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CONTENTS

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SOME ANATOMICAL AND PHYSIOLOGICAL CHANGES IN CITRUS PRODUCED BY BORON DEFICIENCY[1]

A. R. C. HAAS[2] AND L. J. KLOTZ[3]

INTRODUCTION

Recent investigations (Haas[(4)]) have shown that traces of boron are essential for the normal growth of *Citrus,* even though comparatively small additional amounts are toxic. *Citrus* trees make excellent growth when the culture solution contains 0.2 parts per million of boron but show pronounced physiological disturbances when boron is absent. A concentration of boron less than that required for the normal growth of *Citrus* brought about the following symptoms of decline: Leaves curled along the midrib with the tip of the leaf curling downward; leaves colored a brownish or yellowish green, often with a yellowing along the midrib; midrib or veins conspicuous, corky and split; and a progressive loss of affected leaves in the basipetal direction. In severe cases there is a tendency towards 'multiple bud' formation due to new twigs dying when barely visible. This and other symptoms are similar to those found in exanthema. When the bark of the internodes of the twigs, or in severe cases that of the trunk, splits, an amber-colored gum oozes out. Eventually the cracks may widen so that the woody tissue is exposed. In severe cases the apical portion of the branch dies back. The roots become dark brown in color and fail to elongate, and in advanced cases the rootlets decay. Upon the addition of a suitable concentration of boron to the culture solution, the symptoms of decline disappear.

A study of these anatomical changes and their effect on the translocation of sugars from the leaves forms the basis of this paper.

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METHODS

The material examined was obtained from two-year-old budded grapefruit (*Citrus maxima* Merrill), and Valencia orange (*Citrus sinensis* Osbeck) trees grown in sand cultures in galvanized-iron containers. The containers were approximately 20 inches in diameter and 24 inches deep, and were heavily coated inside with asphalt paint prior to being filled with pure silica sand. Each series of cultures contained budded grapefruit and Valencia orange trees.

Decline symptoms were obvious when no boron was added to the culture solution employed, regardless of the presence or absence of traces of other elements in the solutions (Haas[4]).

A solution (Haas[4]) containing the salts ordinarily used, without addition of boron and without traces of any of the other elements, was employed in one series of cultures until the symptoms of decline had reached a stage so severe that many of the shoots had died and it seemed impossible that the trees could survive much longer. Whenever any of the multiple buds began to grow the growth was short-lived, or if it progressed, it soon took on obvious symptoms of decline. At this stage a pathological study of the tissues concerned in the decline failed to show the presence of an organism in the disintegrating material.

One part per million of boron as boric acid was then added to the culture solution. Notwithstanding the fact that for more than a year prior to the boron addition no healthy growth was made by the trees, within several weeks after the boron addition the trees responded with new growth, much of which was healthy in appearance. Shoots which were badly split or gummed died back to the end of the growth cycle, but practically all of the live parts above ground sent out new growth. As growth progressed, the trees took on a more normal appearance; many of the split twigs callused so that in some cases the split portion was scarcely discernible. When recovery was obvious and directly attributable to the addition of boron to the culture solution, the trees were removed from the cultures and an anatomical and physiological study made of the changes induced in the trees by the presence or absence of boron in the culture solution. The methods employed in the determination of the sugars of the leaves are given in detail under "Internal Condition of Shoots and Its Effect on Sugar Content of Leaves."

LEAVES

The gross effect of boron deficiency on the leaves of budded *Citrus* is the same as that previously described and illustrated (Haas,[2] and Haas and Reed[5]) for *Citrus* in sand cultures. The midrib or veins become conspicuous, corky, and split, but there is no gum exudation. Plate 1, figure 1 is a cross section of a *Citrus* leaf in a region of close proximity to a vein and illustrates the manner in which cork formation may proceed and cover over the palisade tissue for some distance from the vein. The vein, which is at the extreme right in the photograph, shows no cork formation on the ventral surface of the leaf. Such boron-deficient leaves are brittle and when crunched they readily fall into pieces. They are not, however, deficient in water content as might be expected; for two lots of leaves tested contained 57 and 61 per cent of water (fresh-weight basis), respectively, which is not abnormal. The brittleness may be related to a lack of proper lignification (Johnston and Dore[7]) since boron has been found to occur most abundantly in bark and lignified parts (Brenchley[1]).

It may be seen in plate 2, figure 1, that cork formation about the region of the vein does not involve the ventral leaf surface. The section shows how completely cork tissue has developed in and about the fibrovascular system. It appears likely that the conduction of materials from the leaves may be interfered with by this abnormal development. It is remarkable that a deficiency or absence of boron should bring about such marked changes in the conducting system of *Citrus,* when concentrations of this element but slightly higher than that necessary for the healthy growth of *Citrus* (Haas[4]) bring about extreme toxicity (Haas[3]). The concentration of boron that permits normal growth of *Citrus* lies between, but relatively close to, two toxic points, namely, one approaching deficiency and the other an excess. The total range between the two toxic limits, if we may call them that, may in sand cultures be only five parts per million or less, depending upon the plant used.

EXTERNAL CONDITION OF SHOOTS

The bark of the internodes of the shoots may split and an amber-colored gum ooze out. After the gum either becomes dry and brittle and breaks away, or is partly dissolved by rain, the crack widens and exposes the woody tissue. The lower row in plate 2, figure 2, shows portions of shoots having the bark split and gummed to varying degrees. The trees from which these shoots were taken were those that received one part per million of boron as boric acid in their culture solution after having been given a boron-free solution for several years previously in the sand cultures. Many of the split portions had begun to callus over and the external gum had gradually disappeared.

The only parallel material ever found in the field comparable to the experimentally grown shoots has been obtained from a certain district in central California, but as yet it has not been shown that boron deficiency is the cause of the splitting, gumming, and eventual death of these shoots. These shoots are shown in the upper row of plate 2, figure 2. For purposes of comparison it should be here pointed out that in exanthema, or 'Florida die-back,' there are gum pockets that occur in the shoots usually at the region of petiole attachment. No splitting of the shoots has been observed. In cases of exanthema one or more of the usual symptoms may be present; sometimes the gum blisters are difficult to find, or are absent, even though the fruit symptoms and type of foliage indicate the presence of the disease. In the present experiments with boron deficiency the splitting and gum exudation of the shoots do not occur until the trouble becomes acute. No flowers were produced on the boron-deficient trees and therefore no fruit was available, but check trees of the same age produced flowers.

In a similar study made for a period of several years by the senior author eighteen four-year-old budded orange trees were grown in sand cultures in tanks 4 feet in diameter by 4 feet deep. The culture solution employed was kept free from traces of certain elements such as boron and copper. Typical gum blisters developed on the shoots at the base of the leaf petioles as occurs in exanthema. A shortage of distilled water in the summer season necessitated the use of tap water. It was noted then that the gum blisters disappeared. The tap water having come from wells, brought traces of a variety of elements including boron into the sand cultures and as a consequence the gum blisters vanished, never to put in an appearance again. Although there was some flowering, these trees produced no fruit. It is not known which element deficiency was responsible for the occurrence of the symptoms of exanthema nor is it known which element or elements present in low concentration brought about the cure. From this it appears reasonable that exanthema may be the result of a deficiency of one or more elements in the culture solution. It is seen therefore that there is some similarity in the symptoms of exanthema and those due to a deficiency of boron. No exanthema has thus far resulted from excessive use of nitrogen in the sand cultures, and the instances above noted were the only ones observed among hundreds of sand and soil cultures receiving various solutions.

INTERNAL CONDITION OF SHOOTS AND ITS EFFECT ON SUGAR CONTENT OF LEAVES

The deficiency of boron has a pronounced effect on the internal condition of the shoots. Plate 1, figure 2, shows the normal condition of the cross section of shoots from control sand cultures. Plate 1, figure 3, shows a rather thick cross section of a shoot typical of the boron-deficient trees. The cambium region of the cross section shows disintegration and gum accumulation. This gum separates the cortex from the woody cylinder and usually oozes out of the split in the bark.

It was mentioned that some of the trees in sand cultures received no boron in their culture solution until the symptoms of boron deficiency had become extreme. When such trees received one part per million of boron in their culture solution they showed marked recovery. Many of the split portions of the shoots tended to callus over and the new leaf growth soon approached normality. Plate 1, figure 4, shows a cross section of such a recovering shoot taken a few months after the trees had been given the one part per million of boron. The gum indicates the time in the growth of the shoot when boron deficiency became extreme. There were some cambial cells that survived the boron starvation and when boron became available these continued the growth of the shoot in diameter. That the region of disintegration of the cambium and the formation of gum is not necessarily continuous is seen in plate 1, figures 3 and 4. Disintegration and gum formation progressed laterally between the cortex and woody cylinder for considerable distances from the longitudinal lesions in the bark.

When gummed shoots of trees that have received no boron are cut longitudinally, they show the gum as separating the bark from the wood. The shoots of trees that have received boron after the period of boron deficiency show in longitudinal section the formation of wood between the gum and the bark, which is typical of recovery (pl. 1, fig. 5). Longitudinal sections show also that the gumming is more severe in certain portions of the shoot than in others, and when more or less continuous in a longitudinal direction, the gum may be of varying density.

It is of interest to note that in plate 1, figures 3 and 4, the gum is localized in the cambium and in portions of the phloem, as will be seen more clearly later. The gum deposits in the xylem were produced when the cambium was at that location and was being affected by a deficiency of boron, the large deposits of gum being due to the disintegration of many cells of the cambium. It may be seen that the individual xylem vessels do not contain gum. It has been shown for Vici faba (Warington[9]) that although the xylem and in certain cases the phloem may degenerate, it is the cambium that more or less completely breaks down when boron is absent from the culture solution. We cannot assume that gum formation has resulted from desiccation of the tissues as has heretofore been commonly assumed in cases of gum formation in *Citrus;* it is more likely that the changed organic metabolism that is brought about by the deficiency of boron has tended to induce the gum formation.

The relative freedom of the xylem vessels from gum deposits is seen in figure 1. The gum is seen protruding through the wide split in the cortex, following along the outer edge of the vascular cylinder and occupying the region formerly occupied by the cambium and phloem. Some of the gum is impregnated with a dark substance, as shown in figure 2, probably a disintegration product, entirely lining the exposed surfaces of the split bark. The section shows the initiation of recovery as soon as boron has been supplied to boron-deficient cultures. The inroads of the gum along the xylem, the production of new wood after the application of boron, and the relative absence of gum from the xylem are evident.

Figure 3 illustrates the separation of the cortex from the woody cylinder as the cambium and portions of adjoining phloem disintegrate and are replaced by gum. In the mouth of the split is seen a lighter gum. There appear to be very few, if any, gum deposits in the cells of the outer bark that survive under a boron-deficient culture solution.

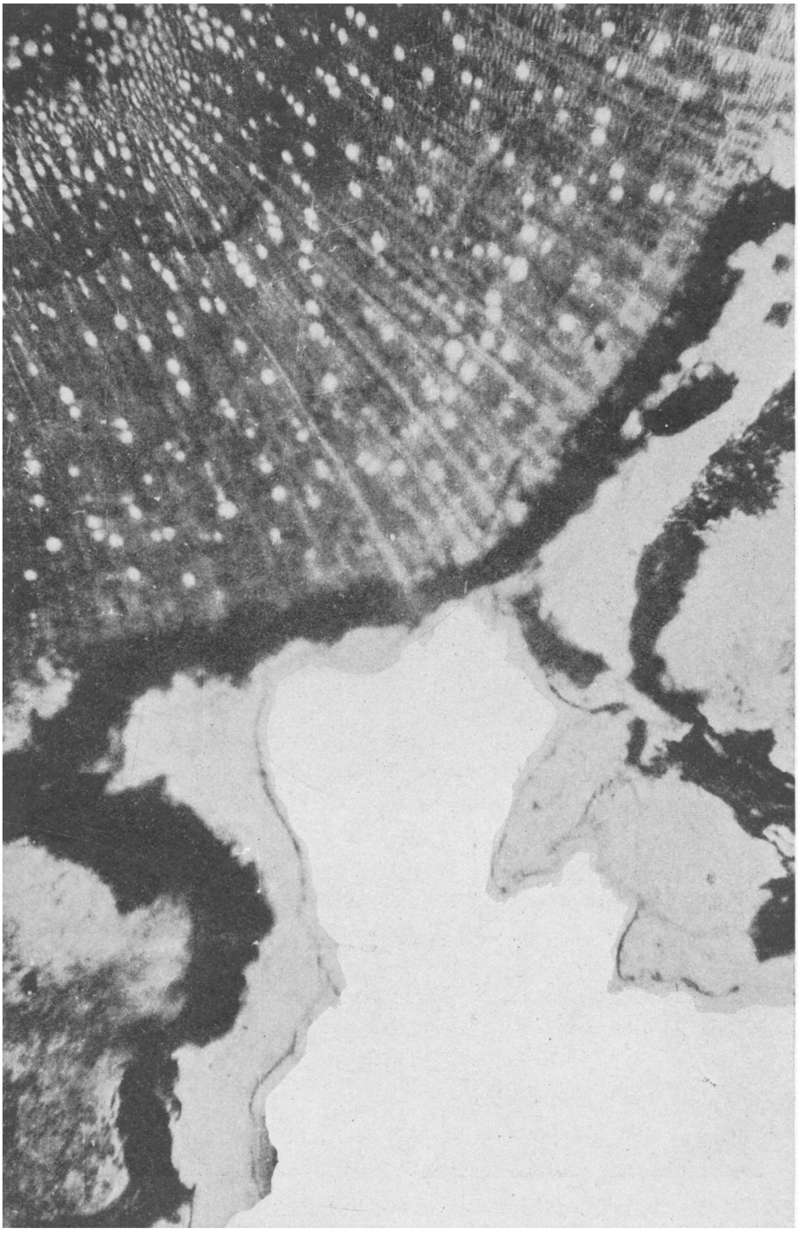

Fig. 1. The xylem vessels are shown to be free from gum deposits.

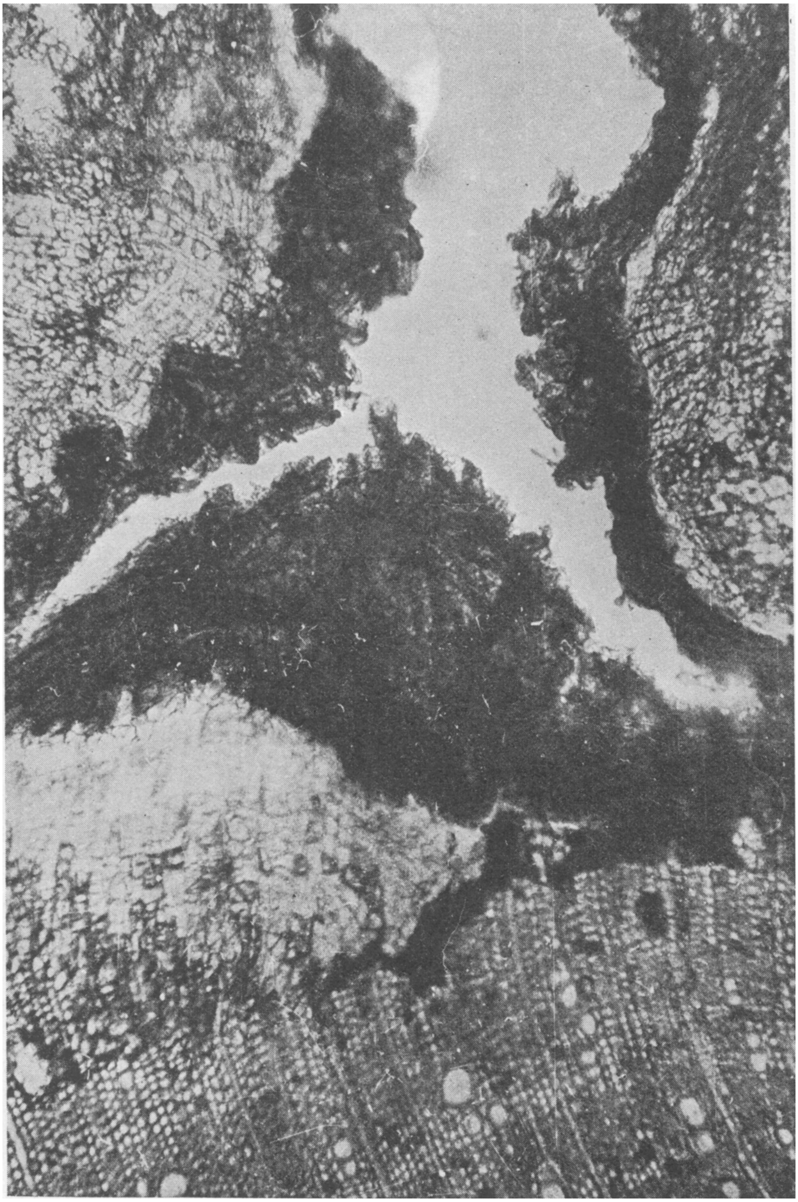

Fig. 2. The split in the bark is shown to extend to the vascular cylinder. The xylem is relatively free of gum. New xylem is being produced after boron has been supplied to a boron-deficient tree in sand culture.

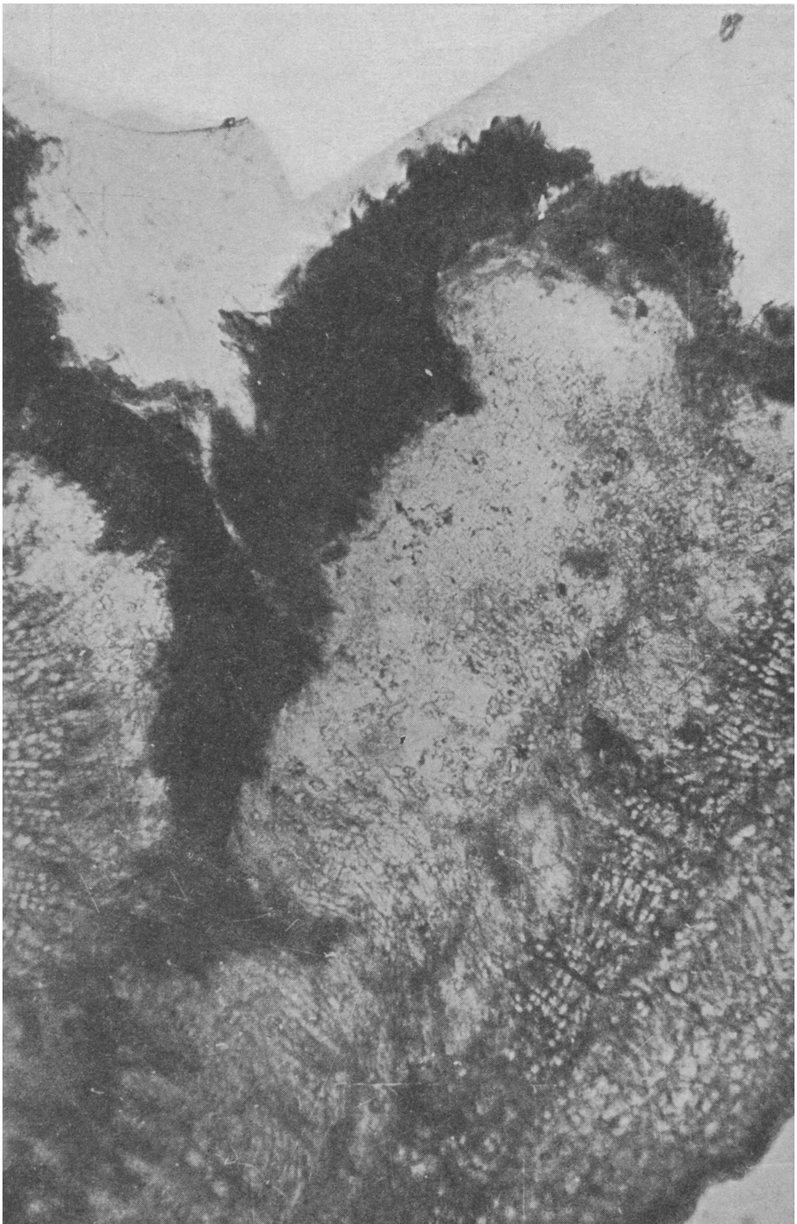

Fig. 3. The separation of the bark from the woody cylinder by dark-colored gum.

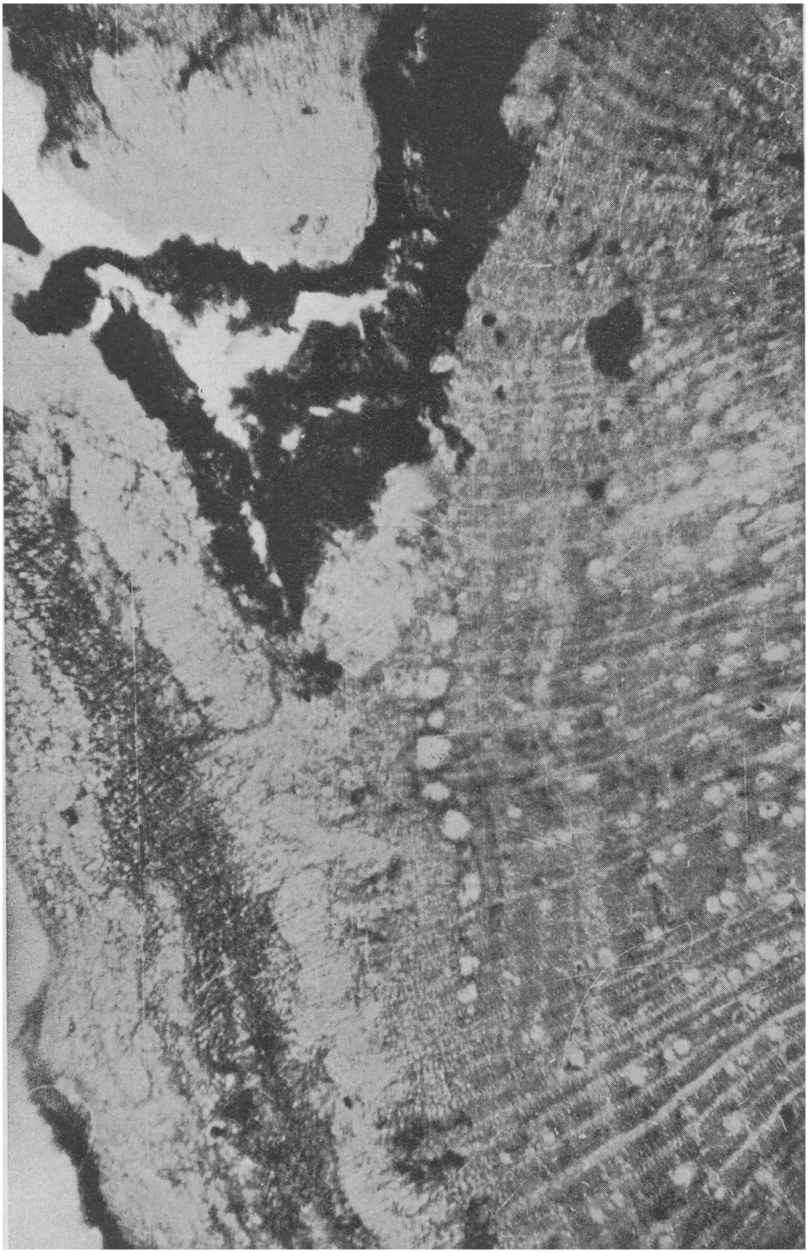

Fig. 4. The disintegration of cambium and phloem as a result of boron deficiency.

The nature and procedure of the disintegration process in the cambium and phloem is seen in figure 4. The changes induced by the deficiency of boron, when the concentration of boron required for normal growth is so very small, leads one to suggest that, after all, boron deficiency may also be considered a form of toxicity, because injury and death are ultimately produced. It may well be that without sufficient boron, the tissues are unable to transform certain metabolic products or conduct them away, or that with a boron deficiency the metabolic processes lack control or an equilibrium and run unhindered in one direction with increasing toxicity as a consequence.

In boron-deficient *Citrus* the upward passage of water and salts in solution may not be appreciably interfered with, at least in the early stages of the deficiency disease. With the cambium and portions of the phloem disintegrated and gummed, an impairment in the conduction of the elaborated foods to and from the leaves might reasonably be expected to occur. A deficiency of boron has been shown in the case of the tomato leaves and stems (Johnston and Dore[7]) to bring about an increase in total sugars and starch as compared with normal plants receiving boron in their culture solution.

Since the phloem elements of the stem, which are the conduits for the food elaborated in the leaves, became so severely disorganized in the absence of boron, it was of interest to determine the effect of this changed anatomy on the movement of sugars from the leaves. Accordingly we have made limited analyses of mature leaves from *Citrus* in sand cultures which received boron and also from those which were in sand deficient in boron. Leaf samples were also obtained from soil cultures and from trees in the field. The total sugars were determined in the dried, ground leaves, using neutral lead acetate as the clarifying agent, hydrochloric acid as the inverting agent, and the Shaffer and Hartmann method[8] for the copper determination.

A sample of mature Valencia orange leaves taken from a sand culture that had not received boron in its culture solution and having all the symptoms of boron deficiency, showed 11.98 per cent of total sugars (dry-weight basis) on June 12, 1929. On that date mature Valencia orange leaves taken from a large thrifty tree in a soil culture showed 7.07 per cent. A sample of mature Valencia orange leaves taken August 22, 1929, from a sand culture that received boron continuously in its culture solution showed 5.0 per cent.

The boron-deficient tree was given one part per million of boron in its culture solution a few days after the sample of leaves was taken on June 12 and thereafter received this concentration of boron at intervals of a few weeks. Leaves that still showed symptoms of boron deficiency were collected from this tree on August 22, 1929. These affected leaves showed 7.45 per cent in contrast with the leaves of the control tree taken on the same date, which showed 5.0 per cent.

The addition of the small amount of boron to the culture solution brought about not only new growth but greatly improved the nature of the new growth and resulted in rapid recovery from gumming. It seems very likely from such preliminary total sugar determinations that even the boron-deficient leaves were benefited by the boron that was supplied when the leaves had about reached their maturity. The delayed supply of boron no doubt improved the conducting system of the tree as is shown by the growth recovery (pl. 1, fig. 4). As a consequence of this improvement in the conducting system some of the excess sugars may have been translocated. Possibly of considerable importance, also, is the regulation by the boron of the metabolic processes, as for example, enzymatic action concerned with the manufacture and transport of carbohydrates. Such enzyme and carbohydrate studies are to be undertaken as material becomes available.

A similar study of the boron-deficient grapefruit tree revealed 13.32 per cent of total sugars in the dried leaves, whereas leaves collected from field trees in Tulare and Orange counties showed 8.10 and 6.43 per cent respectively.

TRUNK

When the *Citrus* trees grown in culture are seriously affected by a deficiency of boron, the bark may split not only on the shoots and at their junction with the trunk, but the bark of the trunk itself may also show long, narrow splittings. These are shown in a tree (pl. 3, fig. 1) that received one part per million of boron in the culture solution after having had no boron supplied for a year or more. It will be seen that some of the split portions have healed over as a consequence of the addition of boron to the nutrient solution. Cultures made of tissue taken from the bark lesions showed them to be free from organisms that might possibly be involved in such bark splitting and gumming.

No gum is visible on the exterior but if we gradually whittle away the outermost bark we pass through a layer of gum-stained tissue before reaching the white wood beneath. Plate 3, figure 2, shows the bark whittled in such a way as to expose both the gum-stained tissue and the lighter-colored wood beneath. Chips showing dense deposits of gum have been placed on the surface of the whittled-off trunk piece in plate 3, figure 2. It appears that the gum concentrates in certain areas before it finally has an exit made for its passage to the surface by a split or lesion forming in the cortex. If we examine a cross section of a trunk of a tree which was given boron in its culture solution after having been without boron until typical symptoms were evident, we find that recovery is shown by the production of new wood outside of partial or discontinuous portions of rings of gum-stained wood (pl. 1, fig. 6). The cross section of the trunk, therefore, is an excellent indicator of the periods in which boron was present in either deficient or adequate amounts.

ROOTSTOCK

The badly affected trees were shown by the colorimetric method of Halma and Haas[6] to be on lemon and on sour orange stocks. There is no reason to assume that either stock is more resistant than the other to a deficiency of boron. Much would depend on the size of the root system and that of the top and the cultural environment, in addition to the actual magnitude of the boron supply. The scion and stock shown in plate 4 were Valencia and sour orange respectively, the tree having been given one part per million of boron in the culture solution after symptoms of boron deficiency were severe. The scion shows a callusing over of the woody cylinder after the boron was supplied. The stock likewise shows the callusing over of the exposed wood, below which are seen incipient stages in the bursting of the cortex, which failed to progress after boron was supplied. It is of interest that the lowermost lesions which failed to go to completion in their destructiveness, were below the surface of the sand in the culture, as may be seen by the lateral rootlet at the left in plate 4. When the bark of the stock was cut away, the gum was evident just as in the scion. Gum was also found on cutting the small rootlets. Many of the rootlets had a brownish unhealthy color.

SUMMARY

1. Boron deficiency is marked by a gradual reduction in the size of the shoots produced, which in extreme cases finally results in the formation of 'multiple buds.' Boron appears to be essential for cell division in the meristematic tissue of growing points, such as buds, but it is likewise essential for cambial activity.

2. When boron is deficient the cambium and portions of the phloem disintegrate. The xylem tissue disintegrates to a much smaller degree, if at all. A copious amount of gum is formed, which finds its way to the exterior through a split in the cortex.

3. Growth is related to the presence of boron. When growth has ceased and gum has formed as a result of a boron deficiency, the addition of boron to the culture solution has been the means of bringing about recovery.

4. The abnormal accumulation of carbohydrates in the leaves of boron-deficient *Citrus,* coupled with the fact that the phloem tissues are destroyed, show that translocation is seriously interfered with.

5. Reduction in the total sugar content of the leaves accompanies recovery of the tissues brought about by the addition of boron to the culture solution.

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PLATE 1

Fig. 1. Formation of cork on dorsal surface of a *Citrus* leaf. The cork may extend out over the palisade tissue for a short distance from the vein.

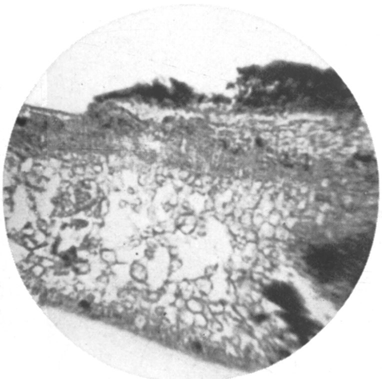
Fig. 2. Cross section of control shoot. Compare this with figures 3 and 4, which show the changes induced in the internal condition of *Citrus* shoots by a boron deficiency.

Fig. 3. Disintegration in region of cambium, and gum formation as a result of continuous boron deficiency.

Fig. 4. Gum formation and partial disintegration of cambium as a result of boron deficiency, followed by recovery and normal growth when given one part per million of boron.

Fig. 5. Gum formation and recovery (longitudinal sections).

Fig. 6. Gum formation beneath the outer bark with recovery when boron was supplied, as seen in cross section. See also plate 3, figure 2.



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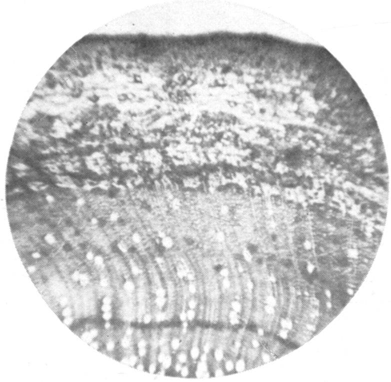

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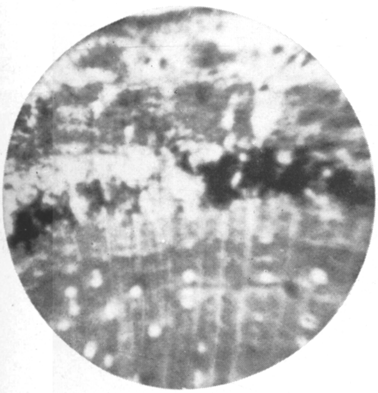

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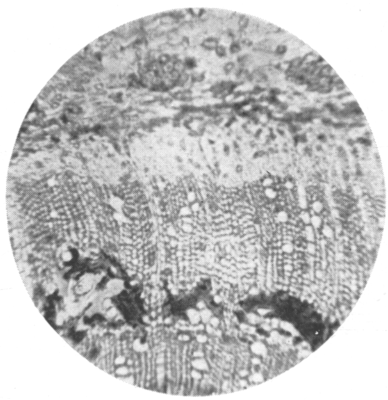

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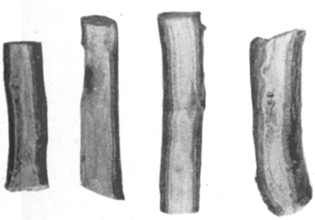

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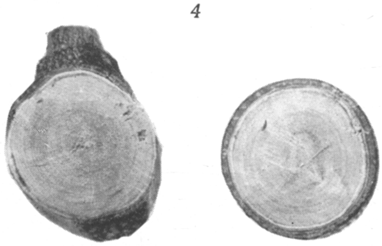

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PLATE 2

Fig. 1. Cork formation and changes induced in the fibrovascular system of a *Citrus* leaf by a deficiency of boron.

Fig. 2. Splitting and gumming of shoots of *Citrus:* upper row, field material; lower row, material experimentally produced in sand cultures by boron deficiency; many of the split portions had begun to callus over after one part per million of boron was added to the culture solution.

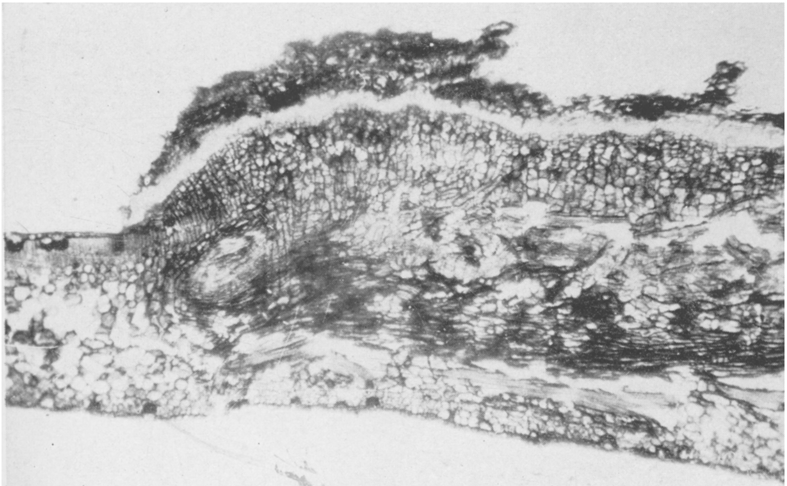

Fig. 1.

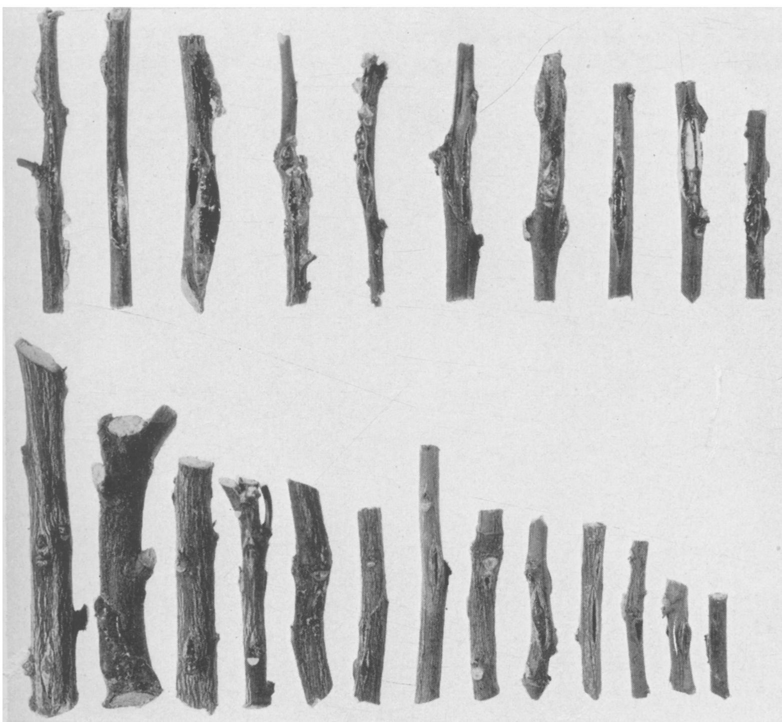

Fig. 2.

PLATE 3

Fig. 1. Splitting of bark of trunk as a consequence of boron deficiency. The healing of the lesions resulted after boron was supplied.

Fig. 2. Gum formation beneath the outer bark when *Citrus* is grown in a boron-deficient sand culture. Chips containing large amounts of gum have been placed on the surface. Compare with cross section in plate 1, figure 6.

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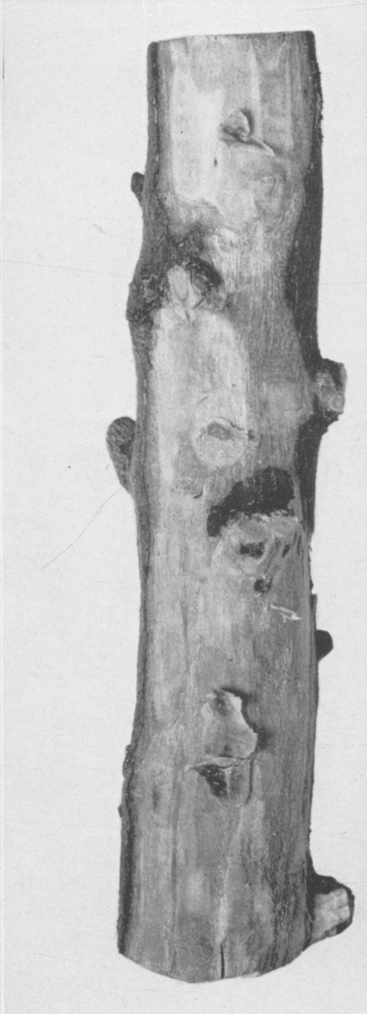

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PLATE 4

Effect of boron deficiency on scion and stock. Recovery when boron was supplied is seen by the callusing over of exposed wood and the arrest of growth of new lesions.

The titles of the Technical Papers of the California Agricultural Experiment Station, Nos. 1 to 20, which HILGARDIA replaces, and copies of which may be had on application to the Publication Secretary, Agricultural Experiment Station, Berkeley, are as follows:

1. The Removal of Sodium Carbonate from Soils, by Walter P. Kelley and Edward E. Thomas. January, 1923.
4. Effect of Sodium Chlorid and Calcium Chlorid upon the Growth and Composition of Young Orange Trees, by H. S. Reed and A. R. C. Haas. April, 1923.
5. Citrus Blast and Black Pit, by H. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.
6. A Study of Deciduous Fruit Tree Rootstocks with Special Reference to Their Identification, by Myer J. Heppner. June, 1923.
7. A Study of the Darkening of Apple Tissue, by E. L. Overholser and W. V. Cruess. June, 1923.
8. Effect of Salts on the Intake of Inorganic Elements and on the Buffer System of the Plant, by D. R. Hoagland and J. C. Martin. July, 1923.
9. Experiments on the Reclamation of Alkali Soils by Leaching with Water and Gypsum, by P. L. Hibbard. August, 1923.
10. The Seasonal Variation of the Soil Moisture in a Walnut Grove in Relation to Hygroscopic Coefficient, by L. D. Batchelor and H. S. Reed. September, 1923.
11. Studies on the Effects of Sodium, Potassium, and Calcium on Young Orange Trees, by H. S. Reed and A. R. C. Haas. October, 1923.
12. The Effect of the Plant on the Reaction of the Culture Solution, by D. R. Hoagland. November, 1923.
14. The Respiration of Potato Tubers in Relation to the Occurrence of Blackheart, by J. P. Bennett and E. T. Bartholomew. January, 1924.
16. The Moisture Equivalent as Influenced by the Amount of Soil Used in its Determination, by F. J. Veihmeyer, O. W. Israelsen and J. P. Conrad. September, 1924.
17. Nutrient and Toxic Effects of Certain Ions on Citrus and Walnut Trees with Especial Reference to the Concentration and Ph of the Medium, by H. S. Reed and A. R. C. Haas. October, 1924.
18. Factors Influencing the Rate of Germination of Seed of Asparagus Officinalis, by H. A. Borthwick. March, 1925.
19. The Relation of the Subcutaneous Administration of Living Bacterium abortum to the Immunity and Carrier Problem of Bovine Infectious Abortion, by George H. Hart and Jacob Traum. April, 1925.
20. A Study of the Conductive Tissues in Shoots of the Bartlett Pear and the Relationship of Food Movement to Dominance of the Apical Buds, by Frank E. Gardner. April, 1925.