EFFECT OF THE DWARF DISEASE ON THE ALFALFA PLANT

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INTRODUCTION

In previous papers (6, 7) the writer has discussed the nature of the dwarf disease of alfalfa (Medicago sativa L.) and the effect of certain environmental factors on its spread and development. That the disease causes a considerable disturbance in the normal histology and physiology of the alfalfa plant is indicated by the dwarfing of the tops and the extensive discoloration in the roots. The results of certain histological and physiological studies made to obtain a better understanding of the nature of the effect of dwarf on the alfalfa plant form the basis of this paper.

HISTOLOGICAL METHODS

For the most part fresh material was used, although many roots and stems were fixed in formal-acetic alcohol, embedded in paraffin, sectioned, and stained. Some material was stored in fixative and sectioned with the sliding microtome without being embedded in paraffin.

The varieties studied were Hairy Peruvian and Chilean, and the plants were collected in the field at different times of the year.

Various stains were used, but Heidenhain’s iron-alum haematoxylin and carbolfuchsin were the most helpful. Certain points, such as the yellowing of the vessels, could be studied best without staining.

THE DISEASE IN THE ROOT

When the bark is removed from diseased taproots in the winter and spring the yellow color observed during the growing season (5) usually is found to be more or less overgrown with a thin layer of new white wood (fig. 1). With the advent of cool weather in the autumn the disease becomes less active and gum formation ceases, so that by late spring a layer of white wood 1 mm or more in thickness is present between the cambium and the yellow wood produced during the previous summer. The thickness of this new layer varies greatly in different roots and in different parts of the same root. A relatively thick layer of new wood may form on one side of the root, where the disease has not made such inroads, and little or none on the other side. A macroscopically perceptible layer of new wood may be present as early as December. The disease becomes active again the next
spring and by midsummer has involved much of the new wood, and the plant slowly succumbs.

A microscopical study of cross sections of diseased roots shows clearly the parts affected. Little or no evidence of trouble of any kind appears in the bark, cambium, wood rays, or fibers of affected plants until the late stages of the disease, when there is usually some yellowing caused by a soluble stain. The parenchymatous cells contiguous to or near plugged vessels are sometimes affected, as is shown by the yellow color in their walls and their staining reaction. Sometimes there is a small amount of plasmolysis in these cells also. How-

![Image of cross and longitudinal sections of three alfalfa roots affected with dwarf. The dark-colored streaks are regions where the vessels are plugged with gum. These roots show the early spring condition, when the discolored areas are covered by a layer of new growth. In cross section A the affected tissues form a complete dark-colored ring about 1 mm beneath the cambium. Cross section B shows a much wider band of discolored cells on the right and upper sides than elsewhere, and cross section C shows only a very small discolored region in the upper left-hand part. Compare with figure 3, which shows not only the unaffected region of winter growth covering the affected ducts beneath but also the layer of plugged cells nearer the cambium, developed during the current season. About natural size.

ever, in many cases, at least in the earlier stages of the disease, the parenchyma cells stain and appear quite normal. No X-bodies have ever been seen; the visible evidence of the disease is restricted for the most part to the vessels of the xylem.

Not all of the xylem is affected. The plugging at first may be limited to one or a few ducts in one or more bundles. Even in fairly advanced stages of disease certain bundles may be entirely unaffected while others are badly plugged (fig. 2). In fact, usually only a com-
paratively small proportion of the total number of ducts of a bundle are involved (figs. 2 and 3). The affected region in roots in which the disease is most active, as indicated by the formation of gum, lies just within the cambium; that is, in the part of the xylem that is functioning most actively in transporting water and nutrients. In advanced stages of the disease so many of the ducts in this region may be plugged that water cannot reach the tops in sufficient quantity to prevent wilt-

![Figure 2](image-url) - Cross section of alfalfa root having medium stage of dwarf. One bundle (a) has no plugged vessels, while the adjacent bundle on the left has several and the one on the right has two. Although unaffected bundles are often found, more frequently some ducts in all the bundles are more or less plugged, especially in the later stages of the disease. The affected region is several cells back from the cambium, a condition commonly seen during the winter and spring months. × 35.

ing during the middle of the day in bright weather even when the soil moisture is very high.

The youngest vessels next to the cambium may be plugged but usually they are not except in the late stages of the disease. The plugging is most commonly in the second and third layer of ducts from the cambium. The width of the affected region increases as the disease develops. In the later stages the affected area usually occupies a zone not more than 2 or 3 mm in width, or, roughly, the outer third
of the distance from the center of the root to the cambium (fig. 3). Not much consistency is shown in the way the ducts are plugged. Sometimes the larger, sometimes the smaller, and sometimes all the ducts are involved. A single plugged duct or a group may be surrounded by ducts that apparently are unaffected. Likewise, one bundle may have a number of plugged ducts while an adjacent one

has only a few or none at all (fig. 2). Many ducts lying end to end may be plugged, forming long strands of apparently continuous gum. The gum often shrinks and pulls away from the walls. Tyloses are sometimes present in the ducts.

THE DISEASE IN THE STEMS

It was pointed out in a previous paper (5) that the tops of affected plants show a gradual dwarfing which continues until the plant dies. It might be expected that the yellowing and plugging of the vascular system of the diseased roots would extend into the stems, but a study of many sections of stems has shown little if any stain and only a
small amount of plugging. Such little plugging as there is in the vessels of the green stems is found chiefly in the late stages and then only for a distance of an inch or two above the crown (fig. 4). The vessels of the stem that are plugged are usually though not always near the pith. The lack of extensive plugging in the stem tissue may be due, at least in part, to the fact that the disease progresses very slowly and that the stems are cut frequently (once every 28 to 34 days) during the growing season, when the disease is most active.

CHARACTER OF THE GUM

The gum may be homogeneous in character (figs. 5 and 6); it may have what appears to be a flocculent or granular precipitate; or it may contain numerous coccuslike or rod-shaped bacterialike bodies (fig. 7). The homogeneous and granular gum is comparable to that formed in many other plants. The bacterialike bodies are not present in the ducts of all affected plants; in fact, they have not been found at all in about 25 percent of the plants examined. Nevertheless they constitute one of the most reliable microscopical diagnostic characters of the disease.

From the beginning of these investigations the question whether or not these bodies are bacteria has presented itself. All efforts to prove by cultural methods that they are living entities have failed (7). Some bacteriologists and pathologists who have examined the material have hesitatingly pronounced these bodies bacteria; others equally competent to judge have said that they were not bacteria. These bodies are limited to the ducts of the xylem. Pathogenic bacteria usually, if not always, sooner or later pass out of the vessels into the intercellular spaces of the parenchyma. The bodies in question are

![Figure 4](image-url)
embedded in a gum which holds them in clumps and keeps them from undergoing Brownian movement. When this gum is dissolved away by 50-percent chromic acid the bodies float free, showing that they are individuals and have a solubility slightly different from that of the gum. At times they appear yellow like the gum in which they are embedded.

The outer portion of these bacterialike bodies differs from the interior as seen both in stained and unstained preparations. The outer portion takes the stain while the inner remains largely or entirely unstained. This has been interpreted by some authorities as indicat-

![Image](image-url)

**Figure 5.** Cross section of alfalfa root affected with dwarf showing group of plugged vessels in which some of the walls have been so changed or impregnated with gum that they have practically lost their identity. Adjacent wood parenchyma cells have been affected to a limited extent. × 190.

ing the presence of a spore (figs. 7 and 8). In certain stages this outer portion takes a red color when treated with phloroglucin and hydrochloric acid, just as does the gum, indicating its similarity to wound gum. Neither these bodies nor the gum takes on this color in the early hyaline stage. So far as ascertained, no bacteria give this reaction for wound gum. Of course, it is possible that the outer portion of these bodies is impregnated with gum and so gives the characteristic gum reaction to the phloroglucin-hydrochloric acid treatment. These bodies are gram-negative and do not take many of the stains that most bacteria do, but rather stain like the gum
near or surrounding them. This fact suggests that these bodies are of a material similar to gum but that they differ from it slightly, at least in their solubility in chromic acid.

In the early stage these bodies are embedded in a hyaline material more or less creamy or semifluid in consistency. This was determined by stirring the material in a single duct under the microscope by means of a capillary glass needle manipulated by an apparatus similar to that described by Roberts (4). This material very soon hardens so that it can be moved about in a cell as a unit and the needle cannot penetrate it. Later the contents of the vessel turn yellow and give the wound-gum reaction as previously described. In the semifluid state the contents of the ducts are more or less readily washed away in the staining process. A few of the bodies were sometimes found isolated, lying along the wall of the duct and apparently free of gum. These often gave the red color when treated with phloroglucin and hydrochloric acid, which suggests that this reaction is due to the nature of the bodies themselves and not to the gum in which they are embedded. These bodies give a negative reaction for tannins, chitin,
Figure 7.—Cross section of alfalfa root stained with carbol-fuchsin, showing bacteriakike bodies. These may be so numerous that they entirely plug the lumen of the vessel. X 1,100.

Figure 8.—Longitudinal section of a vessel in an alfalfa root stained with carbol-fuchsin, showing bacteriaklike bodies scattered about. X 1,200.
protein, and fats and do not react to polarized light as crystals do. Knowledge of their exact chemical nature will have to await further study.

**ORIGIN OF THE GUM**

To determine with certainty the source of the gum plugging the vessels of alfalfa plants affected with dwarf is difficult. In the early stages of the disease certain portions of the walls become impregnated with gum, which eventually collects along their inner margins (figs. 9 and 10). Later the ducts may be partly or completely filled with gum (figs. 2, 3, 5, and 6). The most probable explanation of the origin of this gum is that it is formed in the adjacent parenchyma cells and is forced through the pits into the ducts. There is sometimes evidence of a slight change in the affected vessel wall or in the region of the middle lamellae, but this is so restricted that it hardly seems probable that it serves as a source of much if any of the gum. The walls are never completely disintegrated and gum pockets are never formed as happens in many diseased plants. It seems more probable that sugars, starches, or other carbohydrates in solution in the cell sap in the adjacent parenchyma cells are the source of the gum.
WATER CONTENT OF DISEASED PLANTS

The comparative amount of water in diseased and healthy alfalfa plants was determined in an experiment set up on August 20, 1931. From a field of Chilean alfalfa in its fourth year, diseased and healthy plants having about half of their blossoms open were selected. The plants all came from the same place in the field, and therefore should have been comparable in every way except for the presence of the disease in some of them. Five plants in a fairly late stage of the disease and five healthy plants were dug, wrapped well in newspaper to prevent the loss of water, and brought to the laboratory. After the adhering soil had been removed from the roots with a brush and cloth without wetting, composite samples were made of the healthy tops, diseased tops, healthy roots, and diseased roots. The roots and tops were cut into small pieces, placed in tared weighing bottles, weighed, dried to constant weight at 100° C., and reweighed. The field had been irrigated 2 days before the plants were dug, and the soil was still quite moist. A second experiment, conducted on August 25, 1931, was an exact duplicate of the first except that the tops had been cut in the meantime and the stems were only 3 to 4 inches tall and consequently were young and succulent. The soil was damp, as the field had been irrigated the preceding day. Only the new stems and leaves and about a foot of the taproots were used. This was true for both experiments.

On September 1, 1931, six diseased plants and six healthy plants from the same field were treated in the same manner as in the two preceding experiments except that the plants were held with their taproots in water for 24 hours before being prepared for the dry-weight determinations. The tops were turgid at all times. In this experiment the yellow crown tissue was placed with the roots, and all green tissue was included with the tops. The affected plants were in various stages of disease, but in all cases the disease had advanced sufficiently to cause more or less dwarfing of the tops. The healthy tops were about 1 foot to 15 inches tall. This experiment was duplicated on September 10 and again on September 16, 1931. Of course, the stage of top growth was not the same in any of these tests. On September 1 the tops were perhaps not more than half grown, while
on September 16 they were in what is usually classed as the bud stage, although there was an occasional partly open blossom.

The percentage of water in the roots and tops in these experiments is given in Table 1. These data show that on each date the water content of the healthy tops was slightly higher than that of the diseased tops, while the reverse was true for the roots. The greatest difference between the moisture content of the diseased and healthy tops was on August 25, when the top growth was young and succulent. The greatest difference between the water content of the diseased and healthy roots was in the August collections. No explanation for this can be offered, unless it is the fact that the roots of the September samples stood in water before they were prepared for drying. The difference between the average percentage of water in the diseased and healthy tops and roots for all experiments is quite small and probably is not significant. Nevertheless, the tops of the healthy plants were considerably larger than those of the diseased, and it may be that the larger tops withdrew slightly more water from the roots during the period which of necessity elapsed between the removal of the plants from the soil or water and the determination of the wet weight. Perhaps the slightly lower water content of the diseased tops was due to a partial water deficit. That such a deficit does exist at times is suggested by the fact that diseased plants often wilt even when growing in wet soil.

<table>
<thead>
<tr>
<th>Date</th>
<th>Tops Healthy</th>
<th>Tops Diseased</th>
<th>Roots Healthy</th>
<th>Roots Diseased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 21</td>
<td>70.1</td>
<td>68.5</td>
<td>69.5</td>
<td>74.4</td>
</tr>
<tr>
<td>Aug. 25</td>
<td>62.5</td>
<td>72.2</td>
<td>66.2</td>
<td>70.9</td>
</tr>
<tr>
<td>Sept. 1</td>
<td>82.2</td>
<td>79.2</td>
<td>70.5</td>
<td>70.7</td>
</tr>
<tr>
<td>Sept. 10</td>
<td>82.7</td>
<td>80.6</td>
<td>68.8</td>
<td>69.3</td>
</tr>
<tr>
<td>Sept. 16</td>
<td>77.8</td>
<td>75.2</td>
<td>67.4</td>
<td>70.1</td>
</tr>
<tr>
<td>Average</td>
<td>80.0</td>
<td>77.3</td>
<td>68.7</td>
<td>70.5</td>
</tr>
</tbody>
</table>

TRANSPIRATION

A study was also made of the rate at which dwarfed and healthy plants transpire. Twelve 4-year-old Chilean alfalfa plants, six dwarfed and six healthy, which had received the same treatment throughout, were used for each experiment. The plants were collected at about the same time in the morning (8 a.m.), wrapped well in newspaper, and taken to the laboratory, where the roots were placed at once in water. As much of the taproot as could be pulled conveniently, usually about 8 to 12 inches, was left on each plant. The length of the tops varied in the different experiments. The healthy tops ranged in height from 15 to 16, 14 to 18, and 26 to 31 inches in the experiments conducted on September 1, 10, and 16, 1931, respectively. The tops were of different ages and different stages of maturity, since they were all cut on August 24. The healthy
plants had reached the bud stage by September 16, when the last experiment was conducted. As soon as possible after the plants were brought to the laboratory the taproots and crowns were washed free of adhering soil, and after the lower end of the taproots had been cut off, taproots and crowns were placed at once in 100-cc graduated cylinders filled with tap water. Sufficient water was added to or withdrawn from the graduates to bring the water level to the 100-cc line. The graduates were placed on a table in two rows, so arranged that the tops of the plants did not touch and no two diseased or healthy plants were adjacent. In this way it was hoped to have the diseased and the healthy plants subjected to the same average conditions. The room temperature ranged from about 22° to 30° C. during the experiment, and there was some air circulation from open windows, but no wind was blowing directly upon the plants. The space between the roots and the mouth of the graduate was left open, since preliminary trials showed that not enough water evaporated from the surface in 7 hours to vitiate the results.

After the graduates had been filled as described, a measured amount of water was added each hour to bring the level back to the 100-cc mark. In this manner the total amount of water transpired by each plant in 7 hours was determined. The averages of these values, each representing the average for six plants, for the three experiments are given in table 2. Considerable variation was shown in the amount of water transpired both by the different plants and from hour to hour by the same plants. As might be expected, the greatest water loss occurred during the warmest part of the day.

**Table 2.—Comparative rate of transpiration by dwarfed and healthy alfalfa plants**

<table>
<thead>
<tr>
<th>Healthy</th>
<th>Diseased</th>
<th>Healthy</th>
<th>Diseased</th>
<th>Healthy</th>
<th>Diseased</th>
<th>Healthy</th>
<th>Diseased</th>
<th>Ratio of diseased to healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cc 67.1</td>
<td>Cc 28.8</td>
<td>Cc 85.3</td>
<td>Cc 68.6</td>
<td>G 10.7</td>
<td>G 7.5</td>
<td>Cc 0.80</td>
<td>Cc 0.42</td>
<td>Cc 0.34</td>
</tr>
<tr>
<td>Cc 58.1</td>
<td>Cc 23.3</td>
<td>Cc 74.5</td>
<td>Cc 50.0</td>
<td>G 8.3</td>
<td>G 5.8</td>
<td>Cc 0.78</td>
<td>Cc 0.47</td>
<td>Cc 0.40</td>
</tr>
<tr>
<td>Cc 66.4</td>
<td>Cc 26.7</td>
<td>Cc 99.5</td>
<td>Cc 63.7</td>
<td>G 15.5</td>
<td>G 8.4</td>
<td>Cc 0.67</td>
<td>Cc 0.50</td>
<td>Cc 0.38</td>
</tr>
</tbody>
</table>

6 dwarfed and 6 healthy plants were used in each of the 3 experiments.

The data presented in table 2 show that the healthy plants transpired more rapidly than the diseased plants in all experiments. The tops, however, varied considerably in size; hence, to get the comparison on a more uniform basis, the volume of tops as well as their dry weight was determined. The volume of the tops was first obtained by the water-displacement method, after which they were dried to constant weight in an oven at 100° C. When the volume of tops is used as the criterion, a comparison of the rate of transpiration in the three experiments shows that the healthy tops transpired 0.80, 0.78, and 0.67 cc, and the diseased tops 0.42, 0.47, and 0.50 cc per cubic centimeter of tops, respectively. The corresponding values, obtained
by using the grams of dry weight as a basis of comparison, are 6.27, 7.00, and 4.28 cc for the healthy plants and 3.84, 4.02, and 3.18 cc, for the diseased plants, respectively. When the ratio of diseased to healthy plants was calculated, the values in the last column were obtained. These values show a fairly close agreement between the results obtained by the two methods. In general, they indicate that the diseased plants transpired on an average only 53 to 75 percent as fast as the healthy plants, or, expressed in another way, the healthy plants on a volume basis transpired on an average, about 1.6 times as fast per unit of top tissue, as did the diseased plants.

**RATE OF WATER FLOW THROUGH DISEASED ROOTS**

After it had been determined that alfalfa plants affected with dwarf transpire much more slowly than do healthy plants, the rate at which water could pass through diseased roots was studied. It is known that the tops of diseased plants sometimes wilt during the warm part of the day even when the soil is wet. This indicates that the tops cannot obtain water rapidly enough to offset that lost by transpiration. Likewise, it has been shown by microscopical examination that many of the vessels of affected plants are plugged with gum. That the slowing down of the rate of transpiration and the plugging of the vessels were somewhat interrelated seemed probable.

The rate at which the water can pass through 4-inch segments of the taproots of diseased and healthy plants was determined by a method similar to that described by Melhus, Muncie, and Ho (3). Water was pulled through segments of the roots of 4-year-old alfalfa plants under a constant suction pressure. The diseased and healthy plants were obtained from the same place in the field, were collected at the same time, and were held under comparable conditions throughout.

**Table 3.—Average amount of water pulled through 4-inch segments of healthy and diseased alfalfa roots of different diameters in 10 minutes**

<table>
<thead>
<tr>
<th>Diameter of root at small end</th>
<th>Healthy roots</th>
<th>Diseased roots</th>
<th>Healthy roots</th>
<th>Diseased roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plants</td>
<td>Average amount of water pulled through in 10 minutes</td>
<td>Plants</td>
<td>Average amount of water pulled through in 10 minutes</td>
<td>Plants</td>
</tr>
<tr>
<td>Mm</td>
<td>Number</td>
<td>Cc.</td>
<td>Number</td>
<td>Cc.</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>19.5</td>
<td>1</td>
<td>4.7</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>10.1</td>
<td>1</td>
<td>8.4</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>24.8</td>
<td>4</td>
<td>11.3</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>24.0</td>
<td>5</td>
<td>24.5</td>
</tr>
</tbody>
</table>

1 Each segment was taken from a different plant.

The plants required for the day's testing were brought to the laboratory in the morning and kept in water until used. For the most part, a diseased and then a healthy plant was studied in order to minimize any error that might be due to a change in the plants on standing in water. The segment of root used was taken just below the crown. The diameter of the smaller end was used as a basis of
comparison, since it was assumed that the maximum amount of water that could pass through the root was limited to the amount that could enter the small end. The number of cubic centimeters of water that passed through the 25 segments of the diseased and the healthy roots in 10 minutes was determined, and the values are recorded in table 3. The data in this table show considerable variation in the amount of water that passed through roots of different diameters. Individual healthy roots of the same diameter seemed to vary considerably in this respect. Perhaps this is to be expected of roots of this age and character. Seldom do any two plants have crowns or tops of the same size, or indeed taproots of the same length. Taproots likewise vary greatly in diameter even in a distance as short as 4 inches.

This experiment, however, had only one object, namely, to compare diseased and healthy plants, and it was thought that if any appreciable difference existed it would be brought out by a study of 25 plants of each group. This proved to be the case, as table 3 shows. Although the differences were sometimes slight, as in the case of the roots 7 mm in diameter, for the most part they were rather striking. In the case of roots 9 mm in diameter, the healthy roots conducted more water than the diseased except for one diseased root, which conducted water more freely than any of the healthy roots and was responsible for the slightly higher average for the diseased roots. That the passage of water through the diseased plants should vary greatly in rate is to be expected, not only in view of the variation found in its passage through the healthy plants but also because of the difference in the stage of disease and consequently in the number of ducts plugged with gum. It was impracticable to use roots entirely comparable in every way, owing to the difficulty of obtaining them and to the impossibility of telling by macroscopic examination the exact amount of plugging in the diseased root. The average amount of water pulled through the healthy and diseased roots was 40.6 cc and 25 cc, respectively. This means that on an average water passed through the healthy root segments 1.6 times as fast as through the diseased roots. The fact that the healthy plants transpired approximately 1.6 times as fast as did the diseased plants and permitted the passage of water 1.6 times as fast as the latter suggests that there is a close correlation between these two factors. The data presented in this paper seem to indicate that the inability of the affected alfalfa roots to conduct water may have much to do with the dwarfing of the tops and the ultimate death of the plant.

HYDROGEN-ION CONCENTRATION AND TOTAL ACIDITY OF HEALTHY AND DISEASED PLANTS

It is a well-known fact that plants contain various acids which are more or less highly ionized. The formation of these acids appears to be closely associated with the vital activities of the plant. Anything that alters appreciably the normal processes of a plant may effect a corresponding change in the acidity of its sap. Hurd (2) found that the expressed juice of corn plants varied greatly in both hydrogen-ion concentration and titratable acidity, these being influenced largely by environmental factors which affected the vegetative vigor of the plants. A number of investigators have shown that the hydrogen-ion
concentration of the sap of plants can be changed by applying certain fertilizers to the soil in which they are growing.

Since the rate of growth and transpiration of the alfalfa plant are affected by the dwarf disease, it seemed quite possible that its acidity also would be affected. To test the validity of this assumption, the hydrogen-ion concentration and total acidity of healthy and diseased alfalfa plants were determined. Four-year-old alfalfa plants of the Chilean variety, which had been given comparable treatment throughout, were used. The diseased and healthy plants came from the same area in the field; those for each experiment were collected at the same time and were given the same treatment.

The tops of diseased plants, although of the same age, were not so far advanced as those of the healthy plants. In the first two experiments the tops of the latter had made a vigorous growth and were in the early bud stage, while those of the former were somewhat less advanced, the stage of advancement depending upon the stage of the disease. The method of determining the total acidity was largely that described by Haas (1). Twenty-five cubic centimeters of juice from diseased and 25 cc from healthy plants were obtained by grinding the tissue in a meat grinder and then pressing the juice out through cheesecloth. The samples of juice were kept in a refrigerator until ready for use. The pH values were first determined with the quinhydrone electrode and then N/20 NaOH (1 cc at first and 2 cc later) was added until a value above 8.3, the turning point for phenolphthalein, was reached. The pH values calculated from the successive potential differences thus obtained were plotted against the volume of NaOH required to produce them. From these curves the volume of alkali required to bring the reaction to pH 8.3 was determined and taken to represent the titratable acidity of the sample.

The results thus obtained are presented in table 4. It is evident from the values shown that in the first two experiments the pH, as well as the titratable acidity, of the healthy tops checked closely. This was also true for the diseased tops and roots. The chief disagreement was in the titratable acidity of the healthy roots in the second experiment. Since the second experiment was conducted on the day following the first, the plants were approximately the same age. The last experiment, however, was performed 12 days after the second, and the tops, which had been cut on October 4, were quite young. These younger tops, in the case of both the healthy and the diseased, had a higher pH value and a lower titratable acidity than the corresponding older ones used in the first two experiments. The pH values of the healthy and the diseased roots differed little from those in the other experiments. The titratable acidity of the diseased roots differed considerably, that in the third experiment requiring nearly 10 cc more alkali than did those in the two previous experiments. It is difficult to evaluate the 25.3 cc of NaOH used for the healthy roots. Since it agrees so closely with the comparable figure for the second experiment, it seems probable that the 22.8 cc in the first experiment is the variable one and that there was no appreciable change in the healthy roots because of the new growth. A comparison of the healthy and diseased tops shows that the former had slightly higher pH values than the latter. The differences are so small, however, that they probably are not significant. On the other hand, the diseased tops in each experiment had a higher titratable acidity than
the healthy tops, although that for the third experiment was identical with that for the healthy tops in the first experiment. There was almost no difference between the pH values of the healthy and diseased roots, but the healthy roots had a higher titratable acidity in the first two experiments.

**Table 4.**—The pH and titratable acidity\(^1\) of healthy and diseased alfalfa tops and roots, 1931

<table>
<thead>
<tr>
<th>Date</th>
<th>Tops</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Diseased</td>
</tr>
<tr>
<td></td>
<td>pH  N/20 NaOH</td>
<td>pH  N/20 NaOH</td>
</tr>
<tr>
<td>Oct. 1</td>
<td>6.10  6.05 Cc</td>
<td>6.45  6.20 Cc</td>
</tr>
<tr>
<td>Oct. 2</td>
<td>6.15  6.00 Cc</td>
<td>6.45  6.10 Cc</td>
</tr>
</tbody>
</table>

1 Titratable acidity is expressed as cubic centimeters of N/20 NaOH required to bring 25 cc of juice to pH 8.3.

In general, it may be concluded that the diseased tops had a slightly higher hydrogen-ion concentration and a higher titratable acidity than did the healthy tops, that there was practically no difference in the pH values of the roots, and that the diseased roots had a lower titratable acidity than the healthy ones except in the last experiment where the reverse was true. It is also shown that the increased vegetative vigor of the tops in the third experiment resulted in decreased acidity, the greatest difference being in the healthy tops, which were growing most vigorously. Since the disease is most evident in the roots, at least in the earlier stages, and the pH value of the diseased and healthy roots is almost identical, it seems evident that the disease does not affect the pH value of the plant, the slight difference in the pH value of the tops being attributed to the difference in the stage of their development, that is, the less vigorous growth of the diseased tops is reflected in the lower pH. Whether the slight variation in the titratable acidity of the diseased and healthy roots was due to a difference in vigor or whether it signified the presence of a diseased condition is not known. Since such a great variation existed in the titratable acidity of the diseased roots between the first two experiments and the last, seemingly owing to a change in the stage of growth of the tops, it appears probable that the condition of growth also was responsible for the smaller differences between the diseased and healthy roots.

**ASH CONTENT OF HEALTHY AND DISEASED PLANTS**

On July 10, 1931, healthy and diseased alfalfa plants were taken from the same place in a 4-year-old stand, the tops were removed, washed in distilled water, and wiped dry. The roots were washed in tap water and distilled water. After being cleaned, the tops and roots were divided into two samples, placed in paper bags, and dried in an oven at 75° C. The dried samples were then ground fine and ashed, and the ash was calculated as percentage of dry matter. A second
set of samples was obtained on July 24, 1931, and treated in the same manner, making four samples for each date. The data obtained (table 5) show that the ash content of both the healthy tops and roots is considerably lower than that of the diseased. In fact, the healthy tops and roots had an average of only 88 and 67 percent as much ash as the diseased tops and roots, respectively.

Table 5.—Ash content of healthy and diseased alfalfa tops and roots, 1931

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>Ash content of—</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tops</td>
<td>Healthy</td>
<td>Diseased</td>
<td>Roots</td>
</tr>
<tr>
<td>July 10</td>
<td>Percent</td>
<td>10.10</td>
<td>11.13</td>
<td>Percent</td>
</tr>
<tr>
<td>July 24</td>
<td>7.73</td>
<td>9.10</td>
<td>3.43</td>
<td></td>
</tr>
</tbody>
</table>

1 The writer is indebted to Dr. A. R. C. Haas, of the California Agricultural Experiment Station for these determinations.

2 Expressed as percentage of dry matter.

STARCHES AND SUGARS

The differences in the rate of transpiration and in ash between diseased and healthy plants show that there is an upset in the normal physiology of the alfalfa plants affected with dwarf. Further evidence of this is supplied by a study of the carbohydrate relations of the diseased plants. Although an analytical study was not made, a few observations, especially on the starch content of the affected roots, show clearly that a marked change in the carbohydrate content of the roots takes place as the disease progresses. Many diseased and healthy roots have been sectioned, stained with iodine, and examined. These studies show that in the early stages of the disease the affected roots are gorged with starch just as are the healthy roots. As the disease progresses the starch becomes less abundant, and by the time the disease is well advanced there is little or no starch left. The only observation made on the sugars of the diseased root is that some reducing sugar is present in roots from which the starch has entirely disappeared.

SUMMARY

Alfalfa roots affected with dwarf have more or less yellow color in the wood, owing largely to the presence of gum in the vessels. In the late stages of the disease there also is present a yellow stain, which diffuses into the surrounding tissues. The gum, which is similar in character to wound gum, is limited almost entirely to the vessels in the outer xylem, the other tissues being largely unaffected. In the early stages of the disease the gum is limited to a few vessels in one or more bundles in the upper part of the taproot or crown, but before the plant is killed the whole root system is involved. Gum is found in the green stems, if at all, only in the basal 1 or 2 inches. Some of the gum evidently is forced from adjacent cells into the ducts, where it appears in the form of globules or thin sheets along the inner surface of the
walls. Eventually many of the ducts become completely filled with gum and are rendered functionless.

Many bacterialike bodies are present in some of the vessels. Microchemical tests indicate that they are chemically very similar to the gum in which they are embedded and unlike bacteria in several respects.

The water content of the tops of healthy plants was slightly higher than that of diseased tops; the reverse was true of the roots. Whether this difference is sufficiently great to be significant is questionable.

Healthy alfalfa plants transpired about 1.6 times as fast as did diseased plants.

Water could be pulled through segments of healthy roots about 1.6 times as fast as through diseased roots of approximately equal diameter, indicating a close correlation between transpiration and the rate at which water could pass through the root segments.

The diseased tops had a slightly higher hydrogen-ion concentration and a higher titratable acidity than did the healthy tops, there was no difference in the pH value of the roots, and the diseased roots had a lower titratable acidity than the healthy roots except in one experiment. It seems probable that the differences in acidity between the healthy and diseased plants were due to a difference in growth.

Roots and tops affected with dwarf had a higher ash content than did healthy plants grown under similar conditions.

Qualitative tests showed that the starch in diseased roots gradually diminishes and that it disappears altogether just before the plant dies.

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(6) ———

(7) ———