiency of a given fumigation as previous investigation (3) had shown that it is more difficult to eradicate than the cyclamen mite (*Tarsonemus pallidus* Bks.), the onion thrips (*Thrips tabaci* Lind.), or the black grain thrips

TABLE III

PERCENTAGE CONTROL OF RED SPIDER MITE BY NAPHTHALENE VAPOR

Exposure to naphthalene vapors, hrs.	Per cent naphthalene	Temperature		Relative humidity, per cent	Number of specimens	Per cent control
		Maximum ° F.	Minimum ° F.			
Naphthalene-sulphur						
13	90	78	65	88	179	95
14	90	86	68	88	502	99
14	90	76	60	68	200	99
15	20	74	70	88	368	80
15	25	80	76	85	115	80
15	20	84	72	88	212	89
15	75	82	68	80	113	93
15	75	100	68	88	229	91
18	75	100	68	88	204	90
15	100	89	80	60	253	99
14	100	100	68	88	200	100
Naphthalene-oil						
16	6.7	95	65	—	211	89
16	6.6	69	62	—	222	96
16	7.9	80	60	—	250	100
16	9.5	100	77	70	200	100

(*Heliothrips femoralis* Reuter). Counts of living and dead mites were made 24 hours after treatment. The results are presented in Table III. The per cent of mites killed ranged from 95 to 100 when solid solutions of naphthalene (90 per cent) and sulphur (10 per cent) were used. Solid solutions made up of lower percentages of naphthalene were not effective, while pure naphthalene was impractical because of injury to foliage. Equally successful fumigations were obtained with the liquid solution method.

Plants infested with a species of *Tarsonemus* mite resembling the cyclamen mite, the specific identity of which has not yet been established (4), were included in a number of fumigations. This species seems to have about the same tolerance for naphthalene vapor as the cyclamen mite and was readily eradicated at the concentration required to kill the red spider mite.

SUMMARY

A method of fumigating with naphthalene is described which permits the control of the concentration of naphthalene vapor, and which ensures that the desired concentration will be maintained throughout the fumigation period.

This method involves the continued recirculation of the greenhouse air through a saturator containing a solution of naphthalene in an inert solvent. The concentration of the naphthalene in the solvent determines the maximum concentration which can be reached in the greenhouse air.

A saturator is described and illustrated for use with solutions of naphthalene in oil, which brings about intimate contact of the greenhouse air with the solution used. In addition to solutions of naphthalene and oil, experiments were performed with a solid solution of naphthalene in sulphur using an apparatus described in a previous publication. It was found that satisfactory control of red spider mite could be obtained by both these methods by fumigating for a period of 14 to 16 hours without injury to plants usually considered sensitive to naphthalene fumigation.

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CARBON DIOXIDE STORAGE. V. BREAKING THE DORMANCY OF POTATO TUBERS

NORWOOD C. THORNTON

The forcing of dormant buds into activity has been investigated intensively during the past half of a century. For this purpose light, high and low temperature, oxygen, vacuum, hot water, wounding, and treatments with various chemicals have been employed. For a review of these treatments the reader is referred to papers by Appleman (1) and Denny (5). A survey of the literature indicates that tests of the effect of carbon dioxide with oxygen have not been made.

Weber (11) studying the effect of acetylene on dormant buds considered an oxygen-free atmosphere as the important factor in shortening the rest period. To verify this idea other gases such as nitrogen, hydrogen, and carbon dioxide were employed to give the oxygen-free atmosphere, and with each treatment the rest period of *Syringa vulgaris* was shortened. He did not, however, consider carbon dioxide as a factor in promoting the growth of the buds.

This paper presents the results that have been obtained in shortening the rest period of dormant potatoes (*Solanum tuberosum* L.) by storage in various concentrations of carbon dioxide with oxygen. Determinations were made on the altered rate of respiration of the tubers and changes in pH and reducing properties of the juice as induced by the treatment.

MATERIAL AND METHODS

Dormant potatoes obtained from South Carolina, Maryland, New Jersey, and the Institute garden were treated three to seven days with various concentrations of carbon dioxide immediately after harvesting and at periods of 7 to 14 days later. The varieties used were Bliss Triumph, obtained only from the Institute garden, and Irish Cobbler, from all sources of supply. The tubers, 6 to 12 in number, were placed in 8 or 18-liter tin cans having tight-fitting lids that were sealed with a paraffin and vaseline mixture. The procedure of treating the potatoes with the mixture of 12 to 60 per cent of carbon dioxide with 20 per cent of oxygen and nitrogen, as well as the gas analyses of the storage atmosphere, was the same as used in a previous study (10). The treatments were carried out at 25° C.

Gas analysis of the storage atmosphere at the end of the treatment showed that the tubers had developed, on the average, 7 per cent of carbon dioxide in the control, 15 per cent in the 35 per cent carbon dioxide treatment, and from 17 to 20 per cent in the treatments with 50 per cent or higher concentrations of carbon dioxide. The respiration data were calcu-

lated as milligrams of oxygen uptake per kilogram of tissue per hour rather than on the basis of the carbon dioxide produced. The concentration of carbon dioxide given in the discussion of the results represents that present at the beginning of the experiment.

After obtaining the respiration data the tubers were removed from the cans and one to three one-eye pieces were cut from each and planted in fertile soil in flats. The cut pieces were covered approximately one and one-half to two inches with soil. The flats were placed in the open in the garden during June to October and in the greenhouse and basement during November to January. Data on number of sprouts were taken every few days and records were made of the time necessary for 50 per cent of the pieces to show sprouts above the soil and for maximum sprouting to be attained.

Tissue not used in planting was taken for analyses. After removing the skin the tissue was ground through the nut cutter of a food chopper. The ground tissue was placed in cheesecloth and the juice was squeezed out by hand and poured at once into centrifuge tubes and centrifuged. Determinations of the reduction of methylene blue, reduction of iodine in acid solution, and tests with sodium nitroprusside were carried out on the centrifuged juice according to the methods used by Denny, Miller, and Guthrie (6). The pH measurements were made on the extracted juice with a quinhydrone electrode using a saturated calomel half cell as reference electrode. The glutathione content of the treated and untreated tubers was determined by the method of Guthrie and Wilcoxon (7).

RESULTS

SPROUTING OF DORMANT POTATOES

Treatment of whole tubers. The effectiveness of the carbon dioxide treatment in hastening the sprouting of the dormant buds of freshly-harvested potato tubers is shown by the data in Table I, columns 3 and 4. The tubers treated with 57 per cent of carbon dioxide for six days sprouted and 50 per cent of the sprouts appeared within 19 days after planting. At the end of an additional 11 days, 100 per cent of the tubers had produced sprouts above the soil. Upon reducing the treatment to three days it was found that 29 days were required for 50 per cent emergence. In contrast to the carbon dioxide treatment the control tubers required more than 60 days (flats discarded at this time) from time of planting for 50 per cent of the tubers to produce sprouts above the soil.

Photographs of these potatoes taken 28 days after planting are shown in Figure 1 A and B. The potatoes shown in Figure 1 A were quite dormant at the time of the treatment so that the carbon dioxide concentration had to be greater than 30 per cent to promote sprouting. The potatoes shown in Figure 1 B were of the same lot but the treatment was begun two weeks

TABLE I

EFFECT OF CARBON DIOXIDE ON THE SPROUTING, RESPIRATION, AND PROPERTIES OF THE EXPRESSED JUICE OF DORMANT POTATO TUBERS

Remarks	Per cent CO_2	Days for 50% emergence	Per cent above ground in 30 days	Mg. O_2 used per kg. per hr.	pH	Reduction of methylene blue, minutes	Cc. N/100 iodine absorbed	Sodium nitroprusside test	Mg. glutathione per 100 g. tissue	
									At planting	6 days after planting
South Carolina Irish Cobbler Received June 2, 1932 Treated June 4–10	Control*	60+	12		6.02	30+	1.00	−		
	Control**	60+	6	15.2	6.34	30+	1.20	−	7.2	
	13	60+	0	14.1	6.36	30+	1.75	−		
	30	60+	12	21.6	6.58	30+	1.80	+	8.0	
	46	24	71	36.8	6.70	10	2.00	+	4.8	
	58	19	100	35.6	6.75	10	2.00	+	4.3	
South Carolina Irish Cobbler Treated June 17–20	Control*	60+	0		6.03	30+	0.9	−		
	Control**	60+	0	16.2	6.27	30+	1.05	−	7.5	3.4
	15	60+	0	14.7	6.32	30+	1.15	−	7.5	3.6
	30	60+	18	23.4	6.61	7	1.25	−	7.6	4.1
	59	29	50	30.0	6.63	5	1.30	−	5.4	4.2
South Carolina Irish Cobbler Treated June 17–23	Control*	60+	0		6.03	30+	0.9	−		
	Control**	48	6	14.9	6.22	30+	0.9	−	6.4	4.9
	12	45	25	14.6	6.34	25	1.0	−	6.6	5.0
	28	60+	18	19.0	6.53	8	1.05	−	6.2	5.1
	59	21	100	31.1	6.61	3	1.10	+	2.8	8.1

* Control lot stored in room.
** Control lot stored in container.

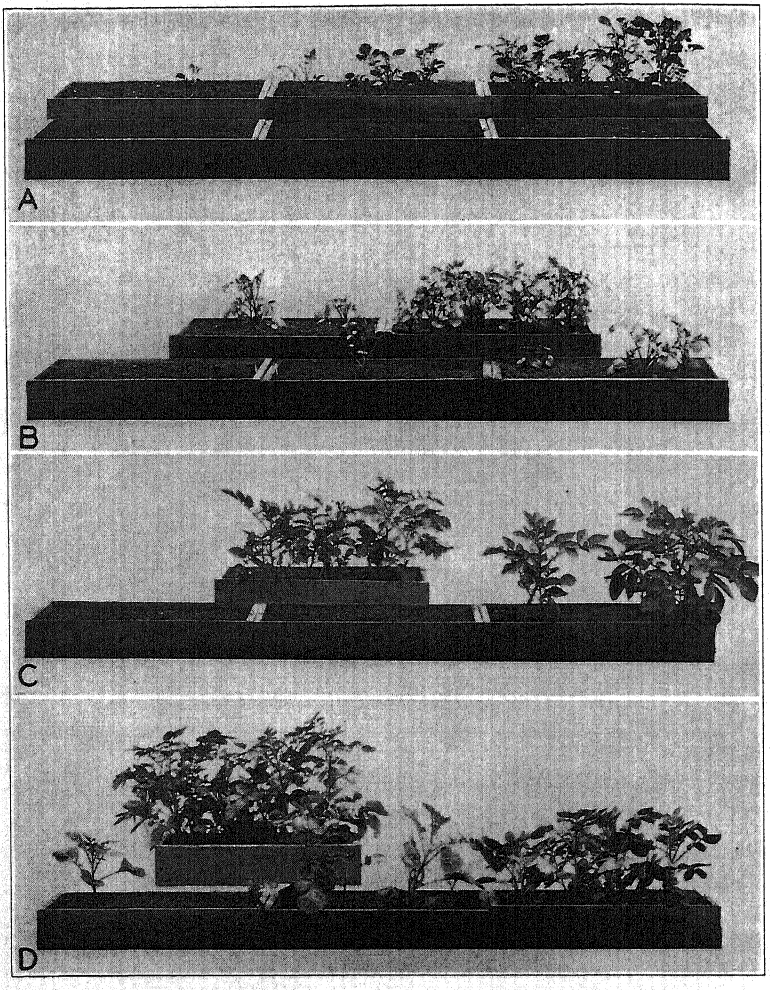


FIGURE 1. The effect on storing dormant potatoes for six days in various concentrations of carbon dioxide at 25° C. (A) Irish Cobblers from South Carolina, treated June 4 to June 10, 1932, photographed July 8. Left to right: front row, control in room, control in container, 13 per cent carbon dioxide; back row, 30 per cent carbon dioxide, 46 per cent carbon dioxide, 58 per cent carbon dioxide. (B) Irish Cobblers from South Carolina, treated June 17 to June 23, 1932, photographed July 21. Left to right: front row, control in room, control in container, 12 per cent carbon dioxide; back row, 28 per cent carbon dioxide, 59 per cent carbon dioxide. (C) Bliss Triumph, treated August 6 to August 12, 1932, photographed October 10. Left to right: front row, control in room, control in container, 35 per cent carbon dioxide; back row, 50 per cent carbon dioxide. (D) Bliss Triumph. Twelve tubers quartered, one piece per treatment from August 17 to August 23, 1932, photographed October 10. Left to right: front row, control planted, control in container, 35 per cent carbon dioxide; back row, 50 per cent carbon dioxide.

after harvesting. In this case an appreciable acceleration in the rate of sprouting was obtained with the application of 12 per cent of carbon dioxide. The tubers that were held as control in the container during the treatment show one sprout which may be attributed to a variation in the dormancy of the buds at this time. However, it is possible that the carbon dioxide accumulating through respiration of the tubers may have had some effect upon the dormancy of these buds, especially in this case, since the dormancy had been lessened somewhat by storage in air for two weeks before the treatment.

Tubers treated with 28 per cent of carbon dioxide (see lower group of data, Table I) required more than 60 days for 50 per cent of the sprouts to appear above the soil. Although the tubers from this treatment were slow to sprout, as compared with the other treatments, they did produce more sprouts above the soil than the tubers held as control in the room. At the end of 60 days, 44 per cent of the tubers that had been treated with 28 per cent carbon dioxide showed sprouts above the soil in comparison with only 11 per cent for the control.

Irish Cobbler potatoes harvested from the Institute garden August 15 were treated for six days beginning on August 20. The treatment with 57 per cent of carbon dioxide promoted sprouting of the tubers so that 50 per cent produced sprouts above the soil within 35 days after planting. At the end of 60 days only 8 per cent of the control tubers had produced sprouts.

Tests were next made with the Bliss Triumph variety immediately after harvesting. Figure 1 C shows the degree of sprouting and growth of the sprouts 59 days after being planted. The data collected upon the rate of sprouting show that with the 50 per cent carbon dioxide treatment half of the tubers had sprouts above the soil within 30 days after planting. The tubers that were treated with 35 per cent of carbon dioxide produced 28 per cent emergence at this time. The control tubers, however, did not produce sprouts within 60 days.

Bliss Triumph potatoes treated ten days after harvesting gave results similar to those obtained with the Irish Cobbler variety in Table I and Figure 1 B. A treatment of the tubers with 50 per cent of carbon dioxide for six days resulted in 50 per cent emergence within 26 days. The tubers treated with 35 per cent of carbon dioxide sprouted and showed 50 per cent of the sprouts above the soil within 38 days. At this time 20 per cent of the tubers that had been held as control in the container produced sprouts above the soil. Four per cent of the tubers that had been held as control in the room during the treatment showed sprouts above the soil at the end of 38 days and 12 per cent within 60 days.

These results have been replicated many times during 1932 and 1933 with dormant potatoes. In general, the results show that a treatment with

TABLE II

EFFECT OF CARBON DIOXIDE ON THE SPROUTING OF DORMANT POTATOES*

Variety and source	Date harvested 1932	Date treated 1932	No. days for 50 per cent emergence				Per cent above ground in 30 days			
			Control**	Control†	35% CO_2	50% CO_2	Control**	Control†	35% CO_2	50% CO_2
Bliss Triumph Yonkers	Aug. 6	Aug. 6 to Aug. 12	71+	71+	71+	38	8	0	8	25
Bliss Triumph Yonkers	Aug. 6	Aug. 17 to Aug. 23	59+	55	20	16	0	0	50	83
Irish Cobbler Yonkers	Aug. 15	Aug. 20 to Aug. 26	83	88+	29	26	0	0	66	57
Irish Cobbler New Jersey	Oct. 26	Oct. 28 to Nov. 2	64+	64+	50	38	0	0	8	16

* Potatoes quartered and one-quarter placed in each treatment.
** Control lot stored in room.
† Control lot stored in container.

40 to 60 per cent of carbon dioxide for three to six days will shorten the rest period of the potatoes and cause 50 per cent of the tubers to show sprouts above the soil within a period of 19 to 35 days after planting as compared with 60 or more days for the control.

Treatment of quartered tubers. In addition to the treatment of whole tubers, experiments were carried out whereby the tubers were cut into quarters before exposing to the carbon dioxide treatments. For this test four treatments were employed in order to use one-quarter of the potato tuber in each. The data given in Table II show that it is possible to treat cut tubers with carbon dioxide and obtain similar sprouting response as with whole tubers. From 16 to 38 days were required for 50 per cent emergence with the treatment of 50 per cent of carbon dioxide. Figure 1 D shows the Bliss Triumph potatoes, treated August 17 to August 23 (Table II), photographed 48 days after planting.

Treatment with other gases. Preliminary experiments, started 6 to 15 days after harvesting, have been carried out with Irish Cobbler potatoes in oxygen concentrations ranging from 0 to 100 per cent for both three and six days. The results show no acceleration of sprouting of the treated tubers over the controls due either to low or high oxygen content. In addition to these tests, tubers were treated with 100 per cent carbon dioxide and 100 per cent nitrogen which in both cases gave a much accelerated rate of sprouting over 100 per cent oxygen. Tubers exposed to normal and reduced percentages of oxygen with 60 per cent of carbon dioxide for three days gave some interesting results; much better and quicker sprouting was obtained with the use of 20 per cent than with 0, 5, or 10 per cent of oxygen. At this time the experiments on these phases of the problem have not been completed.

CHANGES IN THE POTATO WITH THE CARBON DIOXIDE TREATMENT

Increase in respiration. The effect of the carbon dioxide treatment upon the respiration of the potato is comparable with that of many other chemicals effective in shortening the rest period which have been studied by Miller (9). The rate of respiration, as measured by the rate of oxygen uptake, may be more than doubled during the period of the treatment. The data in Table I show an actual increase of from 85 to 134 per cent over the control. This increase in the rate of respiration of the tubers apparently does not in itself bring about the increased tendency to sprout. A test of this assumption was made by cutting the tubers in half and treating one-half with 30 per cent of carbon dioxide for three days while holding the remaining half as control. The rate of respiration of the tubers increased with wounding, but the treatment increased the rate only slightly over that of the control. Upon planting, the treated pieces sprouted and 50 per cent

appeared above ground within a month while the control pieces did not sprout within two months.

Increase in pH. Changes in the pH of the tissue with the carbon dioxide treatment are shown in Table I. Treatments of the tissue with the weak acid resulted in a more alkaline juice as shown by a change of 0.6 to 0.7 of a pH unit toward the alkaline direction. These data have been substantiated by measurements on other plant tissues as well as on non-dormant potatoes. As the potato tuber ages, the difference in pH between the treated and control may at times fall as low as 0.4 of a pH unit. The pH data in Table I do not show, however, the extent of the alkaline reaction produced since even a higher pH value may be obtained by aerating or boiling off the dissolved carbon dioxide from the extracted juice.

Reduction of methylene blue. The reducing properties of the juices extracted from treated tubers are greatly increased as shown in Table I. From the data at hand it appears that the lower concentrations of carbon dioxide, 10 to 15 per cent, exert a slight effect upon the reducing properties of the juices, but higher concentrations have a very decided effect. This was especially true of the juice from tubers treated with 80 per cent of carbon dioxide since 5 cc. of it in many cases reduced 1 cc. of methylene blue within 1 minute as compared to more than 30 minutes for the control.

It is known that the pH value of the solution in which the methylene blue reduction is carried out is sometimes of importance; the less acid the solution the more rapid the rate of reduction. Adjusting the pH of the treated or control juice to the same value with 0.1 N NaOH or 0.1 N HCl showed that this factor did not play a part in the observed difference in the rate of reduction of methylene blue since the same difference was obtained as when the originally expressed juice was used.

Absorption of iodine. Further evidence of the reducing power of the juice extracted from the treated potato is its power to absorb appreciable amounts of iodine in the presence of trichloroacetic acid. Five cc. of the juice were titrated with 0.01 N iodine with starch as an indicator. The juice extracted from freshly-harvested potatoes that were treated with 58 per cent of carbon dioxide for six days absorbed 2 cc. of the iodine as compared with 1 cc. for the control. In this study even the control in the container gave an increase of 0.2 cc. in the iodine titration. As the tubers became less dormant such marked differences were not obtained between the treated and control juices.

Test with sodium nitroprusside. Sodium nitroprusside was used as a qualitative test of the sulphydryl content of the juice extracted from the treated and control tubers. Positive tests were obtained on juices from potatoes that had been subjected to a six-day treatment with 30 to 60 per cent of carbon dioxide, but not with the three-day treatment (Table I). As the potatoes became less dormant the test was increasingly harder to

obtain. Finally, only a treatment with the highest concentration of carbon dioxide would give a slight coloration upon addition of the sodium nitroprusside.

The sodium nitroprusside test is given by reduced glutathione, but not by the oxidized form. Although it is generally accepted that the glutathione present in tissues is the reduced form, it is oxidized in the process of extraction, due to the presence of oxidase and the unavoidable aeration involved in squeezing out the juice. However, as the juice is allowed to stand, the oxidized glutathione may be converted to the reduced form if a suitable reducing system is present. The presence of a system in the treated juice which reduces methylene blue is shown, although it is not known whether this system is effective in reducing glutathione. The nitroprusside test in the juice is, therefore, not a measure of the total amount of glutathione present, but rather of the power of the juice to change glutathione from its oxidized to its reduced form.

Glutathione content of the tubers. Analysis of the tissue for glutathione was made by the sulphur reduction method of Guthrie and Wilcoxon (7). This procedure avoids oxidation of glutathione during the extraction, since oxidase is first destroyed by dropping the tissue into boiling water. The data in Table I show a decrease in the glutathione content of the high carbon dioxide-treated tubers at the end of the treatment. When tubers were cut and planted for six days previous to the analysis, an increase in the glutathione content was observed.

DISCUSSION

The results of these experiments show that carbon dioxide is effective in shortening the rest period of dormant buds. It is effective in either the presence or absence of oxygen. However, an initial supply of oxygen is desirable to prevent the breakdown of the tissue during a long period of treatment. Storage in nitrogen has also resulted in shortening the rest period, possibly due to the accumulation of carbon dioxide during anaerobic respiration. Such treatments were found more effective in promoting growth of the dormant buds than storage in oxygen.

Weber (11) and Boresch (2) have considered low oxygen supply as an important factor in shortening the rest period of dormant buds. Weber obtained oxygen-free atmospheres to bring about suffocation of the tissue by storage in various gases, while Boresch employed a warm water bath which he considered led to a high rate of respiration with a low oxygen supply. Probably in the experiments of Weber and possibly in those of Boresch the carbon dioxide accumulating within the tissue played an important part in promoting the growth of the resting buds. It is likewise true that the favorable results obtained by Denny (4) and Miller (9) with ethylene chlorhydrin were due in part to the carbon dioxide accumulating

in the tissue because of the greatly increased respiration which results from the ethylene chlorhydrin treatment. This is especially applicable in those cases in which treatments were carried out in closed containers which facilitated the accumulation of the carbon dioxide.

The storage of non-dormant potatoes in carbon dioxide has been reported by Kidd (8) and Braun (3). Kidd, working with artificially produced atmospheres, found that 10 per cent carbon dioxide retarded somewhat and 20 per cent inhibited growth of the buds. Braun employed closed containers where the gas accumulated as a result of respiration and found that up to 12 per cent carbon dioxide hastened the sprouting of non-dormant potatoes. The most favorable concentrations were 5 to 8 per cent and any increase above this lowered the total growth to some extent.

SUMMARY

1. Dormant potato tubers treated for 3 to 7 days with 40 to 60 per cent of carbon dioxide with 20 per cent of oxygen at 25° C. sprouted and 50 per cent appeared above the soil in 19 to 35 days as compared with 60 or more days required for the control to appear. These results were obtained with the treatment of either cut or whole tubers.

2. Dormant potatoes subjected to a 3 or 6-day storage in 100 per cent nitrogen or 100 per cent carbon dioxide, and then planted, sprouted more rapidly than when held in 100 per cent oxygen.

3. A 60 per cent carbon dioxide treatment for 3 days with 20 per cent oxygen was more effective in producing sprouting than with either 0, 5, or 10 per cent oxygen.

4. Carbon dioxide increased the rate of respiration of potato tubers as measured by the oxygen uptake.

5. The treatment with 50 to 70 per cent carbon dioxide induced changes whereby the pH of the juice shifted from 0.6 to 0.7 of a pH unit toward alkalinity over that of the control.

6. The reducing properties of the potato juice are greatly increased by the treatment as shown by tests with methylene blue, sodium nitroprusside, and absorption of iodine.

7. The glutathione content of the tubers was decreased during the carbon dioxide treatment, but greatly increased over the control after the tubers were planted.

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TRANSPIRATION OF TOBACCO PLANTS IN RELATION TO RADIANT ENERGY IN THE VISIBLE AND INFRA-RED

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INTRODUCTION

It is difficult, if not impossible, to determine the effect of various single environmental factors such as light intensity, temperature, and humidity on the rate of water loss in plants growing under natural conditions. In nature both the factors themselves and their effects on plants are interrelated. New York Weather Bureau records show that on an average day in June and July total solar radiation increases from sunrise until noon and decreases again toward sunset. Air temperature follows a similar curve except that the maximum occurs normally about 2:00 or 3:00 p.m. The curve of average relative humidity is at a minimum at the same hour at which the temperature is maximum. Many workers have reported for an average day a rise in the transpiration rate of plants until noon or slightly later, followed by a decrease toward evening. It is probable, therefore, that the transpiration rate is closely correlated with one or more of these environmental factors. Before dismissing the subject of transpiration from our minds as either harmful to the plant or a necessary evil, the relation of water loss in plants to each factor in the environment must be most carefully studied. This study was started to determine approximately the effect of each factor on the rate of water loss in a single species, the tobacco plant, *Nicotiana tabacum L.*, variety Turkish. With the development of a more accurate control of environmental factors and an extension of the study to other species, it is hoped that more detailed information will be forthcoming at some future time. The relation between water supply and available solar energy is of considerable economic importance since this equation is without doubt closely related to the ecological distribution of plants. The whole subject, therefore, merits a more detailed study than is included in the present paper, but since some general relations have been established, it is believed advisable to publish an account of them at this time.

METHODS

The studies were made in the constant light room which has been previously described (2). By means of standard air-conditioning machinery, both temperature and humidity were controlled during these experiments. In the first series of experiments the temperature range was 73° to 78° F. in regular cycles varying from 15 to 45 minutes depending upon outside air temperature. The relative humidity range was 47 to 54 per cent. The high temperature range was between 98° and 100° F. with a

correspondingly narrower range of relative humidity. The light source was a 1000 or 1500-watt filament lamp fitted with an aluminum reflector. The labeled voltage of the lamps was 105 except in one experiment with a low efficiency lamp, labeled voltage 120. All lamps were burned on the regular 118 to 120-volt supply line. The lamps were raised and lowered to produce any intensity desired upon the soil in which the plants were grown. Energy measurements were made both with the Weather Bureau pyrheliometer (8) and the Shirley radiometer (11). In the infra-red tests a composite filter made up of nine pieces of Corning's heat-transmitting glass was used to absorb the visible region. This experiment will be described in greater detail later. The amount of water lost per unit area of plant leaf was determined in the following way.

Young tobacco plants having five or six leaves were transferred from four-inch pots to porcelain enamel cups approximately five and one-half inches in diameter by five inches high. The plants were sealed in by means of a layer of warm paraffin and tallow mixture. A glass tube about 7 mm. in diameter was inserted through this layer and into the soil. Measured amounts of water were added as required through this tube. All except three, or rarely four, large leaves were removed. The plants were allowed to grow usually with artificial light for a period of 72 to 96 hours, until they had recovered from manipulation and transfer. They were then placed under experimental conditions. The amount of water lost by each plant was determined by weighing the plants with containers at 12-hour intervals. The containers were brought up to the original weight when they had lost sufficient water to warrant the addition of 50 or 100 grams.

In the first tests leaf areas were determined at the end of each test by cutting off the leaves, printing the outline of each on blue print paper, and estimating the areas with a planimeter. On account of the rapid growth of leaves the areas obtained in this way were not accurate for any time period of the test except the last 48 hours. A method was therefore developed for determining leaf areas rapidly without detaching them from the plant so that the same series of plants could be studied under different conditions over a considerable time period. By making a determination of area every second or third day on attached leaves it was possible to calculate the water loss per unit area as the leaf area increased from day to day. A photograph of the spring clip apparatus used for this purpose is shown in Figure 1. It was made by flattening the jaws of a brass wire test tube holder, attaching the lower jaw to an aluminum plate and the upper jaw to a thin sheet of glass. The aluminum plate used was five by eight inches. The glass plate was four by eight inches. The aluminum plate can be attached to the spring clip by means of small bolts. The glass plate is conveniently held to the clip by De Khotinsky cement. The areas were printed by first placing the plant on a table in a dark room. The spring clip

was opened by grasping it in the right hand and pushing down on the upper wire with the thumb. A piece of photographic paper was laid on the sheet of aluminum. The leaf, still attached to the plant, was placed over the paper and the thumb pressure released. The spring clip held the leaf firmly between the aluminum and glass plates while an exposure of a few seconds was made to a 50-watt lamp housed in a deep sheet metal reflector. The lamp and reflector were suspended by a cord so as to swing freely below the table when not in use. The paper was then developed and the area determined by a planimeter.



FIGURE 1. Spring clip apparatus for determining leaf area on attached leaves.

Certain precautions were found necessary in order to obtain consistent results in water losses. All leaves must be exposed as nearly as possible normal to the incident rays. Many leaves including tobacco are often presented at a sharp angle to the main axis of the plant. This is especially true in high intensity illumination. By this means the plant avoids direct exposure of great areas to high energy values. During the course of these tests the leaves were bent down frequently so as to present the maximum surface to the light. This was usually done during periods of darkness by means of a glass plate supported immediately over the tips of the plants. During light exposure periods the leaves were often bent down by hand. In

later studies only three leaves were allowed to remain on each plant. New leaves were removed from the tip of the plant as fast as they appeared. It was found that the fourth leaf when allowed to remain usually cast a partial shadow on one of the lower leaves and interfered with the results. Placing of the plants on the table under the lamp so as not to shade leaves on other plants was found to be important. Five to nine plants were used in each test. When plants were first transferred from clay pots to the porcelain enamel cups for the tests it was found that a period of several days must be allowed before the loss of water per plant under the same conditions reached a constant value. Exposure of plants to high light intensity before this period invariably resulted in wilting of the leaves and greatly decreased water losses. After this period plants could be exposed up to the limit of their endurance with no wilting as long as sufficient water to make up the loss was supplied at each 12-hour period.

The highest energy value used in the tests herewith reported was 0.72 gram calorie per square centimeter per minute. This value was chosen as approximately half of the maximum intensity of noon sunlight during May, June, and July. According to New York Weather Bureau records the month of May 1933 shows a total of 12,252 gram calories of energy. The average per hour for each 12-hour day was 33 gram calories. This is equivalent to about 0.5 gram calorie per square centimeter per minute. The brightest day recorded in May had a total of 683.6 gram calories, slightly less than one gram calorie per minute. The intensity of artificial light used at 0.72 gram calorie is therefore less than the average for the brightest day but greater than the average May day of this year. The distribution of energy per hour is very different. In the case of the brightest May day the energy reaches a maximum at the noon hour of 1.34 gram calories per minute. At the hours 8:00 a.m. and 4:00 p.m. the energy has fallen to a value slightly greater than 0.5 gram calorie. The energy under the lamp is comparatively constant at 0.72 gram calorie per minute for the entire 12-hour period, the intensity changing only with line voltage variations.

RESULTS OF TESTS

The first tests were made with large plants and with most of the leaves still attached to the plants. The results are presented in Table I. The water losses were calculated from weighings after a period of 12 hours in the light condition and again after 12 hours in darkness. The first test was made in the greenhouse on February 15. This was one of the three darkest days in February according to Weather Bureau records and the water loss was very low as compared with the losses in tests 2, 3, and 4. Tests 2, 3, and 4 were made in the constant condition room using artificial light. All three tests were made at a temperature of 73° to 78° F. and about 50 per cent relative humidity. A 1000-watt, 105-volt lamp, fitted with an alumi-

TABLE I

AVERAGE WATER LOSS PER PLANT AND PER SQUARE INCH OF LEAF AREA IN 12 HOURS UNDER CONDITIONS LISTED. LARGE PLANTS USUALLY WITH MORE THAN THREE LEAVES

Conditions of Tests	Av. leaf area per plant; sq. in.	Darkness		Light	
		Loss per plant; g.	Loss per sq. in.; g.	Loss per plant; g.	Loss per sq. in.; g.
1. Greenhouse. February 15. Sunlight 0.28 gram calorie per minute for brightest hour and 94.3 gram calories for entire day.	78	7	0.09	24	0.30
2. 1000-watt, 105-volt lamp at 33 inches. 0.65 gram calorie. 73° to 78° F. 50 per cent relative humidity.	91	5	0.06	75	0.82
3. Conditions the same. Second test. Large plants.	266	7	0.02	189	0.72
4. Conditions the same. Third test.	64	9	0.16	57	0.89

num reflector, placed at a distance of 33 inches above the soil surface, was used. The calculated loss per square inch in all of the tables is based on the area of only one surface of the leaf. The tests show the low losses in darkness as compared with those under the lamp. On account of the larger number of leaves and the failure to present all surfaces to the light the losses are less than those under comparable conditions listed in the following tables.

The first ten tests on plants listed in Table II show the effects of light intensity and humidity on the rate of water loss. Smaller plants were used and all leaves except three were removed before starting the tests. In this series the same set of plants was used at high and low intensities and at high and low humidities alternating usually with 12-hour periods of darkness. The data in the first four tests indicate that an increase in total energy received from 0.28 to 0.65 of a gram calorie per square centimeter per minute results in an increase in average water loss from 0.60 and 0.63 to 1.15 and 1.29, that is, an increase of approximately 2.3 times the energy doubles the rate of water loss under these conditions. This relation is practically the same at a relative humidity of 88 per cent as shown in tests 7 and 8. The changes in relative humidity from 50 per cent to 88 per cent had no significant effects on rate of water loss. The effects, if any, were small and within the individual variations of the tests. It should be pointed out that these tests were all made at a constant low velocity of air movement. It is possible that humidity changes would have more effect on the transpiration rate at greater velocities. It is also possible that wider ranges in humidity would show an appreciable effect.

Tests 11 through 14 listed in Table II were made with a low efficiency lamp, that is, a 120-volt, 1000-watt lamp burning on a line current of 118 to 120 volts. This is approximately the normal operating efficiency of this type of lamp as used in industrial lighting. In tests 11 and 13 only the infra-red output of the lamp was used. This was accomplished by the use of a filter of Corning's heat-transmitting glass 4.8 mm. in thickness. This filter, which has been described in detail in an earlier publication (1), was made up of nine pieces of glass, each six and one-half inches square, set into copper cames. It was mounted on wooden supports 25 inches above the table. Black cloth was stretched on wire supports around the filter in such a way as to protect the plants against extraneous light and still admit air freely. The same set of seven plants was used throughout the tests. The water loss under infra-red in both tests 11 and 13 was 0.18 gram per square inch in the 12-hour period. The water loss under visible and infra-red radiation at the same energy level (0.22 gram calorie per square centimeter per minute) was found to be 0.34 gram (test 14), a value almost twice that under infra-red alone. Test 12 shows the effect on water loss of increasing both visible and infra-red energy from 0.22 to 0.41 gram calorie. This was

accomplished by leaving the lamp at the same distance above the soil (35 inches) and removing the infra-red filter. By comparison of tests 12 and 14 it will be noted that 1.86 times the energy gives, under these conditions, an increase of about 1.6 times the rate of water loss. This is the same rate of increase as was indicated above under the more efficient lamps, that is, doubling the total energy increases the rate of water loss to 1.74.

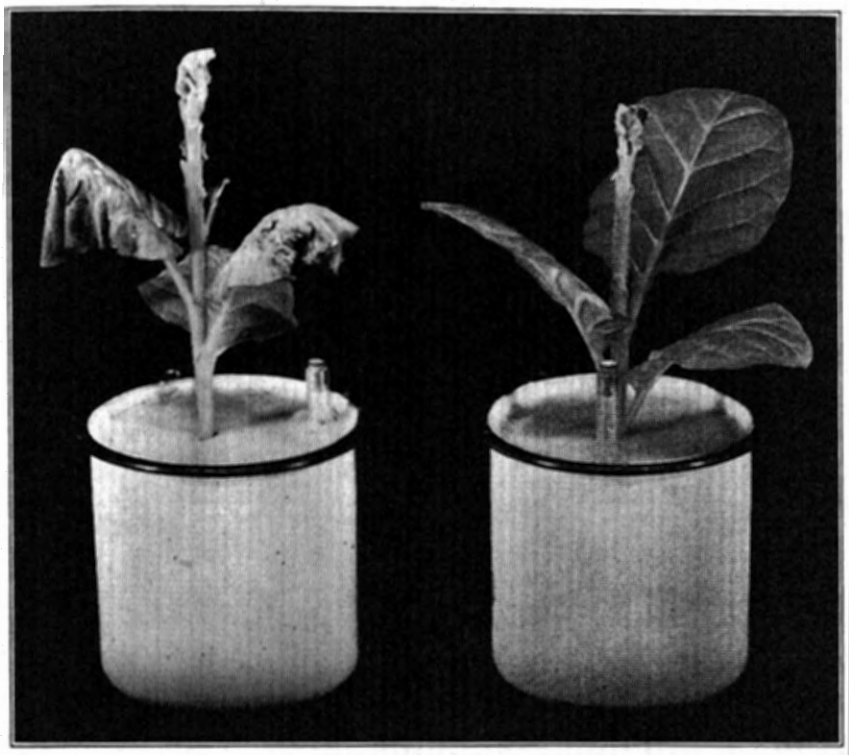


FIGURE 2. Tobacco plants sealed in porcelain enamel cups for transpiration study. Left, plant injured by combination of high infra-red, high humidity, and high temperature. Right, normal plant.

Table III shows the results of tests with nine plants over a period of 18 days during which the water loss was determined under various conditions of temperature, humidity, and light. During this series of tests the average leaf area increased from 22 to 31 square inches. The water losses per unit area were calculated in each case on the area determined on that date nearest to the time at which the loss occurred. Tests 1 through 9 show the losses under both infra-red and visible energy at 0.65 and 0.72 gram calorie per square centimeter per minute at a temperature of 73° to 78° F.

TABLE II

AVERAGE WATER LOSS PER PLANT AND PER SQUARE INCH OF LEAF AREA IN 12 HOURS UNDER CONDITIONS LISTED. TEMPERATURE 73° TO 78° F. SMALL PLANTS WITH THREE LEAVES

Conditions of tests with 1000-watt, 105-volt lamp	Av. leaf area per plant; sq. in.	Darkness		Light	
		Loss per plant; g.	Loss per sq. in.; g.	Loss per plant; g.	Loss per sq. in.; g.
1. Lamp at 32 inches. 0.65 g. cal. High intensity. 50 per cent relative humidity.	30	2.0	0.07	34	1.15
2. Same plants as #1. Lamp at 55.5 inches. 0.28 g. cal. Low intensity. 50 per cent relative humidity.	30	—	—	18	0.60
3. Lamp at 55.5 inches. 0.28 g. cal. Low intensity. 50 per cent relative humidity.	23	2.0	0.05	15	0.63
4. Same plants as #3. Lamp at 33 inches. 0.65 g. cal. High intensity. 50 per cent relative humidity.	—	—	—	26	1.29
5. Lamp at 33 inches. 0.65 g. cal. High intensity. Low humidity. 50 per cent relative humidity.	30	3.0	0.09	32	1.00
6. Same plants and same intensity as #5. High intensity. High humidity. 88 per cent relative humidity.	—	1.0	0.03	35	1.17
7. Lamp at 33 inches. 0.65 g. cal. High intensity. High humidity. 88 per cent relative humidity.	40	1.6	0.04	44	1.09
8. Same plants, same as #7 except lamp at 55.5 inches. 0.28 g. cal. Low intensity. High humidity. 88 per cent relative humidity.	—	2.0	0.05	25	0.56
9. Lamp at 33 inches. 0.65 g. cal. High intensity. High humidity. 88 per cent relative humidity.	40	1.6	0.04	36	0.93
10. Same plants and same intensity as #9. High intensity. Low humidity. 50 per cent relative humidity.	42	2.6	0.06	51	1.19

TABLE II (*Continued*)

Tests with low efficiency 1000-watt, 120-volt lamp—Infra-red and visible. Same plants used throughout. Temperature 73° to 78° F. Relative humidity 80 per cent.	Av. leaf area per plant; sq. in.	Darkness		Light	
		Loss per plant; g.	Loss per sq. in.; g.	Loss per plant; g.	Loss per sq. in.; g.
11. Lamp at 35 inches. 0.22 g. cal. Infra-red only.	43	4.5	0.10	7	0.18
12. Lamp at 35 inches. 0.41 g. cal. Visible and infra-red.	43	—	—	23	0.55
13. Lamp at 35 inches. 0.22 g. cal. Infra-red only.	43	5.0	0.11	8	0.18
14. Lamp at 55 inches. 0.22 g. cal. Visible and infra-red.	45	—	—	15	0.34

TABLE III

AVERAGE WATER LOSS PER PLANT AND PER SQUARE INCH OF LEAF AREA IN 12 HOURS UNDER CONDITIONS LISTED. 1500-WATT, 105-VOLT LAMP AT 33 INCHES FOR VISIBLE TESTS AND 23 INCHES WITH FILTER FOR INFRA-RED TESTS. ENERGY FOR VISIBLE TESTS 0.72 GRAM CALORIE AND FOR INFRA-RED TESTS 0.65 GRAM CALORIE. THE SAME NINE PLANTS USED IN ALL TESTS

Conditions of tests	Av. leaf area per plant; sq. in.	Darkness		Light	
		Loss per plant; g.	Loss per sq. in.; g.	Loss per plant; g.	Loss per sq. in.; g.
1. Apr. 25 Infra-red only 73° to 78° F. Rel. hum. 68%	22	2	0.12	10	0.50
2. " 26 Visible " " " " " "	—	—	—	24	1.16
3. " 27 " " " " " " "	—	2	0.12	28	1.39
4. " 28 Infra-red only " " " " " "	25	—	—	10	0.43
5. " 28 Visible " " " " " "	—	—	—	26	1.10
6. " 29 Infra-red only " " " " " "	—	—	—	11	0.49
7. " 30 Visible " " " " " "	29	—	—	36	1.27
8. May 1 Infra-red only " " " " " "	—	—	—	15	0.53
9. " 1 Visible " " " " " "	—	—	—	33	1.15
10. " 3 " 98° " 100° F. " " "	30	6	0.22	83	2.78
11. " 4 Infra-red only " " " " " "	—	—	—	62	2.09
12. " 5 Visible " " " " " "	—	11	0.35	82	2.75
13. " 6 " " " " " " "	—	—	—	80	2.67
14. " 7 " " " " " " "	31	7	0.23	85	2.82
15. " 8 " " " " " " 87%	—	13	0.42	74	2.38
16. " 9 " " " " " " "	—	—	—	81	2.59
17. " 9 Infra-red only " " " " " "	—	14	0.44	60*	1.92

* Plants badly injured by high energy in infra-red with high temperature and high humidity.

The term "visible" energy includes also the infra-red emitted by the lamp without a filter. The average loss under infra-red from tests 1, 4, 6, and 8 was 0.49 gram in 12 hours per square inch of leaf area. The average loss under visible energy from tests 2, 3, 5, 7, and 9 was 1.21 grams. The rate of loss under visible at this temperature is, therefore, almost 2.5 times the loss under infra-red alone. When the temperature is increased to the range 98° to 100° F. in tests 10 to 14 the infra-red rate of loss increases rapidly to 2.09 as compared with an average of 2.75 for the visible (found in tests 10, 12, 13, and 14). The rate in the visible under these conditions is only 1.3 times that of the infra-red. In tests 15, 16, and 17 the same high temperature was maintained and in addition the humidity was increased to 87 per cent relative. The high humidity in this case produced a slight decrease in the rate under both the visible and infra-red conditions and produced great injury on most of the plants. Wilting of leaves and the development later of large necrotic areas in the leaf lamina occurred. This is shown in Figure 2. The water loss in darkness (Table III) was increased first by the increase in temperature and again by the increase in humidity to a maximum of 0.42 and 0.44 gram per square inch for the 12-hour period. At the lower temperature there was no definite increase in water loss during darkness when humidity was increased.

If the average losses per unit area at low temperatures in tests 2, 3, 5, 7, and 9 be compared with the losses at high temperatures in tests 10, 12, 13, and 14 it will be noted that this temperature change of approximately 24° F. increased the transpiration rate approximately 2.3 times.

STOMATAL OPENING AND WATER LOSS

Frequently during the tests small sections of leaves were removed for determining stomatal opening. Special plants were set aside for this purpose. The procedure in this case was to plunge the section as soon as removed into boiling absolute alcohol. It was then examined with a microscope. All stomata were found to be tightly closed under infra-red only and in darkness. In the light the degree of opening varied but some of the stomata were open at all times. It is apparent, therefore, that losses in the infra-red and in darkness are in the main through the cuticle and are not influenced by stomatal opening. Water losses in light are no doubt affected by stomatal movement. In every case water loss under visible energy was greater than under infra-red, but at high temperatures where it is important that the leaf temperature be held down so as not to go beyond the thermal death point the water loss through the cuticle approached the maximum value obtained in light when the stomata were partially open. Greater injury to tissue is produced by infra-red at high temperature and humidity than by visible radiation. This may be because the cooling system is less efficient due to closure of the stomata.

LEAF TEMPERATURES AND WATER LOSS

Leaf temperatures were taken from time to time under the various conditions by means of thermocouples inserted into the leaf lamina. Leaf temperatures in light were always higher than those in darkness. With a room temperature of 73° to 78° F. the leaf temperature range was 89° to 92° F. under visible radiation at 0.72 gram calorie. At the higher temperature range (98° to 100° F.) leaf temperatures were 105° to 107° F. At the lower range of room temperature the leaves under infra-red only maintained a temperature of approximately 103° F. At the higher temperature range the leaf temperature was only slightly higher at 107° F. Some heat was re-radiated to the leaves from the glass filter in the infra-red tests as the plant leaves on tall plants were often within five inches of the glass. In all of the tests leaf temperatures at no time exceeded 107° F. The plant seemed to hold this temperature in the face of increasing air temperature and increasing radiation. The lamp at one time was brought down to a distance of 14 inches, as measured from the tip of the lamp to the leaf holding the thermocouple and kept there all day. The leaf temperature held steadily between 105° and 107° F. The radiation intensity in this case was 1.6 gram calories. In order to check transpiration under these conditions a leaf was painted on both sides with vaseline. This produced a temperature rise of not more than 2° F. This small increase was in no way proportional to that which might be expected by suddenly stopping the high water losses which were found to obtain under such energy and temperature conditions. The alternative possibility remained that vaseline does not decrease transpiration sufficiently to produce a marked increase in temperature.

In order to determine whether transpiration was checked sufficiently by vaseline, another method of accomplishing the same purpose was tried. Two layers of thin cellophane were cut in the same shape as the leaf but approximately one-half inch larger radially. The two pieces were cemented with shellac at the edges and the envelope thus formed was slipped over a new leaf holding the thermocouple. The lamp was turned on and in four minutes the temperature of the leaf in the envelope increased from 87° to 127° F. where it remained at equilibrium. After 15 minutes the cellophane was removed. The inner walls of the cellophane envelope were covered with beads of water which had come from the leaf and condensed on the cooler surface. The leaf had a distinct aromatic odor which was not present in the case of the other leaves on the same plant. After two days in the greenhouse this leaf developed several large necrotic areas as a result of the treatment. It was apparent that the cellophane envelope had checked much of the energy lost by transpiration at once and a rapid rise of 40° F. had resulted—a temperature which was probably well beyond the

lethal temperature for this leaf. The experiment was repeated with a new envelope and another leaf, except that the second leaf was thoroughly vaselined before slipping the cellophane envelope over it. The temperature mounted to about the same value in the same length of time. After 25 minutes the envelope was removed. It seemed to contain about the same amount of water as in the first case although this was only determined by inspection. Figure 3 is a photograph of this cellophane envelope showing the beads of moisture on the inner walls. This moisture apparently came

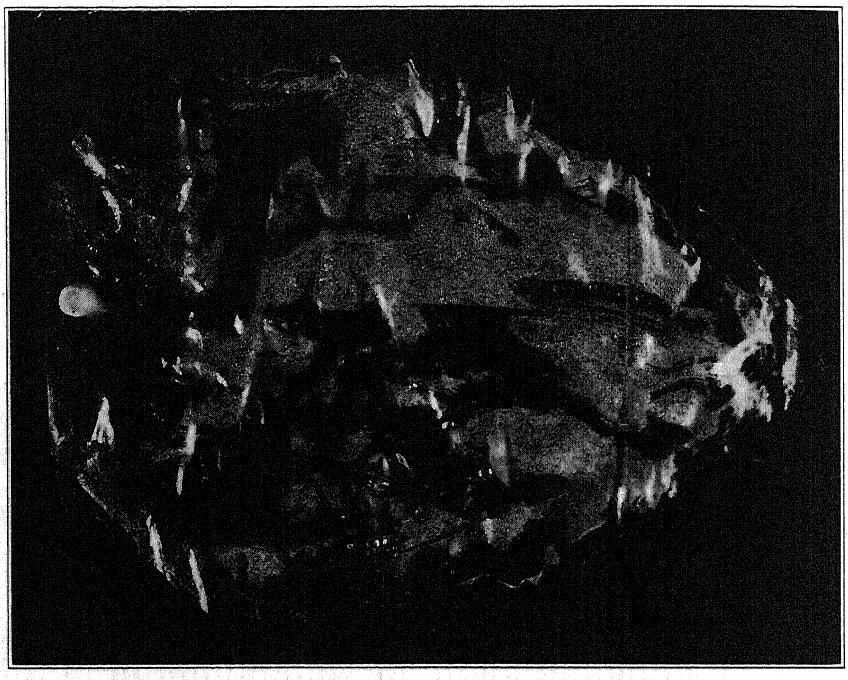


FIGURE 3. Cellophane envelope used to cover a leaf vaselined on both sides, after a short exposure to high intensity visible radiation, showing the beads of moisture adhering to the inner surface of the cellophane.

through the vaselined surfaces of the leaf. It is evident, therefore, that applying vaseline to the tobacco leaf does not stop transpiration. Vaseline also does not produce any considerable increase in temperature. The cellophane envelope is much more effective in checking transpiration and produces immediately in high light intensities a temperature rise beyond the thermal death point.

Plant leaves under infra-red in preceding tests at lower energy values showed a greater increase in temperature than those under visible and infra-red. A plant was exposed through the glass filter to test this further.

The leaves were kept 15 inches below the infra-red transmitting filter in order to be as free as possible from secondary radiation. A 1000-watt, 105-volt lamp was placed six inches above the filter. The infra-red energy received was 0.38 of a gram calorie. The initial air temperature was 78° F. The lamp was kept on until the leaf temperature came to a state of equilibrium, which was about 20 minutes. The leaf temperature was 99° F. The lamp was then turned off and the leaf came to equilibrium again after four minutes at 84° F. The same lamp was then raised to 37.5 inches, after the glass filter was removed, at which level it gave approximately the same total energy value as before. The lamp was turned on again. The equilibrium temperature of the leaf was 88° F. under this lamp without the filter. The air temperature was 80° F. The lamp was turned off again and after four minutes the leaf temperature had fallen to 77° F. The tendency to maintain a higher leaf temperature under infra-red as compared to the visible region of the same energy value is probably due to the lower transpiration rate under infra-red. At higher radiation values the temperature of the leaf when permitted to transpire freely was found never to exceed 107° F. under either visible or infra-red alone conditions.

At the higher values the transpiration rate under infra-red tends to catch up with that under the visible region. Under these conditions the stomata are tightly closed under infra-red and only slightly open under the visible. There is some evidence, therefore, of an increased permeability of the cuticle when this high temperature is reached. The failure to reach the maximum temperature as quickly under the visible radiation as under infra-red may be due to the slight opening of the stomata under the visible radiation.

WATER LOSS FROM ATMOMETERS

Four spherical type porous cup atmometers were used in all of the conditions. Two of these were placed on the table under the lamp. The other two were placed in the room at some distance away and were kept shaded by a black cloth shield placed approximately one foot away from the atmometers. The loss of water from shielded atmometers followed closely the loss from those in darkness. Only the atmometers in light and not shielded are of interest. Those in infra-red only lost water at about the same rate as those under visible radiation of the same intensity. There was no relation between water losses from the atmometers and the plants. The atmometer losses for the 12-hour period corresponding to plants listed in Table II, test 5, were 18 grams for the dark period and 24 grams in light at 50 per cent relative humidity. The corresponding losses for test 6 at the higher relative humidity were 5 grams in darkness and 15 grams in light. The atmometers lost much less water in darkness under high humidity. This was not found to be true of leaves. The loss under light condi-

tions at high humidity is also much less. This was not found true of leaves within this temperature range. The effect of an increase in visible light from 0.28 to 0.65 gram calorie in tests 3 and 4, Table II, was to increase the atmometer water loss in 12 hours from 23 to 30 grams while the loss in darkness was 19 grams. At the higher humidity in tests 7 and 8, Table II, the water loss from atmometers in darkness was 4 grams. In light at 0.65 gram calorie the loss was 14 and at 0.28 gram calorie the loss was 9 grams in 12 hours. The higher humidity greatly reduced the loss from the atmometer cups but had no comparable effect on plant leaves. The water losses from atmometers corresponding to the tests on plants in Table III show the same type of divergence. In tests 2, 3, 5, 7, and 9 at low humidity the average loss in the dark period was 11 grams, while the average loss in light was 22. At the higher temperature in tests 10, 12, 13, and 14 the dark loss was 13 and light loss 30 grams. The increase in temperature had very little effect on water loss from the atmometers while the rate is more than doubled in case of leaves in light and greatly increased even in darkness. In tests 15, 16, and 17 when humidity was increased at the high temperature the atmometer loss in darkness was 11 and in light 30 grams. It is evident from the above that temperature affects water loss from atmometers very slightly or not at all while humidity and light intensity do affect the loss. In the case of plant leaves, humidity has little effect while light intensity and temperature play the important parts in determining water loss under the conditions of these tests.

DISCUSSION OF RESULTS AND OF CLOSELY RELATED LITERATURE

Briggs and Shantz (3) after a two-year study of transpiration under natural conditions in Colorado determined the squares of the coefficients of correlation of plant transpiration with weather factors as follows: wet bulb depression, 0.77; temperature, 0.50; radiation, 0.42; and wind velocity, 0.05. Since the sum of these squares exceeds unity, they point out that the physical factors are evidently intercorrelated. This correlation has already been pointed out in the introduction of the present paper. By a study of transpiration under artificial light, with a close control of temperature and humidity it is possible to determine more accurately the effect of each single factor on the water loss of plants. The present study was made with different intensities of radiant energy, using infra-red alone and the combination of infra-red and visible as emitted by 1000 or 1500-watt lamps along with various combinations of humidity and temperature. Water losses from tobacco plants were determined by the loss of weight method applied after each 12-hour period. The plants were sealed into porcelain enamel containers using a mixture of warm beeswax and tallow. A method was developed which allowed the area of plant leaves to be determined rapidly at any time while still attached so that the study of water

losses from the same plants could be continued over a longer period of time and under several separate conditions of temperature and humidity.

The results indicate that at a temperature range of 73° to 78° F. an increase of 2.3 times the energy doubles the rate of water loss. This relation appears to be independent of humidity within a range of 50 to 80 per cent relative, under the conditions of the tests. A lamp operating at lower efficiency (120-volt lamp on a 118 to 120 line voltage) gave approximately the same rate of increase in water loss. At high temperatures (98° to 100° F.) an increase of humidity from 68 to 87 relative produced a slight decrease in water loss under both visible and infra-red only conditions and caused great injury to most of the plants. At a lower temperature (73° to 78° F.) the rate of loss under the lamp without a filter (visible and infra-red) was about 2.5 times the loss under the lamp with a heat-transmitting filter (infra-red alone). When the temperature was increased to 98° to 100° F., the infra-red rate of loss increased rapidly until the visible rate was only 1.3 times that of the infra-red. The water losses at this high temperature under the visible conditions were 2.75 grams per square inch of leaf area in the 12-hour period as compared with a loss of 2.09 grams per square inch under infra-red only. If this high rate were maintained in large tobacco plants with 3000 square inches of surface at a high temperature and high light intensity, the water loss in 12 hours might amount to as much as 8000 grams. It is probable that the rate would fall off considerably as more leaves were added since not all leaves would be exposed at normal incidence to the light. Under infra-red only conditions the loss from such a large plant might reach a total of more than 6000 grams. The stomata were found to be closed under infra-red. This confirms the work of Sayre (10) who found that stomata of *Rumex patientia* did not open in light of wave length longer than 690 mμ. This indicates that under the most favorable conditions (high temperature and high radiant energy) cuticular transpiration becomes a large part of the total. As the leaf temperature approaches the thermal death point, either because of high radiation or because of high temperature, it loses great quantities of water. This effectually holds the temperature down below the lethal point. High humidity decreases slightly the amount of water which can be lost at high temperatures and results in considerable leaf injury under these conditions. At lower temperatures high humidity has little effect since the leaf has no difficulty in evaporating sufficient water to keep below the thermal death point. Closed stomata under infra-red conditions result in less water loss and, at high temperature and high radiation values, injure the plant. The work of Clum (5, 6) is interesting in this connection. Working with fuchsia and cabbage he failed to find any correlation between the transpiration rate of a leaf and the difference between its temperature and that of air. Painting leaves with vaseline so as to check transpiration caused an in-

crease in temperature of usually less than 2° C. The author concludes (6, p. 229) that, "in a climate such as that of Ithaca, New York, the cooling effect of transpiration is insignificant and never actually protects the leaves of the plants studied from burning in bright sunlight." This failure to get a rapid rise in temperature when leaves are painted with vaseline is believed due to the fact that vaseline does not check transpiration sufficiently. When a leaf was inclosed in a close fitting cellophane envelope and exposed to an intensity of approximately 1.6 gram calories of energy from a 1000-watt lamp the leaf temperature was found to rise from 87° to 127° F. in an exposure of four minutes. Such an envelope effectually prevents all cooling of the leaf by the evaporation of water. By this method it can be easily and quickly demonstrated that a leaf loses water rapidly under such conditions, as large quantities of water are condensed on the inner walls of the cellophane. The rapid rise in temperature above the thermal death point as compared with the low temperature of a leaf not covered with cellophane is also very convincing evidence that the evaporation of water at high temperatures and under high radiation values in the open cools the leaf very effectively and keeps its temperature below the thermal death point. It can be demonstrated that a tobacco leaf even though thoroughly vaselined on both sides loses considerable quantities of water by slipping a cellophane envelope over such a leaf. After 20 to 25 minutes' exposure to the 1000-watt lamp the inside walls of the envelope are again covered with small droplets of moisture which has evaporated through the vaselined surface. The leaf, therefore, has essentially the same means of cooling as the animal has at high air temperatures, that is, by the evaporation of great quantities of water from its surface. If this is checked sufficiently a rapid rise in temperature results. The regulation of temperature in the plant however consists in holding the temperature below a certain maximum in spite of external temperatures and radiation values while in the animal a definite, constant temperature is maintained by means of an internal heating apparatus interacting with surface cooling due to loss of water. Curtis (7) has discussed the possible functions of transpiration in plant leaves. Citing the work of Clum (5, 6) Curtis believes that transpiration has little cooling effect on plants and that other possible functions do not necessitate so great a water loss. He concludes that transpiration seems to be more harmful than beneficial on hot days when the cooling effects are most needed. The failure of Clum to reduce transpiration sufficiently to observe heating effects has already been discussed. This has been a common source of error in many similar studies which have been described in the literature. A second common source of error is the failure to duplicate exactly climatic conditions during two periods of observation of transpiration rates. Unless a plant is held under strictly comparable conditions as regards temperature, air movement, and radiation during the two

periods of observation it is impossible to determine the decrease or increase in transpiration rate brought about by vaselining leaves or otherwise treating or altering the leaf surface. Many workers have used cobalt chloride paper or special inclosed cells attached to leaves to determine the amount of water loss. Unless it can be shown definitely that transpiration from unmodified leaves under accurately controlled conditions, which can be easily duplicated, follows closely the loss from leaves with such modified surfaces these measurements are of little value. Such a correlation has never been shown and from the nature of transpiration it is extremely unlikely that such modified leaves lose water at the same rate under all conditions as those in the open. It is believed that, although there is voluminous literature on the subject of plant transpiration, failure to take into account these two common sources of error outlined above largely discounts the value of many previous observations. Livingston (9) and others have used the water losses from porous cup atmometers of various types as a measure of the evaporating power of the air as it generally affects transpiration of plants. The value of this instrument in indicating water losses from plants under any set of natural conditions is doubtful. Its indication of the evaporating power of the air as it generally applies to a plant was not found accurate under either darkness or light conditions. The plant leaf is a living thing. Water losses from its surface exhibit regulations characteristic of living tissue in response to the changes in environmental factors and cannot be correlated with any simple mechanical system of evaporation. This conclusion is in agreement with the observations of Briggs and Shantz (4). They found that the curve of hourly evaporation from a spherical atmometer cup showed a departure of 43 per cent from the curve of hourly transpiration of *Medicago sativa* plants. These tests were made under outdoor conditions at Akron, Colorado, during a period of hot, dry weather in July. Knowing the conditions which affect the rate of water loss from atmometers and plant leaves, it is possible to devise an artificial climate which will produce a much greater departure in the two curves.

Clum (5) and others have pointed out the fact that plants growing in Arizona, Kansas, and the western plains country have a leaf temperature very near to or only slightly above air temperature, while plant leaves in the New York region are normally several degrees above air temperature. The reason for this is believed to be the fact that plant leaves in hot climates with high light intensity are near the thermal death point where transpiration is highest and cooling effects therefore at a maximum. The present experiments indicate that lower temperatures and lower light intensity tend to give leaf temperatures considerably above air temperatures because of low transpiration rates. As these factors are increased there is no corresponding rise in leaf temperature beyond a maximum as this maximum is held by increased transpiration or evaporation of water.

While no adequate records of radiant energy in Arizona, Kansas, and various parts of the state of New York are available, the data of Briggs and Shantz (3) show that the total solar energy at Akron, Colorado, during part of June and July 1914 had an integrated average value slightly less than 900 gram calories per day. This same figure, representing averages for the last eight years, in the case of the New York City Weather Bureau records is 422 for June and 413 for July. Mean air temperatures in Arizona and Kansas are known definitely to be above the New York region. The probable differences in light intensity and air temperature no doubt account for the observed differences in leaf temperature. It is interesting to note that one factor in the environment of plants, total radiant energy available at various places in the world, has been the least studied. Next to temperature this factor is probably the most important in the life of plants. Our dependence on the energy of sunlight has been known for years yet only recently has enough interest been aroused by the fact to keep a record of it.

SUMMARY

1. The transpiration rates of tobacco plants were studied under both visible and infra-red radiation with temperature and humidity controlled by standard air-conditioning machinery. The loss of weight method was used with whole plants weighed after 12-hour intervals.

2. Using the output of a 1000-watt, 105-volt lamp operating on a 120-volt line, an increase of 2.3 times the energy was found to double the rate of water loss at a temperature range of 73° to 78° F. This relation appears to be independent of humidity within a range of 50 to 88 per cent relative under the conditions of the tests.

3. At high temperatures (98° to 100° F.) high humidity appeared to decrease transpiration slightly. A lamp operating at lower efficiency (120-volt lamp on a 120-volt line) gave approximately the same rate of increase in water loss.

4. At a temperature range of 73° to 78° F. the rate of water loss under the high efficiency lamp without a filter was about 2.5 times the loss under the same lamp with a heat-transmitting filter (infra-red alone).

5. At a high temperature range of 98° to 100° F. the infra-red rate of loss increased rapidly until the visible rate was only 1.3 times that of the infra-red. The amount of water loss at this high temperature under the visible conditions was 2.75 grams per square inch of leaf area in 12 hours as compared with a loss of 2.09 grams per square inch under infra-red only.

6. The stomata were found completely closed under infra-red. This high rate of transpiration is, therefore, believed to be wholly cuticular.

7. Transpiration was effectively stopped by enclosing the leaf in a snug fitting cellophane envelope. Leaf temperatures of inclosed leaves at

high radiation values (1.6 gram calories) rose from 87° to 127° F. in an exposure of four minutes. The leaves were badly injured.

8. A leaf which has been thoroughly vaselined on both surfaces was found to maintain a relatively high transpiration rate and therefore showed very little rise in temperature as a result of this treatment.

9. Water losses from porous cup atmometers were found to have no relation to water losses from plants under the conditions of the tests.

10. Water losses in tobacco plants were found sufficient to hold the leaf at a temperature not to exceed 107° F. under all conditions imposed in these tests.

11. Transpiration of leaves makes it possible for them to exist under high radiation values at high temperatures.

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