Station Bulletin 279 May 1931

A Method for Studying Water Conduction in Plants in Relation to Pruning, Grafting, and Other Horticultural Practices

Agricultural Experiment Station
Oregon State Agricultural College
CORVALLIS

TABLE OF CONTENTS

A Method for Studying Water Conduction in Plants in Relation to Pruning, Grafting, and other Horticultural Practices

By E. M. Harvey

INTRODUCTION

The general problem of water conduction in plants is accepted by plant physiologists as fundamental. Yet it is often considered as both simple and more or less settled. As a result of this latter point of view, there seems to be a tendency among those of us who are dealing with physiological problems in the orchard to take for granted our knowledge of paths of water conduction. In our general conception of these we may be quite correct, but are we clear as to the specific and frequent modifications in the paths of water conduction taking place during the development of a tree, particularly the modifications which are necessitated by our horticultural practices?

Whenever one enters an orchard and observes the individual trees with a desire to understand the detailed processes which they must carry on, he realizes that, regardless of the merits of the cultural practices followed in that orchard, there are numerous situations arising from their application that present specific physiological difficulties to the tree.

Since this Bulletin is concerned with water movement only, the many other phases of the subject will not be mentioned.

As an introduction to the subject-matter to be considered, a few typical questions are presented as follows:

1. A large scaffold branch has been removed. Has the removal of such a branch made its former water-conducting elements available to the remainder of the tree? What benefit or harm has its removal rendered the tree as a whole?

2. A smaller branch, from 3 to 6 cm. in diameter, has been removed. Does the water-conducting system supplying this branch become available to the branches higher in the tree?

3. Small branches have been removed in mild thinning operations. Do their vascular elements become available to the small branches and spurs beyond?

4. In different types of crotches, is there a significant difference in the amount of water-conducting elements shared in common by the members concerned?

5. What effect does a small or large canker have upon water conduction in its vicinity?

6. A vigorous water-shoot is growing on a large branch. How does it affect the water-conducting system of the older branch?

7. When a tree is top-grafted, what vascular elements are available for the young scion?

8. If a large branch is removed, what is the fate of the root or roots that have formerly received their supply of elaborated food from it?

9. If there is cross-transference of water from one hydrostatic unit to others in a stem, is it done with ease or difficulty?

10. What forces direct the cambium in the orientation of the xylem elements which it lays down?

These and many other questions of a similar nature, all relating to the problem of a better practical understanding of water movements in trees, have been before the writer for some time. Of the several suggested methods which might aid in answering them, the use of dye solutions seemed the most feasible.

The employment of dye solutions for demonstrating the paths of water conduction in plants began more than two hundred years ago. During the subsequent period, the records of observations and experiments made with the aid of dye solutions constitute a literature of considerable mass, into which it is unnecessary to enter for the purpose of this report. Suffice it to say, that few refinements have been introduced since the inception of the method.

One of the most critical employments of the method, however, apparently occurs in the work of MacDougal, Overton, and Smith[2]. By the use of dye solutions these men were able to distinguish the water-conducting and gas-containing portions of the annual rings of several species of trees included in the genera, Quercus, Salix, Pinus, Juglans, and Alnus. The results of these authors also indicated how very difficult is the cross-transfer of water between different hydrostatic units of a stem. One phase of this work approaches closely the subject-matter of the present report; namely, the substitution of a suction pump for the pull of transpiration. The base of the cut-off branch was placed in a dye solution and the *apex* was attached to the suction pump. The advantage which the authors claimed for this modification of the dye method was "that one can obtain in a few minutes, results which would require a day or more if obtained by means of transpiration" (page 34).

More recently R. B. Harvey[1], in reporting his observation on the paths of water conduction by means of dye solutions, suggested the dye "Light Green S. F." as very useful for this sort of studies, stating that it is relatively non-toxic and gives rapid penetration and good localization. He suggested also four other dyes having similar characteristics. He did not employ suction or positive pressure to hasten the entry and progress of these dyes, nor did he apply the method to special horticultural problems, except that he drew attention to the effects of two bruised spots upon water conduction in the outer wood of a box-elder branch.

The purpose of this Bulletin is to present a modification of the dye method for studying the paths of water movement in plants and to show a few examples of its application. The principle of the method itself was developed during a study of phyllotaxy of the apple and pear. It was found that if a leafy shoot, of current season, were brought into the laboratory and its basal end connected with a suction force, while a cut-off leaf petiole located approximately twelve or more nodes from the base, was placed in a dye solution, the dye entered and followed down the individual traces of that leaf, plainly outlining them through the shoot. Afterward the course of these traces in successive cross-sections was observed. The orthostichy of a leaf could be determined simply by noting the number of the node (from the leaf in question) in which the branch gap appears to bifurcate the middle trace of the leaf.

The definiteness with which the localization of leaf traces could be outlined by this method of drawing the dye "backward" through the stem, led the writer to try the same principle on cut-off branches to determine the individual water-conducting units of smaller side branches. The methods showed that the path of water conduction of any side branch—i.e., lateral to the larger branch under investigation—could be sharply localized throughout its course in a very brief time. Not only could the water-conducting unit of one side branch be determined, but by using dyes of different colors the individual units of as many side branches as desired could at the same time also be determined. In one set-up, there were fourteen side branches and spurs connected with six different kinds of dyes. When the suction was started, the courses of all their individual water-conducting units were simultaneously outlined throughout the main branch.

If a rather large, normal, uninjured apple branch is set up in this manner, the dye will flow into each connected side branch and then move directly "downward" through a sharply delineated area of the xylem toward the source of suction, and also "upward" directly away from it.* The orderliness and definiteness of the individual paths of water conduction in a normal branch seemed to indicate that the method might be capable of showing up irregularities in the vascular system, caused by abnormal situations, such as mentioned near the beginning of these remarks.

The purpose in suggesting the application of the dye method to this end is to help the investigator to gain a better understanding of the responses that a plant is more likely to make to different cultural treatments.

THE METHOD

General. The principle of the method is very simple. As mentioned above, it consists in applying suction at the basal end, or at a side branch located near the basal end, and a dye solution to any cut-off side branch whose path of water conduction it is desired to study. The drawing of the dye "backwards" through the stem seems to allow a somewhat better differentiation of the individual units of a branch.

In practice, therefore, a branch or trunk bearing a pruning wound, canker, crotch, scion or water-shoot, is cut from the tree and brought into the laboraatory. The specific side branches to be investigated are connected with dye solutions and to the base of the main branch and suction is applied. The negative tension used has been about 700 mm. of Hg, and the time it is allowed to act is 5 to 45 minutes, depending on the distances the dyes must be drawn and other circumstances which the investigator can soon determine.

*An explanation of this movement of the dye solution directly away from the suction force is given by the writer in a paper entitled "The Movement of Water in Plants as Affected by a Mutual Relation between the Hydrostatic and Pneumatic Systems," *Plant Physiology*, July 1931.

Some details of the procedure are presented as follows: (1) the application of suction to the base of the branch or trunk, (2) connecting the dye solutions, (3) the dyes.

The application of suction. Branches having a diameter of not more than 7 cm. are connected with the source of suction by means of one-hole rubber stoppers of the same diameter as the branch. A branch 7 cm. in diameter is then connected by means of a No. 13 rubber stopper, if its larger diameter is placed next to the base of the branch. The surface of the stopper should be against the surface of the branch but numerous intersecting grooves should be cut into the contact surface of the rubber stopper in order to permit equal distribution of negative tension to all portions of the base. A section of rubber tubing approximately the diameter of the branch and stopper is used to make the connection air tight. When branches or trunks exceed 7 cm. in diameter, flower pots of suitable size may be substituted for rubber stoppers, and sections of automobile inner tubes for ordinary laboratory rubber tubing. The pots should be paraffined and the drainage opening in the bottom may need to be enlarged by means of a twist drill before good connection can be made through it. The writer has applied suction, in this manner, to stems up to 16 cm. in diameter. The larger size required a six-inch flower pot and a section of a five-inch automobile inner tube over the stem and pot.

Connecting the dye solutions. If only one dye solution is used, the apex or side branch to receive it is simply submerged in the dye before suction is established. Sometimes it is possible to submerge two or more branches into as many dye solutions, but usually, if more than one connection is desired, all but one must come from the side or above. For the latter, the dye solutions may be supplied from glass funnels or calcium chloride tubes, with rubber tubing connections. Whatever the size of the connection, provision must be made for excluding air bubbles before suction is established. In small connections this is quite easy. After the dye solution is placed in the container it is only necessary to squeeze and release quickly a few times the rubber tubing near the cut surface of the branch to cause the dye solution to enter and exclude the air. When a large branch is involved, a section of large rubber tubing is drawn over both the end of the branch and a one-hole rubber stopper, of about the same diameter as the branch. A space of one or two centimeters should be left between the end of the branch and the stopper. After the dye solution is placed, the rubber stopper is moved toward and away from the end of the branch several times to rid the connection of air bubbles.

The dyes. All dyes to be useful in these experiments must be of an acid reaction. Basic dyes will not penetrate xylem tissue satisfactorily. The writer has tried about forty dyes, and without exception the dyes that penetrated the xylem readily were of the acid sort. The familiar basic dyes such as the saffranins, methyl violet, methyl green, rosnailin, emerald green and methylene blue are of little use in this work. The best acid dyes found for tracing paths of water conduction were: aniline blue, amaranth, acid or light green, acid fuscin, trypan blue, methyl blue, eosin, tartrazine (and most of the yellow dyes), and ponceau red. The yellow dyes, while penetrating readily, are not very desirable for material that is to be kept for later examination, on account of the lack of color contrast with the dried wood.

The question of the toxicity of a dye is not so important in this type of experiment as in the type mentioned by R. B. Harvey[1].

Except in cases of special demonstration, the bark should be left intact while the dyes are being drawn into the stem. Immediately the experiment is over, the bark should be removed to study the course of the dye in the outer wood. When the bark is first removed, the effects are often extremely striking. They must be seen first-hand to be appreciated. Later these intense surface effects fade somewhat, but the internal situations generally remain about the same, so that material can be put aside for future dissection and study. It has been found useful in later dissection to outline the individual water-conducting units at place to place in the stem and determine the cross-sectional areas by means of a planimeter.

APPLICATION

In this division of the report it is intended to present, under a few arbitrary headings, some possible applications of the dye method for observing paths of water conduction in plants.

Unless otherwise mentioned, all material is apple, variety either Ortley or Jonathan. In all figures the bark had been removed before photographing*. In some cases the surface was retouched with a solution of the original dye. This was necessary only when the time elapsing between the completion of the experiment and the photographing allowed too much fading at the surface for proper contrast. It is unfortunate that the specimens could not have been presented by means of color photographs because many are very striking in their natural colors.

1. Normal uninjured branches. It was stated in the Introduction that normal branches showed great regularity of their paths of water-conduction units. Therefore it seems fitting to show a few illustrations of this fact before passing on to a consideration of the more abnormal situations.

When suction is applied to the base of a normal branch and a dye solution to a side branch, as previously described, the dye enters and flows both toward, and away from, the source of suction, but regularly through the xylem tissue which constitutes the individual unit of the side branch receiving the dye. The cross-sectional area of the xylem involved in the main branch is approximately the same as the total cross-sectional area of the side branch. This statement refers only to such area of xylem in the main branch as lies *below* the origin of the side branch. Here the average of 47 comparative determinations, by means of a planimeter, showed 11 percent greater area in the main stem than in the side branch. The total range of difference was 43 to 201 percent, but with one-half of the observations falling within the range of 87 to 136 percent. In some cases an explanation of extreme differences was not difficult to find. The proximity of pruning wounds accounted for some of the lower values, and the presence of other side branches sharing vascular tissue accounted for some of the extremely high values in favor of the main branch.

In regard to the xylem unit of the side branch lying *above* the point of origin, its cross-sectional area, even close to the side branch, varies very greatly. This variation may be related to vigor or demands for water of the particular

*All photographs were made by Mr. W. C. Whitaker of the Botany department. The choice of proper screens to give contrast was in many cases difficult, and the writer gratefully acknowledges Mr. Whitaker's assistance.

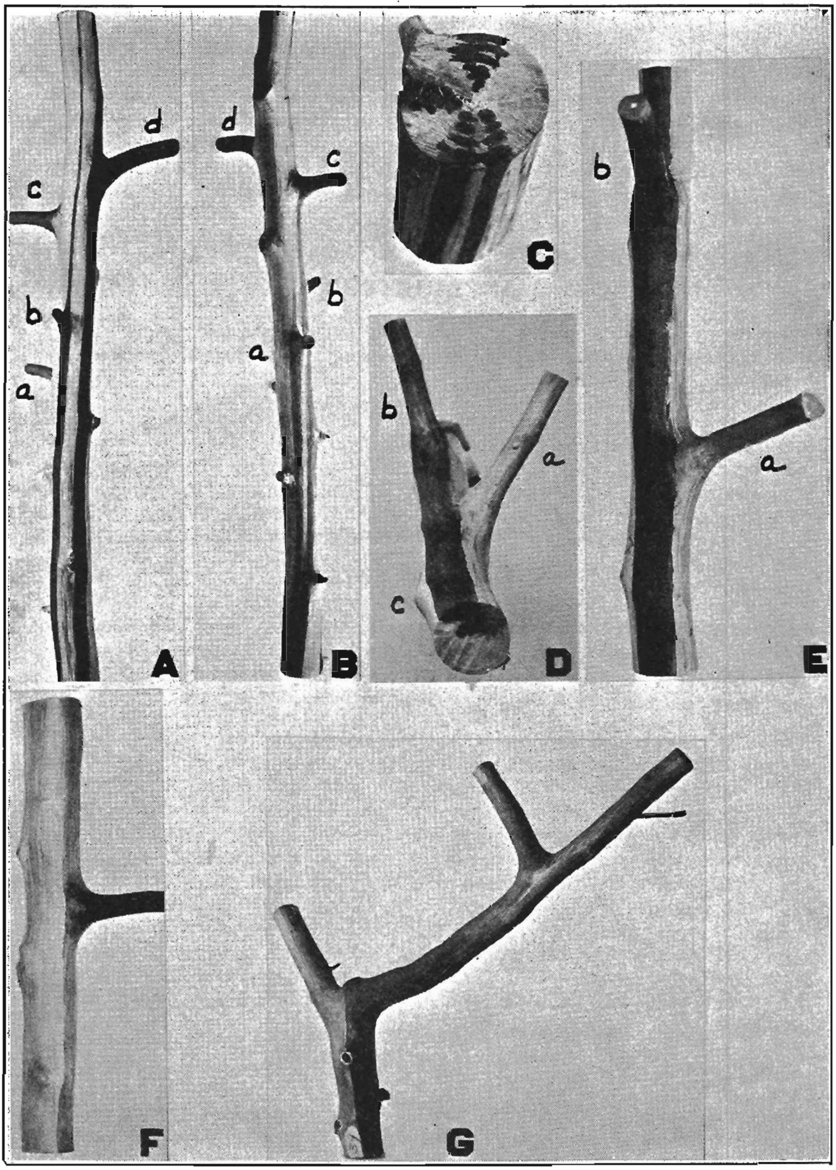

Figure 1. A and B show the upper portion of the "leader" of an apple tree. Dye solutions were admitted to the side branches, a, b, c, and d (×1/4). C is a cross-section at the base of the same branch, showing the individual hydrostatic units of the four side branches (×2/5). D, E, F, and G are referred to in the text (×1/3).

branch. The results of the observations made seem to point to the conclusion that the cambium will lay down tissue directed to a branch in response only to a superior demand. Hence the amount of xylem tissue extending *above* a branch and identified as an integral part of its vascular unit possibly indicates its relative power to remove water from the tissue above it.

Returning to the subject of the typical experiment, it should be stated that the results are so generally uniform that any deviation from the usual situation is assumed to indicate some abnormal interfering factor. When the area of the xylem stained by the dye is irregular in shape, broken up, or suddenly reduced in area, a pruning wound, an old canker, or the competitive activity of some other neighboring branch, is looked for.

There is evidently no fixed directive activity of a given cambium; it seems to respond at all times to the greatest demand made upon it, from whatsoever source. Under unusual circumstances, the cambium at a given point might alter the direction of the vascular elements it is laying down several times in the course of a single season. When growth begins in the spring, the cambium at various points through a tree has to redirect its laying down of new elements in accordance with the changed water demands produced by the pruning-off of branches during the preceding winter.

The paths of water conduction in a tree are not to be thought of as something fixed, therefore, but rather as meandering streams. The cambium each growing season, in adding to them, may lay down the new vessels to the right or left, directed this way or that, and in greater quantities or less, always responding, as Dr. C. A. Shull has suggested to the writer, to the lines of saturation deficit. This matter will be mentioned again in connection with the subject of crotches and the activities of water-shoots and scions.

A typical response of a normal branch is shown in Figure 1, A, B, C. This specimen was 4 cm. in diameter at the base. The four side branches—a, b, c, and d—were connected with the dye solutions of brilliant yellow, aniline blue, eosin, and light green, respectively. The paths taken by the dye solutions were extremely regular within the older xylem of the main branch; and the individual water-conducting units of the side branches were outlined by the dyes, on the outer surface of the wood, as straightly and sharply as any pencil line could be drawn. The photographs were not made until after the stem had thoroughly dried out and split. The surface colors by this time had faded too much for good contrast and in the necessary retouching the different units could not be outlined as sharply as in the original.

Figure 1, D and E, shows another section of a branch in two positions. It may be considered as normal, except that the amount of vascular tissue which the side branches (a) and (b) control above their origins is excessive. The basal section shown in Figure 1 D indicates also that during the growing season immediately following the pruning out at (c) the xylem unit of branch (a) retreated to the right, away from the wound. Figure 1 F also shows a side branch (a) having an unusual amount of vascular tissue above its point of origin.

An interesting interrelationship of six branches of a small trunk is shown in Figure 2, A, B, and C, presenting a combination of both normal and abnormal situations. On the whole, the trunk has almost recovered from some

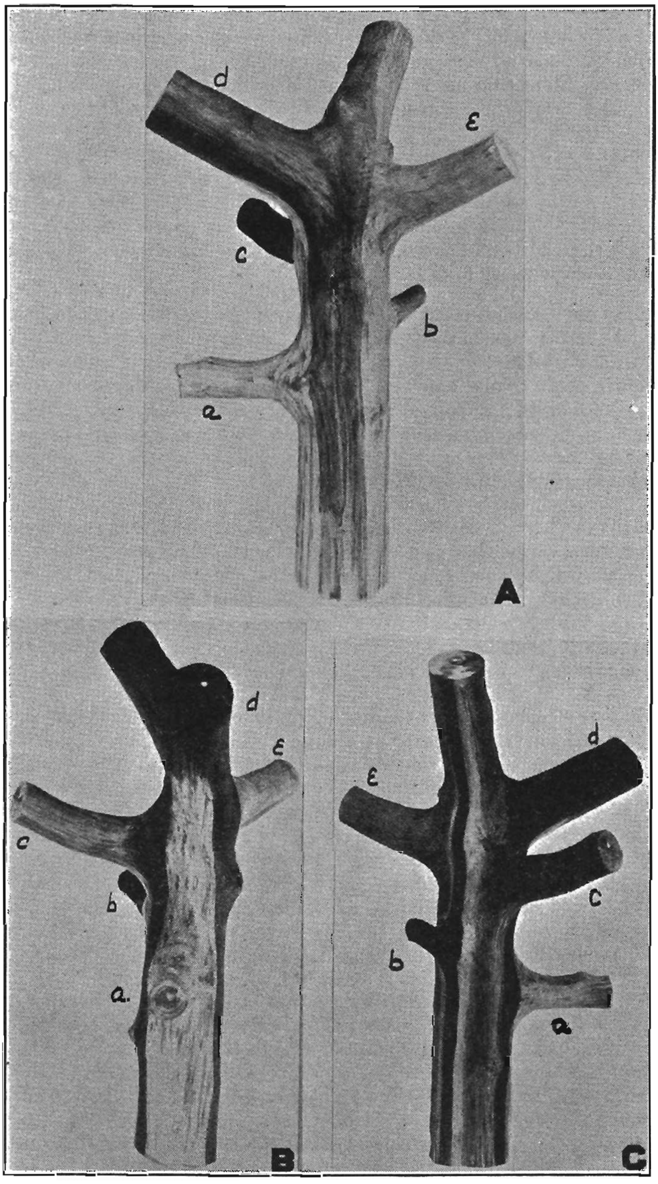

Figure 2. Shows a section of an apple trunk to which dye solutions were introduced into branches a, b, c, d, and e. These letters indicate the corresponding branches in the three exposures A, B, and C. Exposure A was photographed through a different color screen from that used for B and C ($\times 1/6$).

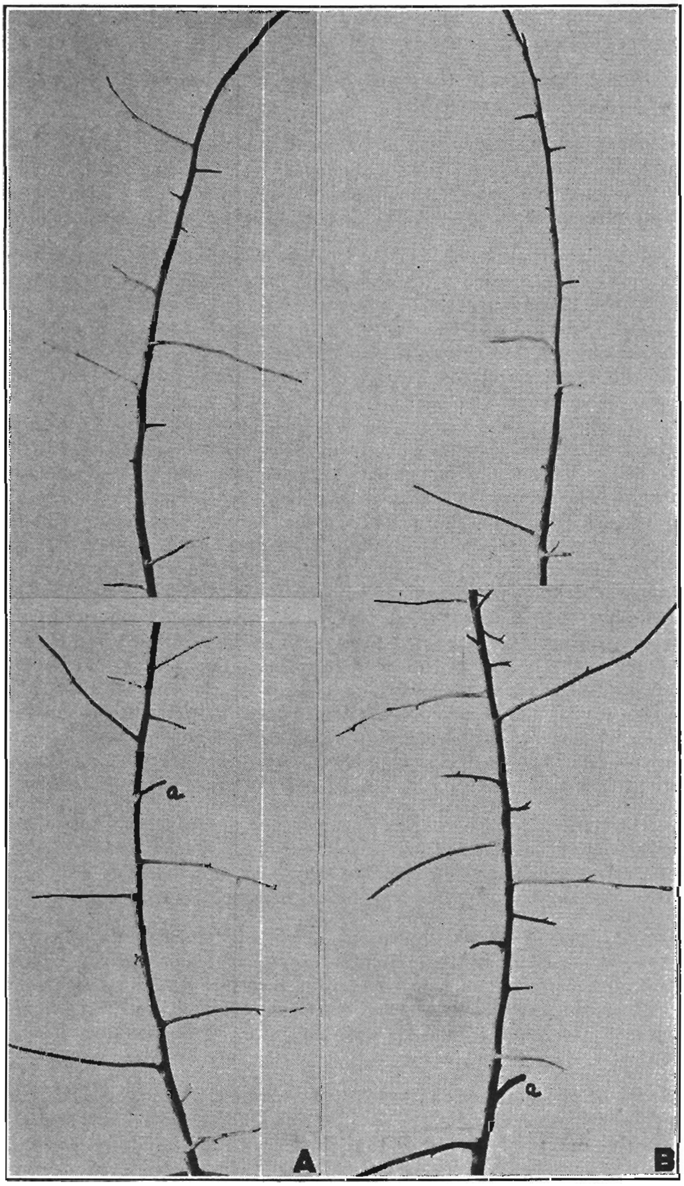

Figure 3. Two apple branches, each three years old, showing the large amount of water-conducting tissue common to young twigs and spurs. Points (a) received the dye solutions. The somewhat spiral course of the hydrostatic units made it necessary to reorient the upper portions of each branch before photographing ($\times 1/10$).

previous pruning wounds. This specimen will be referred to later in the discussion of crotches.

The normal situation in the two younger branches is shown in Figure 3, A and B, and will be discussed in Section 6, page 20.

2. Effects of pruning wounds in medium-sized branches. Whenever observing the outcome of a few experiments of the kind considered in this report, it is scarcely possible to avoid noting the great disturbance which pruning wounds are capable of producing in the normal paths of water conduction.

The following will serve as a fairly typical example. A branch, seven years old, having a partly healed pruning wound, was brought into the laboratory. A side branch, 65 cm. directly above the wound, was selected to receive the dye solution. Suction was then applied at the base 12 cm. below the wound. Previous experience had shown that if conditions were normal the dye would enter the side branch and flow directly and uniformly down its own individual xylem unit to the source of suction. But when the experiment was stopped, examination showed that the negative tension had not been transmitted directly upward to the side branch but from the opposite side of the main branch, owing to the resistance of the wound. Figure 4 shows how the dye entered the main branch and proceeded downward. First, it followed the normal unit of the side branch (A); soon the path began to assume an unusual outline (B); and finally it was broken into numerous parts as it passed the pruning wound (C). This irregular pathway was probably the one through which the side branch had been receiving its water supply. The fact remains that, in spite of the presence of this wound, the side branch in question had continued to receive a supply of water. But to overcome the added resistance incurred by the wound, the leaves of the branches above must have had to use more than the normal amount of energy to obtain it. To provide more than the normal amount of energy, the leaves would have had to allow their moisture content to be lowered sufficiently to release it. The effects of a continued situation of this kind on the activities of the branch, including its development of fruit, would likely be insidious and therefore difficult to evaluate.

The disturbances for which pruning wounds may be held responsible are so numerous that to attempt to present them in detail would exceed the scope of a report designed primarily to suggest a method. Any one employing the method will continually meet examples of such disturbances.

In passing it may be stated that many planimeter determinations were made of the cross-sectional areas of xylem units above, at, and below pruning wounds. It was found that sometimes the apparent restriction of cross-sectional area produced by wounds was almost compensated for by the swellings in the stem in the immediate region, even though the paths of conduction were greatly distorted.

3. Effects of the removal of scaffold branches. The results of the present observations by means of the dye method furnish no evidence that the water-conducting elements of an old, established scaffold branch can ever become available to any other portion of the tree. In fact, all the evidence points to the situation that, upon the removal of such a branch, the xylem elements which have developed with it become non-functioning and remain enclosed in the trunk as mere mechanical structures only. Such strands of non-functioning xylem may be the chief sources of infection by wood-rotting fungi.

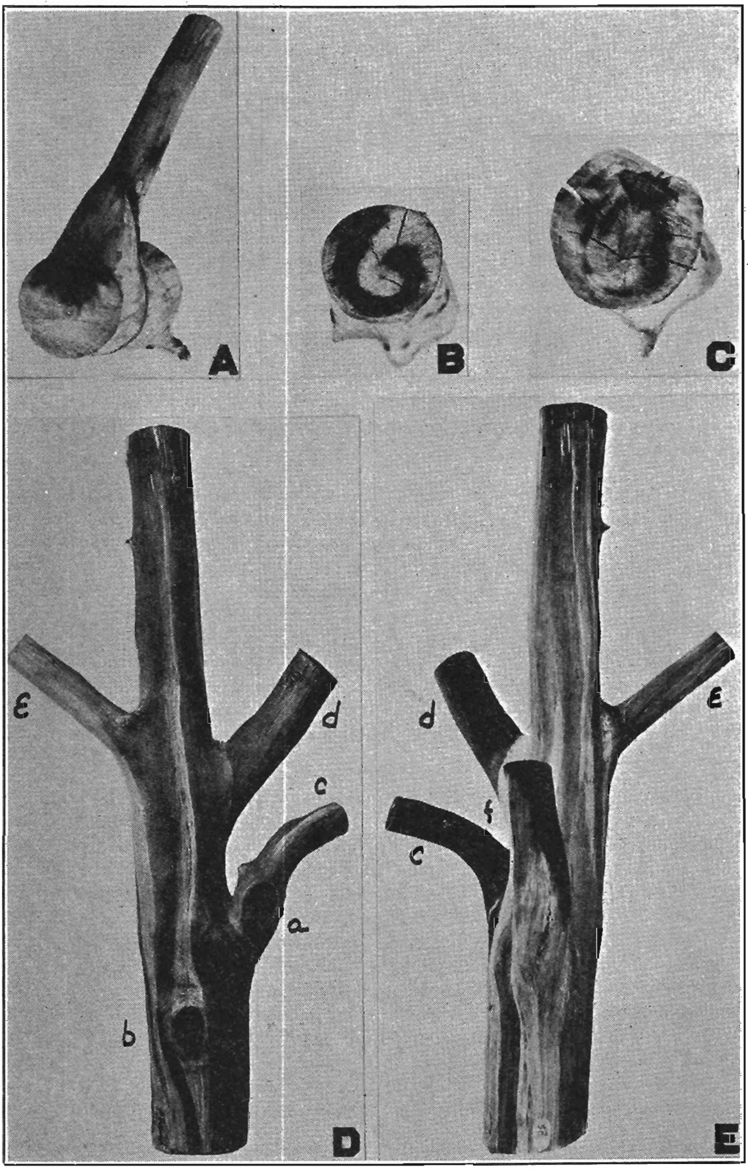

Figure 4. A, B, and C are successive cross-sections (33 cm. apart), showing the disturbance in the path of water conduction of the side branch A caused by the pruning wound shown in C ($\times {}^1/_2$). D and E show the course of dye solutions in a rather large trunk of apple, as described in the text ($\times {}^1/_7$).

Figure 4, D and E, shows an apple trunk with two old pruning wounds; neither of the xylem units of these two branches has been available to the rest of the tree since their removal. This was shown by dissection. But the branches and main trunk above the wounds had utilized the former cambium of the removed branches to such an extent that the outer wood was almost entirely directed toward the remaining branches. The old cambium had not been utilized entirely, however, as shown by the non-functioning strip of outer wood below and above (D b).

Figure 5 C shows a cherry trunk bearing scars from the removal of two large branches. Their xylem elements not only had ceased to function but had soon become infected with wood-rotting fungi. Figure 5 D is a cross-section just below the old branch scars. The small amount of functioning xylem remaining for the live branch above may be observed at (a). Only the last two annual rings carried any of the dye solution.

When large branches are removed by pruning, one must wonder as to the fate of the roots supplying them with water and salts, and receiving in return elaborated food. No information on the subject has been found during the course of the present observations.

Another question arising in connection with the removal of large branches is that of "stimulation" of growth in the rest of the tree. The general idea that there is a "stimulation" probably is derived from two sources; namely, (1) the assumption that the water and other supplies going to a large branch will somehow become available to the rest of the tree if that branch is removed; and (2) certain observed responses of the tree to the removal of large branches. Two of these responses are: the development of vigorous water-shoots in the vicinity of the cuts; and sometimes an apparent general improvement in the remaining branches. From the observations made with the dye method, however, it is felt that there is no direct stimulating effect from the removal of a large branch, for the reason that its vascular tissue is not made available to the rest of the tree.

The benefits observed can be explained on two grounds; (1) The living cambium, which formerly had always laid down tissues for the removed branch, now becomes available to the branches above it. These branches create lines of saturation deficit, directed toward themselves, across and into this band of cambium. The cambium then begins to lay down new vascular elements along these lines. The result of such changes in the activity of the cambium is a distinct gain to the branches remaining above, but the gain comes slowly through this reorientation of the former cambium of the large removed branch. The rapid growth of water-shoots is possible also by reason of this newly available cambium area. A water-shoot is particularly capable of directing to itself the activities of older cambium (see Section VII). (2) When large branches are removed, an obvious gain is derived from the "opening up" of the tree—that is to say, by allowing the remaining branches to have better exposure to light. This "opening up" is often an extremely important factor.

Admitting the gains mentioned above, the writer is inclined to believe that in general the gains do not compensate for the great disturbance the removal of large branches produces and the danger to infection incurred. It seems that there can be no rational system of pruning established unless it be based on a comprehension of the habits of the tree growth such that the person in charge

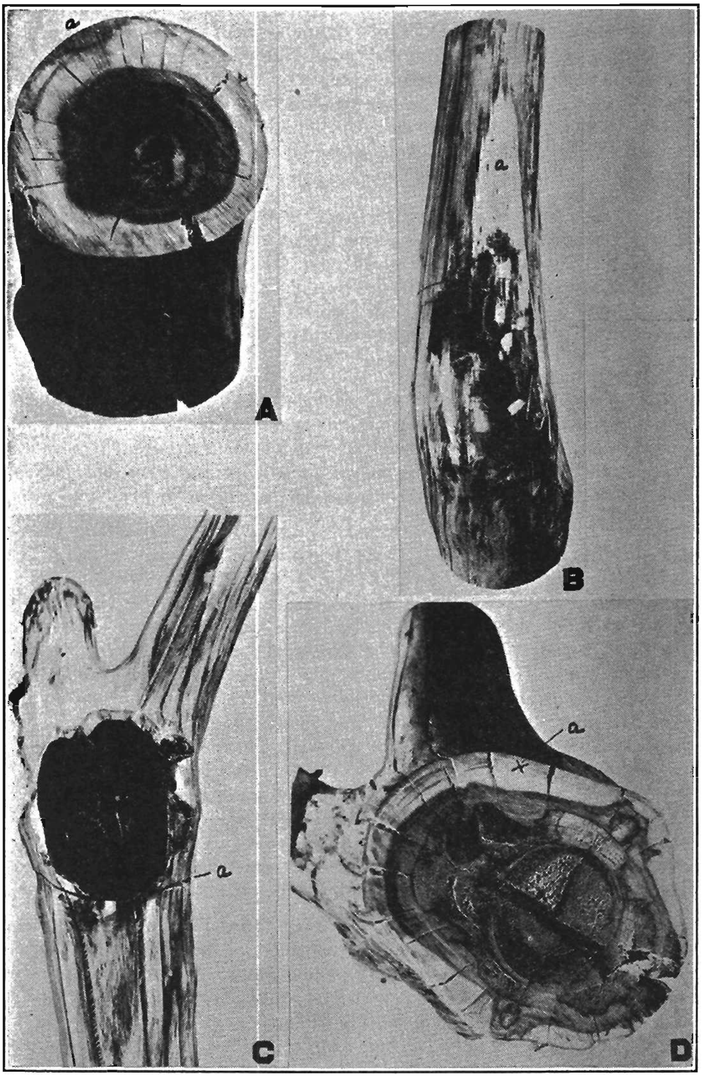

Figure 5. B is a section of a large cherry branch bearing a gummosis area below (a), and is intended to show how the dye solution could not pass through it $(\times {}^{1}/_{5})$. A is a cross-section a short distance below point (Ba). In A the non-functioning area due to gummosis is indicated at (a). The entire center of this branch is also non-functioning on account of a large pruning wound located below the portion shown. C indicates the effects of two large pruning wounds on the passage of dye solutions in the outer wood of a cherry trunk $(\times {}^{1}/_{5})$. D is a cross-section of C. In D, the small amount of xylem tissue through which the dye solution could pass is indicated at (a). The dark central portiou shows the rotted or non-functioning portion caused by the pruning wounds.

of the orchard can picture clearly the form which the tree should have twenty to twenty-five years after it is planted. He must also be capable of controlling this development, so that few scaffold branches will ever need to be removed. If this presupposes an unattainable knowledge of tree growth, then orchards must continue to suffer the consequences of the amputations of major branches, and pruning will continue on the year-to-year basis. It is interesting to note that in the case of a young orchard established in Southern Oregon, the owner has planned the future form of the trees with the hope that no major branches shall have to be removed for many years.

That such planning of the future form of a tree is possible was indicated to the writer when he saw, before the Kofukuji temple in Nara, a pine tree that had been planted more than one thousand years ago. It framed the front of the temple artistically. It was obvious that with great skill every branch had been selected and developed to such an end, and there was no evidence observed that any large branch had ever been removed.

4. Influence of type of crotch. During the course of these studies, one hundred crotches, at least, were examined after they had been injected with dye solutions. Numerous determinations were made of cross-sectional areas of the water-conducting tissue shared by the two members. It was assumed that the greater the amount of such tissue held in common, the more adaptable the crotch would be, and therefore more desirable for practical purposes*.

It was soon realized that there must be many factors affecting the amount of water-conducting tissue shared by the members of any crotch. This conclusion was borne out later when an attempt was made to correlate the amount of common tissue with the size and the angle subtended by the crotch. But from the results of these cross-sectional area determinations, it seems permissible to state that in general the larger the angle, the larger is the proportion of common tissue. The exceptions, however, were numerous. One crotch having an angle of only 30° showed practically 100 percent of common tissue, and many with angles of 90° showed almost none. In Figure 6 B, branch (a), which makes an angle of 90° with the main branch, showed (after dissection) nothing in common with the branch above its origin. Again, Figure 1 G shows two crotches of about 90°, yet neither shares any significant amount of tissue. Branch (a) of Figure 2, A, B, and C, comes out at an angle of 95° but has no water-conducting tissue above, except in the outer annual ring. Some recent event had taken place to cause the cambium above (a) to start the development of common tissue with branch (d).

Deductively, one may assume a few factors that may affect the amount of common water-conducting elements in crotches. One of these is the relative vigor or water-pulling power of the two members. It seems reasonable that if a branch is capable of pulling water strongly from below, it will have a relatively strong tendency also to pull water from above. And to the extent that it can create a saturation deficit above, the cambium in that region will lay down tissue in accordance with this demand. If this be true, it is possible that a branch, leaving the main stem at a wide angle, controls, in general, more water-conducting tissue above, because such an angle would usually

*In many of these experiments the dye solution was applied to one member and the suction to the other. It seemed to make little difference as to the details of the hook-up, however, for the reason that the interaction between the hydrostatic and pneumatic systems causes a rapid movement of the dye in all directions within a given hydrostatic-pneumatic unit regardless of the location of the points of the application of the dye and suction within it. (See Harvey, *Plant Physiology*, July 1931.)

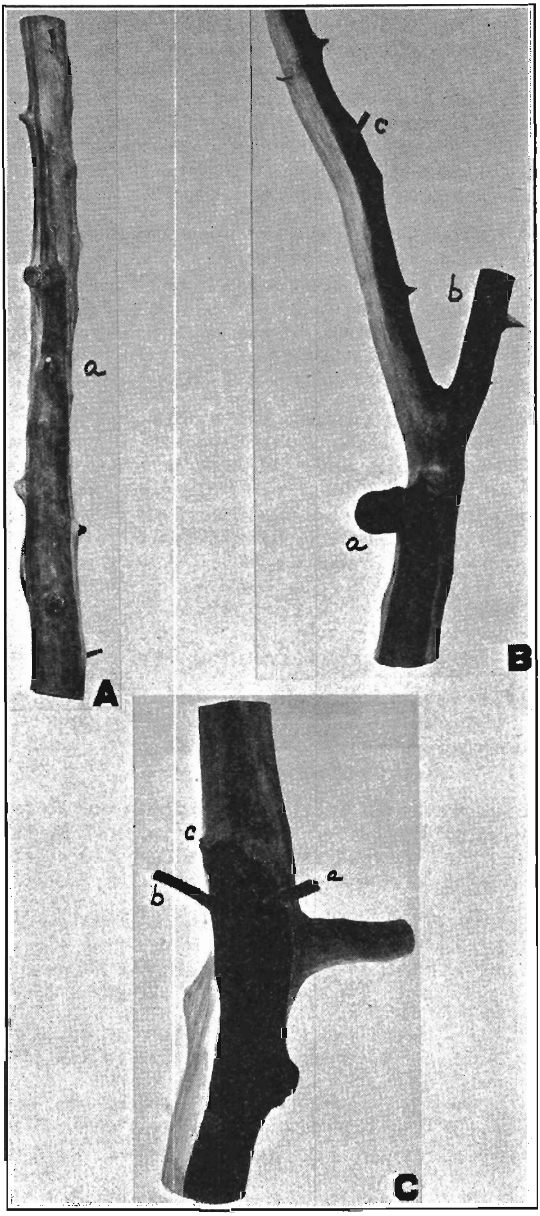

Figure 6. Shows the relatively large area of the current xylem which young water-shoots are able to usurp [note A(a), B(c), and C(a) and (b)]. Branch (a) of B is also referred to in the text (×1/8).

give the branch better exposure to air and light. This question of the water-pulling power on the development of tissue above the branch will be considered again under the topic of water-sprouts. The question might be easily answered experimentally by commencing in the spring to provide additional exposure to evaporation for some branches, and protection for others. At the end of the growing season, examination could be made by means of the dye method to determine the relative amount of upward directed water-conduction tissue produced in the two lots during the summer.

Another factor which modifies the amount of common tissue in a crotch is the close proximity of a competing branch. This situation was frequently noted. Such an effect may be observed in Figure 7 C where branch (a) has diminished the common tissue in the crotch above. On the other side, the common crotch-tissue of branch (b) (see Figure 7 D), extends more than half way around it. Another crotch is shown in Figure 7 A and 7 B, illustrating to a lesser degree the same effect of a third branch (a).

5. Effects of cankers, gummosis spots, and wounds of farm implements. The effects on water conduction of cankers and gummosis spots, etc., were not studied as such. A number of cases, however, of the sort were observed incidentally during other tests. It seemed plain from such chance observations that almost any kind of disturbance, even apparently insignificant ones, may totally block water conduction across the area involved. In Figure 4 E, branch (f) shows an old canker almost healed, yet it prevented the passage of the dye solution. In this case the clear area as shown in the photograph has been non-functioning for at least three years. Figure 5 B shows the effects of a gummosis area in a cherry stem. No dye could be drawn across it, and two annual rings are involved as indicated at point (a) in Figure 5 A.

The failure of the cambium in such areas to carry on its normal duties, at least for a time, throws a considerable extra burden upon the rest of the cambium of the corresponding section of the stem, even though the lost water-conducting area is largely compensated for later.

6. The paths of water conduction in branches one to four years old. It is frequently stated by horticulturists that the greatest stimulation from pruning is derived from "small cuts," well distributed over the tree. The mild-thinning type of pruning seems to be generally considered as beneficial.

The probable harmful effects of the removal of large branches were discussed in Section 3, page 14. But the writer was curious to see whether or not the application of the dye method would furnish any evidence for the value of "small cuts."

The reasonableness of the idea, that there is a "stimulation" by the removal of small branches, was quickly indicated when the interrelations of the water-conducting units of small side branches were examined. It was shown by the dye method that a side branch on wood two to three years old might sometimes share its xylem system with many other side branches and spurs, provided they are located in a relatively wide sector on the same side of the main branch. For example, if a branch three to four years old, and all its side branches and spurs, is brought into the laboratory and connected up with suction at the base and a dye solution at one cut-off side branch (chosen almost at random), the dye will enter and flow upward and downward from the cut-off side branch throughout the entire hydrostatic unit of the latter and

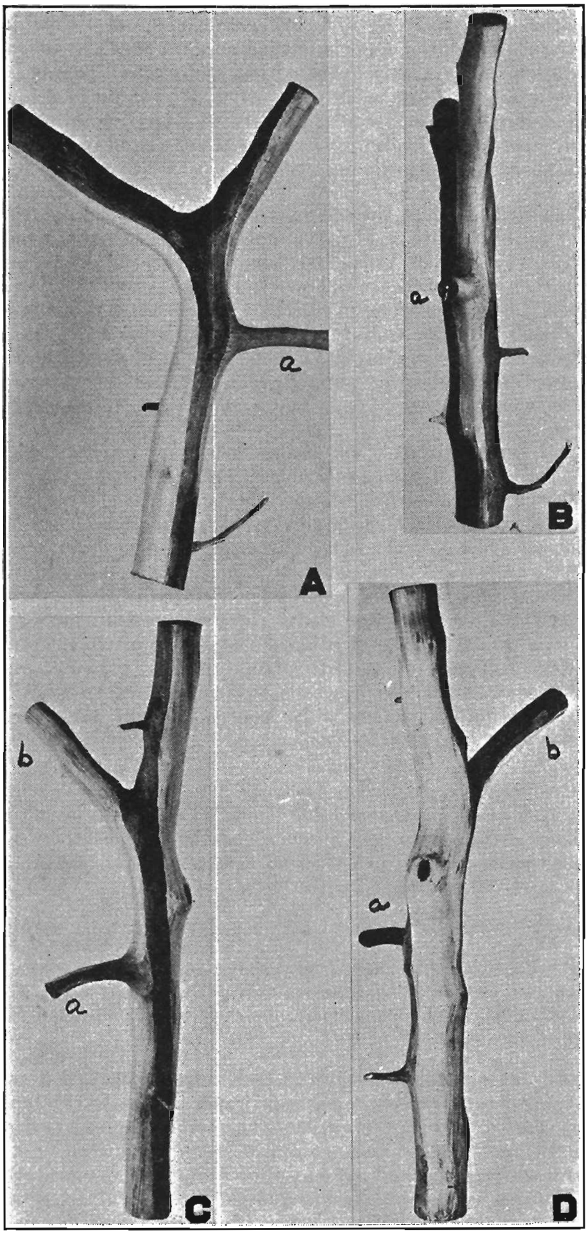

Figure 7. Illustrates the amount of common crotch-tissue as affected by the presence of other branches.

those portions of the units of all other branches and spurs with which it shares conducting tissue. When the experiment is over and the bark is removed from the entire branch, it is quickly evident that many small branches and spurs share their hydrostatic units with each other. The outer portions of a tree thus show considerable possible adaptability to altered conditions caused by pruning. This situation is in striking contrast to the rigid individuality shown by old established scaffold branches.

It is not difficult to see how the removal of a small branch might immediately increase the supply of water and other materials to the other small branches and spurs with which it had previously shared its vascular system. Figure 3 shows two branches three years old where suction was at the base and dye solutions were introduced at points (a). On branch A there were 28 twigs and spurs. It was found on examination that side branch (a) shared, at least to some extent, its water-conducting tissue with 13 of the total 28 twigs and branches shown in the photographs. Branch B had 37 twigs and spurs, and side branch (a) was found to share its water-conducting tissue with 16 of them.

7. Effects of water-shoots. Water-shoots present interesting examples of the seizure of control of old established cambium by young and vigorous branches. When a water-shoot begins development on an old branch, its strong demand for water causes a large area of the cambium to cease laying down water-conducting tissue for the older branch or branches above and to begin directing all tissue toward the new shoot. The extent of the cambium area so involved is oftentimes astonishing. Figure 6 presents three such examples. Figure 6B at (c) is a water-shoot to which a dye solution was attached. It was but 7 mm. in diameter, yet it had been able to usurp the entire current season's activity of nearly one-half the cambium of a branch 45 mm. in diameter. The wide downward and upward sweep of its water-conducting tissue indicates that it could draw not only from below but also from above its attachment, at least in times of stress.

Figure 6 C shows a pair of water-shoots. The remarkable width of their water-conducting tissue is observable. In this case the control of the older cambium stops rather abruptly above. This halting of their control was probably due to the influence of two more water-shoots located at (c). The latter shoots were not connected with dye solutions.

Figure 6 A shows the extensive water system captured by water-shoot (a).

The relatively great water-pulling power that water-shoots appear to possess shows the reasonableness of the horticultural advice that water-shoots ought to be removed as soon as possible unless they are to be used later as permanent structures.

8. The vascular elements available to a newly engrafted scion. From the observations made concerning the relation of the water-conducting tissues of stock and scion, it seems certain that the scion cannot utilize any of the xylem elements previously existing in the stock. When an engrafted scion begins to need water, this need can be supplied only by new water-conducting elements laid down by the old cambium of the stock in direct response to the demand of the scion. In this the relation of scion to stock is very similar to that of water-shoot to parent branch. The scion appears to make an even more sweeping and successful demand on the old cambium. The scion's ap-

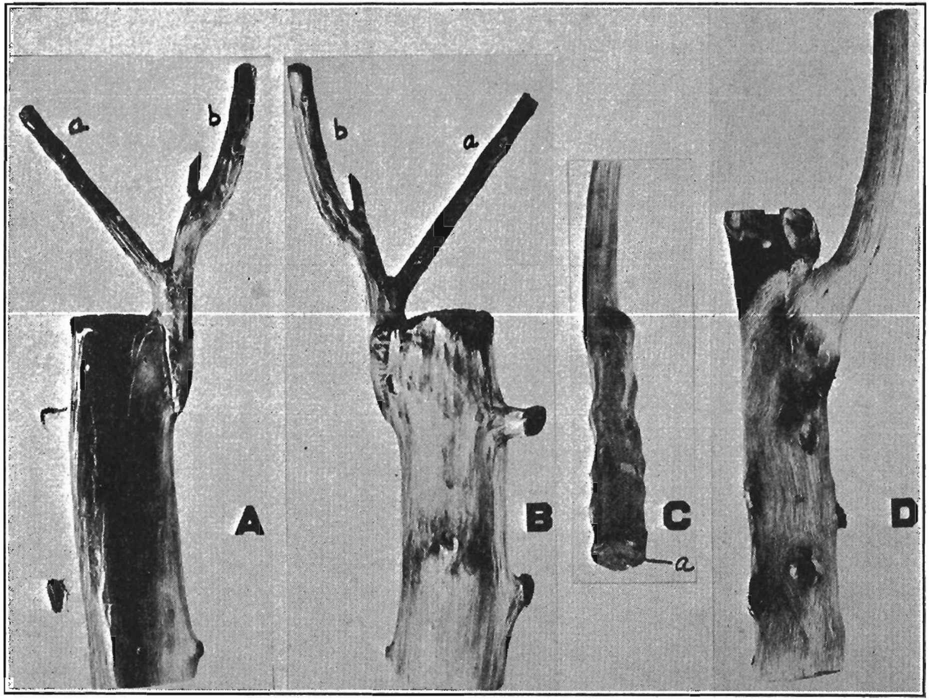

Figure 8. A and B are two views of a one-year-old scion top-grafted upon a branch 7 years old. The two branches (a) and (b) received different colored dyes. The vascular elements controlled by each branch are indicated ($\times 1/4$). C is a one-year-old scion budded on a one-year-old stock. D shows the vascular elements of a stock formed and controlled by a scion.

parent greater success, however, is probably due to the fact that it has practically no competition.

Figure 8, A and B, shows two views of a top-grafted apple branch 7 years old, after one season's growth of the scion. The two branches, originating from axillary buds of the scion, received different dyes. Each branch evidently pulled water from the old cambium independently and created saturation deficits in all directions, but each was more completely successful in the region lying directly beneath it. Each was able to obtain some vascular elements in the other's region, however. The influence of the two branches was noticeable entirely around the stock and on the side opposite. Each branch obtained almost an equal amount of new water-conducting tissue from the cambium.

In budded nursery material, it was found that the previously existing xylem tissue of the stock was unusable by the engrafted bud. Figure 8 C shows a cross-section below such a specimen. The photograph does not show the sharp contrast which in the original the eye could detect between the dye-filled outer ring and the brown, non-functioning inner ring. Figure 8 D also shows the relation of the water-conducting tissue of scions and stock.

Referring again to the control of the cambium of the stock by the immediate water requirements of the scion, an interesting piece of evidence came from the examination of an entire tree, three years old. It had been grafted when two years old, so that the scion had developed through one season. A dye solution was attached to the scion and suction was applied well down on the tap-root. When the tree was dissected after the experiment, it was found that the cambium throughout had started its activity about the same time, but at the base of the scion all the xylem elements of the last season had been directed to, and connected with, the scion. On passing down the stem and into the tap-root, it was noted that the thickness of the xylem so directed became less and less until finally only a few scattered vessels, in the extreme late season's growth, were connected with the scion. On the other hand, the xylem of this annual ring, not connected with the scion, increased from zero at the top to include the entire width of the ring at 30 cm. down on the tap-root. Such a situation seemed to indicate that the changes in the orientation of the cambium activities had been initiated by the scion, and that this influence was gradually propagated downward to the roots, the cambium at any given point responding as soon as the influence reached it.

9. Application of the study of phyllotaxy. It was stated in the introduction to this Bulletin, that it was for the study of phyllotaxy in the apple and pear that the present modification of the dye method was adopted. The writer still believes that the method is well suited for rapid checking of reported phyllotaxies and the determination of others. No particular practical value is claimed here, but the application is suggested for those persons who may be interested in this special subject.

10. Application to herbaceous plants. The general question of the cross-transference of elaborated foods, water and other materials, may be very different in herbaceous and woody plants.

The available information regarding the cross-transference of elaborated foods in woody plants indicates that such transference is slight. It now seems probable also that in woody plants the water moves from the hydrostatic unit to another only against great resistance. But White[3] concluded that the structure of the crown of the strawberry plant is such that cross-transference of elaborated food should take place readily. Recently Dr. G. M. Darrow brought some strawberry plants into the writer's laboratory to check the corresponding movement of water. A plant was selected with two runners, originating on opposite sides of the crown. Each runner was cut off about 12 to 15 cm. from the crown and a dye solution attached to one and suction to the other. The experiment was stopped after ten minutes. When the crown was cut across, it was found that the dye had stained beautifully every portion of its xylem elements. There seemed little doubt of the ready cross-conduction of water in the strawberry plant.

Several preliminary tests were made by means of the dye method on some of the cane fruits. The results indicated interesting possibilities in this direction.

11. Application to teaching. The water-conducting units of a branch can be outlined so quickly and interestingly by the present dye method that it does not seem out of place to suggest its advantage for class-room demonstation in plant physiology and horticulture. The material that is more unusual or more difficult to obtain may be prepared from time to time as opportunity offers and preserved as museum pieces. The simpler demonstrations may be made before the class or may be introduced as student laboratory experiments. The advantage, if any, of introducing this procedure into the student laboratory lies in the rapidity and definiteness with which the most striking details of the paths of water conduction in woody plants can be demonstrated.

CONCLUSION

This report presents a modification of the dye-injection method and offers suggestions for its application to the study of the effects of horticultural practices on the paths of water conduction in plants.

The presentation has been made almost solely in the hope that it may prove to be an aid to a practical understanding of the factors controlling the paths of water conduction.

The general features of the method are not new, but the chief advantage of the present modification would seem to lie in the facility with which it can be applied.

The remarks and discussion that accompany the different phases of the present application of the dye method are intended to indicate the direction taken by the evidence observed, and as suggestive only. The method has not been applied in a quantitative manner and could not be expected to settle the questions brought forward. Many of these questions admit of a quantitative attack by other methods and it is probable that other such questions will be suggested to any one who employs the dye method very extensively.

LITERATURE CITED

[1] Harvey, R. B.—Tracing the Transpiration Stream with Dyes. Am. Jour. Bot. *17:* 657-662. 1930.

[2] MacDougal, D. T., J. B. Overton, and Gilbert M. Smith.—The Hydrostatic-Pneumatic System of Certain Trees: Movements of Liquids and Gases. Carnegie Inst. Wash. Pub. No. 397. 1929.

[3] White, Philip R.—Studies of the Physiological Anatomy of the Strawberry. Jour. Agr. Res. *35:* 481-492. 1927.