Alfalfa Wilt as Influenced by Soil Temperature and Soil Moisture

By Benjamin Koehler and Fred Reuel Jones

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By Benjamin Koehler and Fred Reuel Jones[a]

ALFALFA WILT is a disease that has spread rapidly in Illinois during the last ten years and is now an important factor in limiting alfalfa production. It was first recognized as a bacterial disease in 1924 by the junior author. It was found at this time in Stephenson county in the northern extremity of Illinois. What appears to have been the same disease, however, was observed in Illinois by L. R. Tehon[b] as early as 1921.

A survey by the senior author in 1925 revealed the disease to be generally distributed thruout the state, altho only about 8 percent of the alfalfa fields two or more years old showed infection. No symptoms were found in younger fields at that time. The disease caused serious losses in many fields. Because of its alarming nature and because very little was known about its behavior, it demanded attention. Tho work on the etiology and certain other aspects of the disease was already under way,[7, 8, 9, 15*] it seemed highly desirable to make a study of the relation of the disease to its environment. The present study has been made in an attempt to determine the influence of soil temperature and soil moisture on the development of the disease in wilt-inoculated plants. In addition, records were made of the behavior and composition of apparently healthy control plants grown under the same environmental conditions. The histological work was done at the University of Wisconsin by the junior author.

Characteristics of Alfalfa Wilt

Altho the disease has been called "wilt," the usual symptoms are a blighting and dwarfing of the plants rather than wilting. The stems of infected plants are not only unusually short, but they are also more slender than the stems of healthy plants and sometimes appear to be

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[b]Botanist, Illinois State Natural History Survey, oral statement.

*These numbers refer to literature citations, pages 78 and 79.

greater in number (Fig. 1). The leaves usually are much smaller than normal (Figs. 1 and 2) and are light green. The edges often dry out to a straw color and curl upward. Some plants show these symptoms only to a slight extent or in only one or two stems. With the

FIG. 1.—ABOVEGROUND SYMPTOMS OF ALFALFA WILT

The dwarfed condition of the plant in the foreground is typical of the advanced stage of the bacterial wilt disease caused by *Phytomonas insidiosum.* The plants in the background are normal.

advent of hot weather in the fore part of the season, plants may show wilting or blighting, which is soon followed by death, without first going thru the dwarf stage.

Alfalfa wilt is a disease primarily of the roots. By cutting the roots open the disease can first be detected there by the appearance of a yellow stain beneath the bark in tissue which otherwise is of a white or very pale ivory color. After symptoms appear in the tops, a very decided band of yellow-to-brown wood is found in the roots beneath the bark (Fig. 3). The depth of color and the width of this band vary with the severity of the disease. The discoloration, even at an early stage, extends for a long distance down the root; it is thus distinguished from that near the crown resulting from winter injuries.

Spread of Wilt Infection

In a year-old, healthy appearing stand of alfalfa in which infection has started, plants showing symptoms of wilt in the roots are usually few in number and entirely unsuspected of being infected. In the following year symptoms become evident aboveground, and by the

FIG. 2.—(*A*) Healthy Plant, (*B*) Somewhat Diseased Plant, and (*C*) Severely Diseased Plant

The above sprigs of alfalfa were taken from an old field in autumn. Note the diminutive size and cupping of the leaves in the diseased plant (*C*). The edges of the leaves are dead and have dried to a straw color; the rest of the leaf is a lighter green than normal.

third year considerable loss from the disease is usually experienced. When a new seeding is made in a field where a wilt-infected stand has just been plowed under, the development of the disease may be considerably more rapid.

Infection takes place thru wounds, the bacteria usually being carried from plant to plant in water. Frost cracks which cause little harm in themselves provide excellent openings for the entrance of bacteria. Such cracks develop in most alfalfa fields in an average winter in Illinois, and in a severe winter they are formed very abundantly. After the disease has become established in a field, the bacteria appear to be washed from the openings in the diseased plants to the cracks in the healthy ones, so that wilt disease is likely to be especially destructive after a hard winter.[7, 9*] Apparently the sickle bar also spreads the

disease by transferring bacteria from infected plants to healthy ones in the plant juice.

It has often been observed that the better the stand and the more thrifty the original condition of the alfalfa, the faster the disease spreads over a field after infection has occurred. Wilt infection usually makes very slow advance in thin stands four to six or more years old which contain considerable grass and weeds, tho in the more intensive

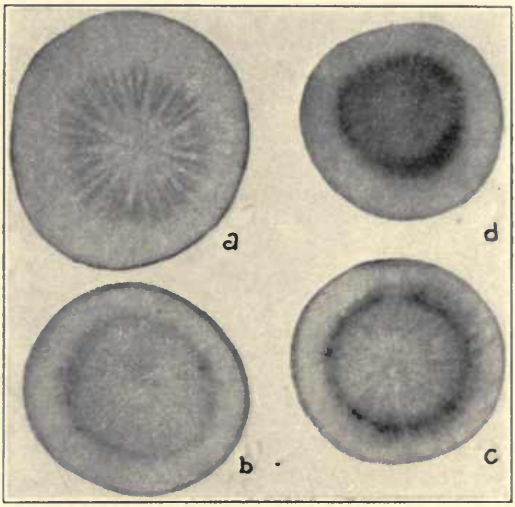

FIG. 3.—SYMPTOMS OF ALFALFA WILT IN CROSS-SECTIONS OF ROOTS
(*a*) Healthy, (*b*) slightly diseased, (*c*) moderately diseased, and (*d*) severely diseased. Enlarged 3 times.

alfalfa-growing regions of the state, such old fields have practically always been found to contain wilt-infected plants. It would be interesting to know when and how wilt infection first entered these fields. Judging from observations of younger, thicker stands which have been completely devastated by the invasion of wilt, these older infected fields would not be expected to have survived if wilt had been introduced while the stand was young and heavy. Possibly such stands were thin and grassy from the start or else infection was introduced after they had reached that condition.

Further light on the above situation was secured from some experiments conducted on the University farm at Urbana. Where alfalfa was sown by itself at the rate of 15 pounds an acre, wilt had destroyed nearly all the plants at the end of three years; but where alfalfa was seeded in adjoining plots at the rate of 7½ pounds an acre as a mix-

ture with timothy in one case and with orchard grass in another, the alfalfa was still in fair condition by the end of the third year, altho the stand here too had been somewhat reduced by wilt.[a]

Present Status of Alfalfa Wilt in Illinois

A limited survey of alfalfa fields in various parts of Illinois, including a considerable number of the more important alfalfa-growing counties, was undertaken in the summer of 1930. Only fields two or

TABLE 1.—PERCENTAGE OF ALFALFA FIELDS TWO OR MORE YEARS OLD INFECTED WITH WILT, AS FOUND IN SURVEY OF 21 COUNTIES IN ILLINOIS, 1930[a]

County	Number of fields examined	Number of fields infected with wilt
Boone	2	1
DeKalb	7	3
Iroquois	8	6
Jersey	7	4
Kane	5	2
Lake	7	4
LaSalle	8	2
Lee	9	7
Livingston	8	4
Madison	16	15
McHenry	8	6
McLean	4	3
Montgomery	7	5
Morgan	3	2
Ogle	5	3
Pike	12	8
Sangamon	4	4
St. Clair	14	8
Vermilion	5	3
Winnebago	3	1
Woodford	5	4
Total number fields	147	95
Percentage infected	...	64.6

[a]L. R. Tehon, Illinois State Natural History Survey, cooperated in making part of this survey.

more years old were considered because the disease is not likely to show much external evidence in younger fields. Examinations were made during and after midsummer in the second or third growth of the season. Experience has shown that the external symptoms are often then more evident than they are in the first growth. Search was made for the dwarfed plants (Fig. 1) and foliage symptoms (Fig. 2) characteristic of the disease. In most cases a portion of the main root of the plant examined was dug up to a depth of about six inches and cut crosswise in order to observe whether the roots also showed the symptoms of the wilt disease (Fig. 3).

The disease was found to be more prevalent in the state than had been anticipated. Of 147 fields two or more years old examined, 64.6

[a]Forage crops experiments conducted by J. J. Pieper.

percent showed wilt infection (Table 1). In some sections apparently all the fields two or more years old were diseased. These conditions were in striking contrast to those observed in the survey made five years earlier, in which only 8 percent of the older fields examined were infected. Altho the first survey was less extensive, involving only about half as many fields, yet the increase in percentage of fields showing wilt infection was at once apparent when the second survey was made. Several severe winters occurred during this five-year period that may have given an unusual impetus to the development of the disease (see page 41). The preceding winter, 1929-30, however, was generally considered favorable for the overwintering of legumes and winter cereals in Illinois.

In some fields only a very small percentage of the plants showed the usual outward symptoms of wilt, and the damage from the disease in these fields at the time of observation was slight. Many fields, on the other hand, showed severe reductions in stand and most of these were no doubt plowed up.

Recovery From Wilt Infection Rare

Recovery from wilt infection seems to occur seldom in Illinois. In some fields showing infection a considerable number of apparently healthy plants, as judged by the foliage, were dug up. Many of these showed signs of the disease in the roots; in most cases the attacks were recent, the yellow band being relatively narrow and appearing in the xylem next to the cambium. In the south-central part of the state, however, a few plants that apparently had recovered were found. These showed a yellow or brown band deeper in the xylem with newer, apparently normal, xylem surrounding it. This condition was the exception rather than the rule.

While a considerable number of wilt-infected plants may die during the summer, observations have shown that a large number also die during the winter. In an alfalfa field on the University farm at Urbana several hundred plants showing various stages of foliage symptoms were marked in the fall of 1927 and in the fall of 1928. In the following spring of each year it was found that none of the marked plants had come thru the winter alive.

It is evident that alfalfa wilt is now well established thruout Illinois and is an important factor in limiting alfalfa production.

Control Measures

In places where alfalfa wilt has not yet been introduced very generally, it is advisable to sow only certified nothern-grown seed, es-

pecially that from the Dakotas, Montana, and Idaho. Surveys in Illinois indicate that the disease has been much slower to establish itself in communities where, in the past, only such seed was used. None of the northern-grown strains, however, show special resistance to the disease where infection has already become established. Grimm and other variegated varieties are no more resistant than hardy common strains.

Because of the slow development of wilt, the disease is not likely to cause trouble where alfalfa is used in a rotation as a short-time crop.[3, 16*] The use of grass in a mixture with alfalfa, as mentioned earlier, may be of value in lengthening the life of the stand. After a wilt-infected alfalfa field is plowed up, it should be planted to other crops for several years before being reseeded to alfalfa.

Wilt-resistant alfalfa varieties discovered during the last few years may prove a means of wilt control.[6, 19, 20, 22*] It now appears that most or all of the strains introduced from Russian Turkestan may be highly wilt-resistant. While hardy enough, most of these strains do not promise to yield satisfactorily under Illinois conditions. Tests of them are now under way, and it is hoped that some good yielders will be found. Seed of resistant strains will probably be commercially available after a few years.

STUDIES ON GROWTH OF ALFALFA AND DEVELOPMENT OF WILT AS AFFECTED BY SOIL TEMPERATURE AND SOIL MOISTURE

Methods Used in Soil Temperature Studies

In this investigation the desired soil temperatures for studying the growth of alfalfa and for studying the development of wilt under controlled conditions were maintained by means of several groups of "Wisconsin tanks"[10, 12*] (Fig. 4). The tanks that were operated at a temperature of 30° C. were located in a greenhouse room kept at 25 to 28° C., those operated at 25° C. were in a room kept at 21 to 24° C., and the tanks operated at 20, 15, and 10° C. were in a room kept at 15 to 18° C. The tanks at the upper three temperatures were operated slightly above room temperature and were electrically heated. The tanks at 15 and 10° C. were operated below room temperature by a mechanism similar to that described by Leukel.[12*] In place of a large ice chest, however, a smaller, well-insulated tank of water kept cold by mechanical refrigeration was used.

The cans in which the plants were grown were 8 inches in diameter and 18 inches deep, of galvanized iron, and painted with an acid-proof

enamel (Probus). There were eight cans of control plants and eight cans of inoculated plants at each temperature in each of the three experiments. In the first experiment ten to fifteen plants were grown per can, but in subsequent experiments the plants were thinned uniformly to eight plants per can at or before the time when the plants were 6 inches high.

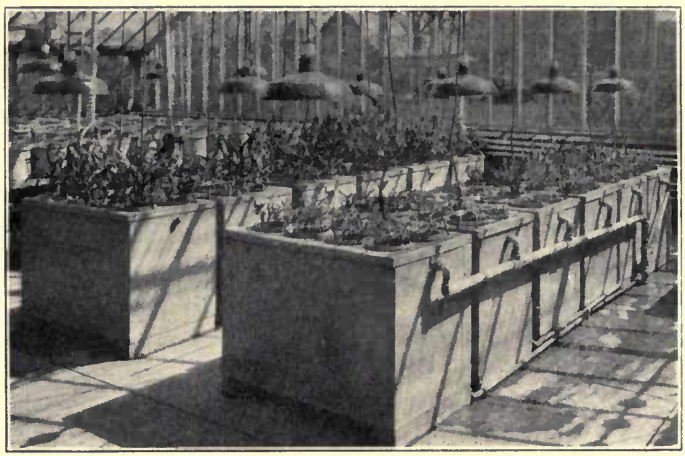

FIG. 4.—SOIL TEMPERATURE CONTROL EQUIPMENT

The six tanks in the group in the foreground were cooled by mechanical refrigeration and were used for the plants grown at 10 and 15° C. soil temperatures. Those in the group in the background were heated electrically and were used for the plants grown at 20° C.

The soil was a dark brown silt loam which had been in sod for more than twenty-five years and which had never been cropped with alfalfa. It had a moisture-holding capacity of 70.4 percent, according to Hilgard's method of determination,[5]* and a moisture equivalent (1,000 times gravity) of 28.1 percent.[2]* The moisture-holding capacity of the soil as compacted into the cans was also determined (see page 56), and the soil moisture was maintained at approximately 60 percent of the moisture-holding capacity as determined by the last-named method. The plants were usually sprayed daily with tap water during the course of the experiments in order to eliminate red-spider injury. This process added some water to the soil; the proper moisture was maintained by weighing three times a week.

During the winter season artificial illumination with 500-watt incandescent lights was used thruout the night. The lights were provided with large reflectors and were placed 30 inches above the tanks and

sufficiently close together to insure even illumination (Fig. 4). As the plants grew in height, the lights were raised somewhat. The plants appeared to grow normally under this illumination, and some that were allowed sufficient time produced seed.

The seed used for all the soil temperature and moisture experiments was of the regional strain known as South Dakota No. 12 (common).

The bacteria producing the wilt disease were introduced directly into the plant by cutting the stems close to the crown with a razor blade dipped in the bacterial suspension. The control plants were cut back in the same manner with a clean blade. This method of inoculation was chosen rather than the application of the bacteria into wounds in the root, presumably the more common method of infection in the field, because it was believed that the latter method involved a study of the effect of environment upon the penetration of the vascular system from such wounds, a problem of less immediate importance and demanding a different procedure in its study. The method of inoculation chosen, while it seemed preferable to that introducing the bacteria into root wounds, was far from satisfactory. From a study previously made of the distance which bacteria may be drawn into cut stems and roots thru the vascular system, it was found that individual stems and plants vary greatly in the amount of inoculum absorbed, and moreover that the distance the bacteria are carried depends largely upon the age and vigor of the stems. Thus the failure to infect all of the plants in these experiments may well be due to the failure to introduce a sufficient quantity of inoculum, as well as to possible differences in resistance in the alfalfa plant population.

Three experiments with variations in temperature were conducted in three successive years. Dates of planting, inoculation, length of experiment, and other details are given in Table 3, page 50.

In Experiments 1 and 2 (first and third years) the plantings were made in the spring and no attempt at temperature control was made until fall. The cans were kept in the greenhouse, and during the heat of summer the glass was whitewashed. In addition to the daily spraying to prevent red-spider injury, nicotine dust was applied when needed to prevent leafhopper injury. The plants were cut off about an inch above the soil when in about the one-fifth bloom stage, and the inoculations were made at that time.

In Experiment 3 (second year) the seed was planted in the fall; temperatures as indicated in Table 3 were maintained from the time of planting, and the plants were cut and inoculated when in the young vegetative stage. Following inoculation, this experiment was carried

on only until the plants reached the one-fifth bloom stage for the first time.

A different procedure in cutting was used in each experiment. In Experiment 1 all plants were grown for the same length of time and all cuttings were made at the same time. At each cutting the plants grown at 30 and 25° C. were in approximately one-fifth bloom, those at 20° C. were just beginning to bloom, those at 15° C. were in the bud stage, while those at 10° C. scarcely showed buds tho the plants were of good size (Fig. 7). In Experiment 2 the plants were again all grown for the same length of time, but all cuttings were made when the plants were in approximately the one-fifth bloom stage. Thus the plants at the two higher temperatures were cut more frequently than those at the lower temperatures. In Experiment 3 the final cuttings were made when the plants reached the one-fifth bloom stage for the first time. This involved different periods of time (Fig. 6).

Each of the above methods presents merits of its own which tend to counterbalance some of the faults of the other methods; by considering all three, therefore, a fairer interpretation of the results can be made. Somewhat different results were obtained by the respective methods, but for the most part the differences were of degree rather than of kind.

In each experiment oven-dry weights were made of the forage removed at each cutting and of the roots after the last cutting. The drying temperature used was about 90° C.

Influence of Soil Temperature on Growth of Alfalfa

The range of temperature in these experiments was limited to that generally prevailing during the growing season in central Illinois, where the average maximum soil temperature is less than 30° C. and the mean daily temperature reaches the 25° C. mark during only a few weeks in the summer (Fig. 5).[17]* It is well known that common alfalfa (*Medicago sativa* L.) is adapted to wide differences in temperature. In North America it is grown all the way from Canada to Mexico.

The percentage of germination in the experiments was nearly equal at all temperatures thruout the range used, altho emergence, as might be expected, was slower at 10° C. than at the intermediate or higher temperatures. The length of time required for the plants to attain the one-fifth bloom stage after cutting varied with the procedure. In Experiment 3, in which the respective temperatures were controlled thruout the test, the time ranged from 58 to 85 days following the early cutting. But when all plants had developed under the same conditions until the one-fifth bloom stage had been reached for the first time and

temperature control was then started, as in Experiment 2, the time required to attain the one-fifth bloom stage ranged from 33 to 50 days respectively from high to low temperature. The roots being already established and carrying food reserves, the growth of tops in this case was much faster, altho the difference in the relative rate of develop-

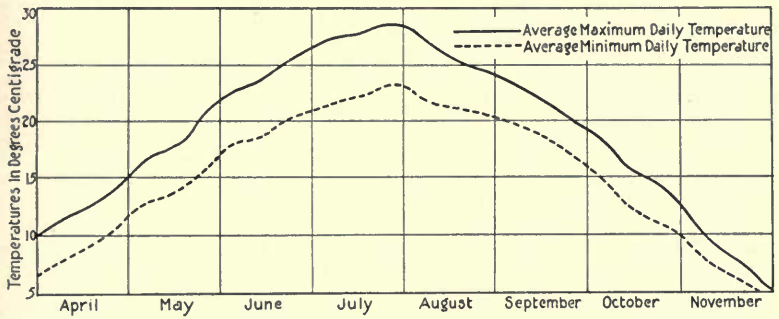

FIG. 5.—TEN-YEAR AVERAGE OF MAXIMUM AND MINIMUM SOIL TEMPERATURES, APRIL TO NOVEMBER, 1918-1927, AT URBANA, ILLINOIS

The temperatures shown above were taken at a depth of six inches. The soil was a dark brown silt loam and was kept free from vegetation.

ment at the extreme temperatures was still fully as great as in Experiment 3. It must be borne in mind that the air temperature no doubt also has an important direct influence on the aerial growth and an indirect influence on the root growth. The range in air temperature in

TABLE 2.—WEIGHTS OF ROOTS AND FORAGE OF ALFALFA PLANTS GROWN AT FIVE DIFFERENT SOIL TEMPERATURES, UNINOCULATED AND INOCULATED WITH *Phytomonas insidiosum*

Soil temperature		Mean oven dry weights of roots and forage per can				Ratio of roots to forage	
		Experiment 2[a]		Experiment 3[a]		Experiment 2	Experiment 3
		Roots	Forage	Roots	Forage		
		Control plants					
°C	°F	gms.	gms.	gms.	gms.		
10	50	8.38	2.99	4.43	5.39	2.80 : 1	.82 : 1
15	59	9.26	3.69	5.33	6.43	2.51 : 1	.83 : 1
20	68	10.89	5.04	6.04	6.85	2.16 : 1	.88 : 1
25	77	7.73	4.28	4.42	5.63	1.81 : 1	.79 : 1
30	86	5.51	3.28	2.29	3.91	1.68 : 1	.59 : 1
		Inoculated plants					
10	50	7.96	2.97	3.12	4.84	2.68 : 1	.65 : 1
15	59	9.06	3.53	3.66	6.34	2.56 : 1	.58 : 1
20	68	7.75	4.98	3.46	6.53	1.55 : 1	.53 : 1
25	77	4.34	3.84	1.01	3.22	1.13 : 1	.31 : 1
30	86	1.85	2.54	.31	1.13	.73 : 1	.27 : 1

[a]Weights of forage are for the last cutting. For other data concerning the conditions of these experiments see Table 3.

TABLE 3.—EFFECT OF SOIL TEMPERATURE AND MOISTURE CONDITIONS ON THE DEVELOPMENT OF ALFALFA WILT WHEN PLANTS WERE ARTIFICIALLY INOCULATED WITH *Phytomonas insidiosum*

Experiment No.	Date				Age of plants			Temperature		Soil moisture	Crops of forage produced after inoculating	Wilt infection		
	Seed planted	Artificial illumination added	Inoculations made	End of experiment	Soil temperature control started	Inoculations made	End of experiment	Air	Soil			Root symptoms	Foliage symptoms	Plants killed by wilt
Effect of temperature on development of wilt														
					days	*days*	*days*	*°C*	*°C*	*perct.*	*number*	*perct.*	*perct.*	*perct.*
1.....	5–15–26	12–3–26	12–3–26	4–18–27	200	202	338	15–18	10	60	4	19.4	0	0
								15–18	15	60	4	74.6	43.7	21.3
								15–18	20	60	4	85.1	67.3	49.2
								21–24	25	60	4	90.5	76.4	64.3
								25–28	30	60	4	93.4	88.4	81.8
2.....	5–3–28	12–10–28	1–30–29	5–10–29	219	275	375	15–18	10	60	2	6.2	0	0
								15–18	15	60	2	9.4	6.3	1.6
								15–18	20	60	2	51.3	29.0	18.4
								21–24	25	60	3	74.1	51.8	33.3
								25–28	30	60	3	85.7	73.5	57.1
3.....	10–15–27	11–15–27	1–14–28	4–8–28	0	91	176	15–18	10	60	1	66.2	33.8	0
				3–7–28	0	91	164	15–18	15	60	1	83.8	43.8	2.5
				3–21–28	0	91	158	15–18	20	60	1	95.0	46.3	17.5
				3–12–28	0	91	149	21–24	25	60	1	98.7	71.2	48.7
				3–12–28	0	91	149	21–24	30	60	1	100.0	91.3	73.8
Effect of soil moisture on development of wilt														
4.....	10–14–27	11–15–27	1–14–28	4–27–28	...	92	196	15–18	15–18	35	1	78.3	63.3	33.3
										50	1	86.7	76.6	43.3
										65	1	91.6	88.3	36.7
										80	1	98.3	88.3	31.7
5.....	5–2–28	12–10–28	1–30–29	6–1–29	...	276	398	21–24	21–24	35	3	18.7	9.4	3.1
										50	3	50.0	34.4	12.5
										65	3	83.3	66.7	33.3
										80	3	81.5	62.9	29.6

all of the experiments (Table 3) was not so wide as the range in soil temperature.

In both Experiments 2 and 3 the highest dry weight of forage per cutting and the highest dry weight of roots in the control plants (plants uninoculated with *Phytomonas insidiosum*) at the conclusion of each experiment were obtained at 20° C. Data for the last cutting

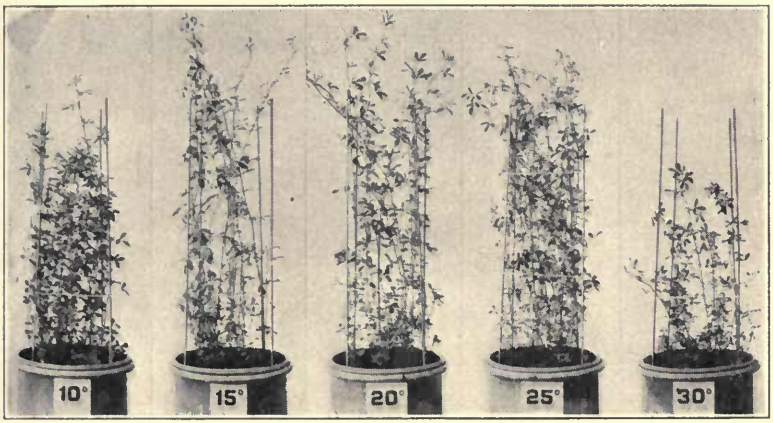

FIG. 6.—HEALTHY ALFALFA PLANTS GROWN TO THE ONE-FIFTH BLOOM STAGE AT DIFFERENT SOIL AND AIR TEMPERATURES

Representative cans of plants from Experiment 3 are shown above. The soil temperatures indicated were maintained from the time the seed was planted. The ages of the plants were respectively from left to right, 176, 164, 158, 149, and 149 days. In the same order the average height of all plants in the eight replicated cans at each temperature was 46.9, 61.8, 64.9, 60.4, and 29.3 centimeters.

are shown in Table 2. Furthermore, growth of roots and forage, as judged by dry weight, was considerably better at the lower extreme of 10° C. than at the upper extreme of 30° C. A comparison of the sizes of the plants grown at the various temperatures is shown in Figs. 6 and 7, *A*.

The ratio of roots to forage, in the case both of uninoculated and of wilt-inoculated plants, was much higher in the plants grown under cooler temperatures than with those grown at the upper temperatures (Table 2 and Fig. 8). The weights given are from the final cutting, so that the effect of the inoculations on the production of forage can be seen. As for the controls, the weights of all cuttings at each temperature were very similar. In Experiment 2 all plants were grown for the same length of time, but as blossoming occurs sooner at warm temperatures than at cool temperatures, those at the warm tempera-

tures were cut one more time than those at the cool temperatures (Table 3). If the total weights of all cuttings were considered, the differences in ratios with respect to temperature would be still greater.

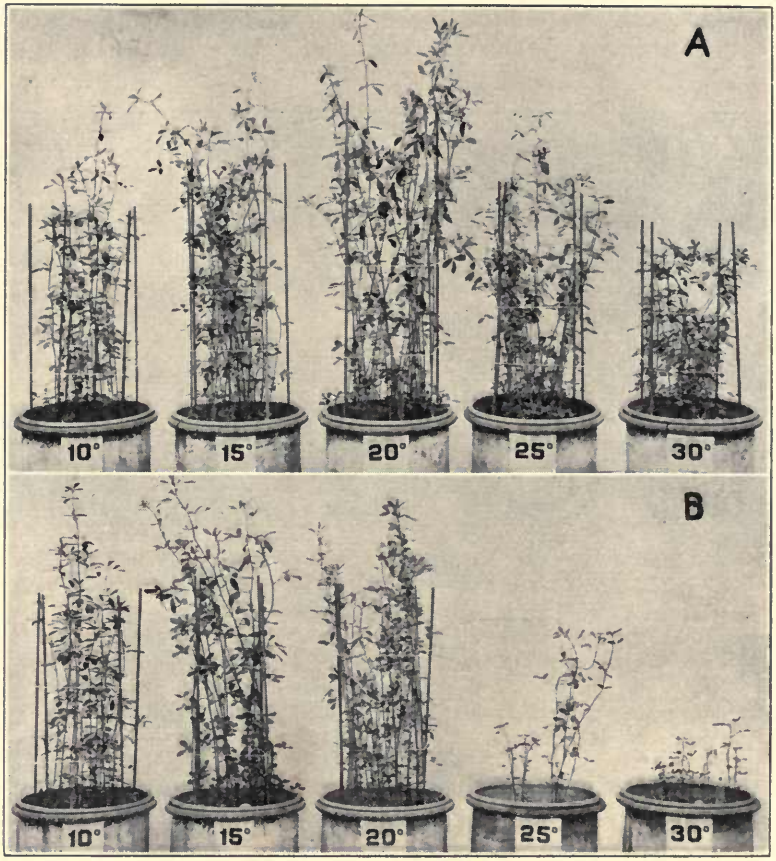

FIG. 7.—REPRESENTATIVE CANS OF ALFALFA PLANTS OF EXPERIMENT 1 GROWN AT TEMPERATURES INDICATED

(*A*) Control, (*B*) inoculated with *Phytomonas insidiosum.* All plants were of the same age and ready for the fourth cutting. The development ranged from buds scarcely showing at 10° C. to one-fifth bloom at 20 and 30° C. Maximum vegetative growth probably had been nearly attained at all the temperatures. Note the severe effect of the disease at the higher temperatures.

In Experiment 3 all plants were grown to the same stage of blossoming. At the different temperatures different lengths of time were required to attain this stage (Table 3). The two experiments (Nos. 2 and 3) thus involve two different methods, but in both cases the

ratios of roots to forage follow the same trend in response to differences in temperature. It thus appears that root growth is more rapid under moderate or cool weather conditions than during the heat of summer. Not only was the ratio of roots to tops highest at 10° C. in these experiments, but the percentage of root reserves was also highest at that temperature (see page 72).

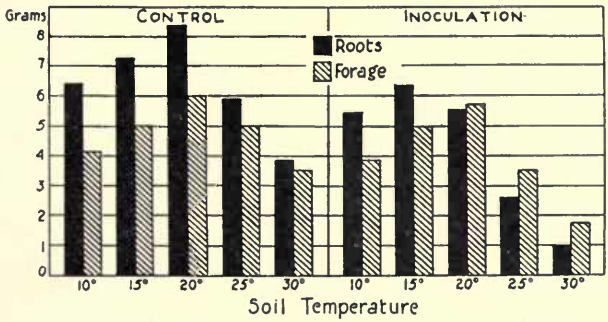

FIG. 8.—EFFECT OF SOIL TEMPERATURE ON OVEN-DRY WEIGHTS OF ALFALFA ROOTS AND FORAGE

These weights are an average of the weights obtained in Experiments 2 and 3 (Table 2). There were eight plants in each can of controls, but the surviving plants in the inoculated cans varied in number. Temperature affects not only the total amount of growth but also the relative amounts of roots and forage.

In plants other than alfalfa it has frequently been found, and appears to be the rule, that the ratio of root growth to top growth is higher at low temperatures than at warmer temperatures.

Influence of Soil Temperature on Development of Wilt

The symptoms of wilt observed in the alfalfa plants grown in the greenhouse were very similar to those observed in the field plants (Figs. 1, 2, and 3 on pages 40, 41, and 42 respectively).

Notes on the appearance of infected plants in the greenhouse were made periodically. At the end of the experiment the roots of the remaining plants were taken up, washed clean of soil, cut open, and examined for signs of wilt disease. Usually a number of inoculated plants which had not shown symptoms in the top growth did show symptoms in the roots. In Experiment 1 some of the control plants showed root symptoms of wilt when harvested. This condition evidently had been caused by the transfer of bacteria from inoculated plants to the controls by the scissors used in cutting back the tops. In Experiments 2 and 3 separate instruments were used for cutting

the control and inoculated plants, and no further evidence of infection in the controls was found.

Wilt symptoms first show in the roots, then in the foliage, and finally the plants die. The extent to which these three features developed in the experiments under discussion, together with certain data concerning the conditions of each experiment, are shown in Table 3, page 50.

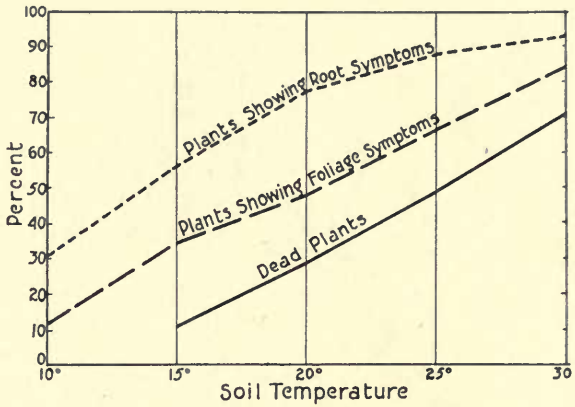

FIG. 9.—EFFECT OF SOIL TEMPERATURE ON THREE ASPECTS OF ALFALFA WILT DISEASE

At a temperature of 30° C. these three aspects of the disease—root symptoms, foliage symptoms, and dead plants—developed in close sequence. The above graph shows the average of the final results from Experiments 1, 2, and 3.

No plants died from wilt at 10° C. in any of the three experiments, and no infections as revealed by foliage symptoms occurred at 10° C. in Experiments 1 and 2. A relatively low percentage of plants at that temperature in each experiment did show wilt symptoms, however, when the roots were examined. The percentage of plants showing symptoms of disease increased rapidly with increases in temperature. The appearance of the plants in representative cans of Experiment 1 is shown in Fig. 7, page 52.

A summary of the extent to which the three aspects of wilt—root symptoms, foliage symptoms, and death—developed at the five temperatures used is charted in Fig. 9. The three curves show that the disease proceeds slowly at low temperatures, while at high temperatures it progresses rapidly, soon ending in the death of the plants.

When inoculations of wilt bacteria were made in young plants, the effect was more severe than when made in older plants. External evidence of wilt occurred as early as 14 days after inoculation in young

plants grown at 30° C. (Fig. 10, Experiment 3). In older plants which had already reached the blooming stage and were cut before inoculating, evidences of wilt did not appear for 22 to 25 days after inoculation (Fig. 10, Experiments 1 and 2). As seen in Fig. 10, the dis-

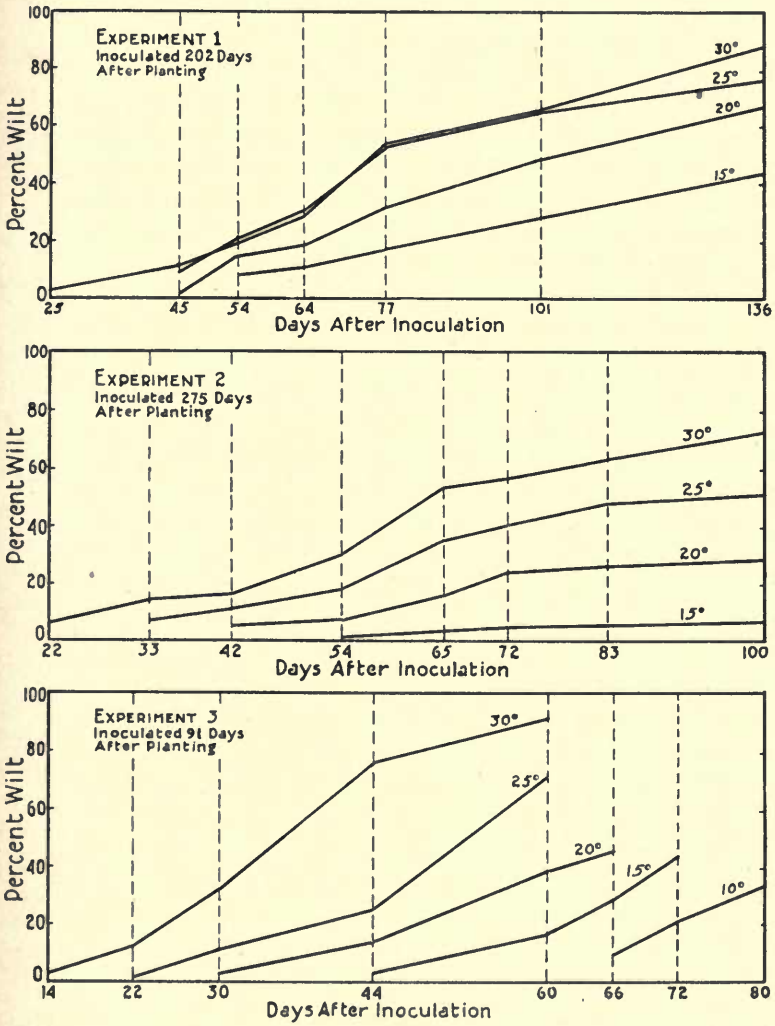

FIG. 10.—INCREASE IN PERCENTAGE OF PLANTS SHOWING WILT SYMPTOMS IN FOLIAGE WITH INCREASE IN SOIL TEMPERATURE

Several cuttings were made after inoculating and before the end of Experiments 1 and 2, but in Experiment 3 the plants at each temperature were harvested when they reached the one-fifth bloom stage. In each experiment wilt increased with increase in temperature within the experimental range used.

ease progressed more rapidly in the young plants of Experiment 3 than in the older plants of the other two experiments. Wilt symptoms occurred in the top growth of the plants maintained at 10° C. in Experiment 3 in 66 days, while no wilt symptoms appeared at this temperature in the older plants of Experiments 1 and 2 even after 136 and 100 days respectively.

Root growth is poorer in infected plants than one would judge it to be by the appearance of the top growth. This fact is shown by the weights and ratios given in Table 2. The effect of disease on root growth is least evident at low temperatures, at which the disease makes slow progress, but becomes more pronounced at medium and high temperatures. Thus root growth in infected plants is especially poor during the hot weather of summer. The consequent prevention of storage of adequate root reserves may be responsible in part for the high mortality from the disease during the winter.

Methods Used in Soil Moisture Studies

The soil used in the soil moisture studies was of the same kind as that used in the temperature studies.

The cans were likewise of galvanized iron painted with acid-proof enamel and were 12 inches wide and 24 inches high. There were four cans of control plants and four cans of inoculated plants at each degree of soil moisture in each experiment. Several small drain holes were punched in the bottom of each can. The soil was tamped into the cans with a 10-pound tamper at every 2 or 3 inches.

Three extra cans were filled, in the same manner as described above, for making a moisture-holding (maximum capillary capacity) determination under the soil conditions of the experiment. These cans were flooded with water repeatedly until water flowed from the drain holes at the bottom. The cans were then covered tightly and were allowed to drain for four days. Moisture determinations of the soil were then made at various depths, and it was found that the moisture-holding capacity in this case was about 44.5 percent, expressed in terms of dry weight of soil, as compared with 70.4 percent when determined with a 1-centimeter-high cup according to the Hilgard method.

The length of time the cans were drained, four days, was chosen arbitrarily. The amount of water draining from the bottoms of the cans decreased from day to day and dripping had nearly ceased on the fourth day. The moisture condition thruout the cans at that time was believed to be very like that in a capillary column immediately above the water table. Later tests showed that water would continue to drain from such cans for a long time; at the end of six months the moisture

percentage had dropped considerably and approached that occurring at the upper end of a capillary column, or "the field-carrying capacity," as the term is used by Shantz.[23*]

The moisture-holding capacity having been determined, water was added to the top of the soil in the cans in order to have eight cans each at 50, 65, and 80 percent of the moisture-holding capacity. Another portion of the soil was spread out to a thin layer and stirred frequently until it had dried to 35 percent of the moisture-holding capacity. Eight cans were filled and compacted with this soil.

It has been shown that soil moistures below the "field-carrying capacity," or minimum capillary capacity, cannot be uniformly maintained by adding water to replenish that lost by evaporation or transpiration.[23, 25*] In preliminary tests the field-carrying capacity of the soil as placed in the above cans was determined at 45 to 50 percent

TABLE 4.—SOIL MOISTURES OCCURRING AT DIFFERENT DEPTHS IN EXPERIMENT 5

(Expressed in percentage of moisture-holding capacity)

Intended moisture	Moistures as they occurred at different depths below the surface					
	2 in.	6 in.	10 in.	14 in.	18 in.	22 in.
perct.	*perct.*	*perct.*	*perct.*	*perct.*	*perct.*	*perct.*
35[a]	34.4	36.7	35.1	36.5	32.8	32.0
35[b]	56.5	54.1	50.5	46.3	40.2	32.2
50	54.9	51.9	50.1	45.3	44.5	44.2
65	71.3	64.5	65.8	62.3	55.6	55.8
80	81.5	79.7	78.2	79.0	79.0	78.5

[a]Moisture determinations made 30 days after watering when weight of soil indicated that soil moisture was down to 35 percent.

[b]Moisture determinations made 2 days after attempting to add sufficient water to percolate to bottom of can.

of the moisture-holding capacity. Therefore, when water was added to the 35-percent cans, a considerable quantity, sufficient to raise the moisture up to 45 percent, was used in order that the water would pass uniformly all the way down. Actually, as shown in Table 4, the moisture did not pass quite all the way down, for the lower few inches in the cans remained permanently dry. While these cans are called 35 percent in the text, the moisture actually fluctuated between 50 and 35 percent. This fact should be borne in mind whenever the lower moisture content of 35 percent appears in this bulletin. The time intervals between waterings in this low-moisture series varied with conditions, the average interval being 30 days, tho at the higher levels the original weight was restored by watering three times a week.

One whole moisture series had to be discarded because water-tight cans were used. It was at first thought that soil water at less than the moisture-holding capacity would remain evenly distributed in the soil.

At 80 percent of the moisture-holding capacity it did not do so but passed downward by gravity. This downward movement is slow, and if some fast-growing crops were grown in the cans so that the roots would grow to the bottom before the bottom soil became saturated, the factor of distribution probably could be ignored because the upward movement of water by root absorption would be faster than the downward movement by gravity. But alfalfa plants grow slowly in the early stage. When borings were made at the end of four months, the upper part of the soil was found to be fairly dry while the lower one-fourth was saturated. On further examination it was found that the alfalfa roots were located only in the drier soil and would not penetrate the saturated portion. The same conditions were also noticeable, but to a less extent, in the 65-percent cans. Having openings at the bottoms of the cans overcomes this difficulty because the slow downward seepage drains away and is replaced by the addition of water at the top. After the soil is permeated with roots, there probably is no longer any seepage under the conditions of the experiments here described. It is evident also that the addition of water at any place, except the top of the soil, would not be advisable, at least for the higher soil moisture.

No surface mulch was used on the soil in any of the cans. The daily sprayings already referred to kept the soil surface damp during part of the day. Furthermore it has been demonstrated that loss of water from the soil surface by evaporation is very slight[25*] compared with that lost by transpiration by plants growing therein. The shade from the plants and the daily sprayings usually prevented a surface crust from developing on the soil.

As it seemed best in these experiments to add the water directly to the soil surface, none of the special watering devices, such as the auto-irrigator,[14*] potometer,[11*] or inverted flower pot beneath the soil surface with a tube leading down to it, were used. Furthermore none of these devices works at moistures below the field-carrying capacity.

The cans in both Experiments 4 and 5 were placed on greenhouse benches. Experiment 4 was conducted at a soil and air temperature of 15 to 18° C. Experiment 5 was conducted at an average greenhouse temperature of 21 to 24° C., tho the temperatures often went higher during the summer months before inoculations of wilt bacteria were made and before an attempt at temperature control was started. Data on time of planting, inoculation, harvest, and artificial illumination are given in Table 3, page 50.

After inoculation, the plants in Experiment 4 reached the one-fifth bloom stage and were cut only once before the end of the experiment; in Experiment 5 the plants, after the second inoculation,

reached the one-fifth bloom stage three times before the experiment was concluded.

Influence of Soil Moisture on Growth of Alfalfa

In the soil moisture experiments when soil was maintained at 65 percent of its moisture-holding capacity, the highest dry weight of both roots and forage was attained in the control, or uninoculated, plants (Table 5 and Fig. 11).

TABLE 5.—WEIGHTS OF ROOTS AND FORAGE OF ALFALFA PLANTS GROWN UNDER FOUR DIFFERENT CONDITIONS OF SOIL MOISTURE, UNINOCULATED AND INOCULATED WITH *Phytomonas insidiosum*

Soil moisture	Mean oven-dry weights of roots and forage per can				Ratio of roots to forage	
	Experiment 4[a]		Experiment 5[a]		Experiment 4	Experiment 5
	Roots	Forage	Roots	Forage		
Control plants						
perct.	*gms.*	*gms.*	*gms.*	*gms.*		
35[b]	7.59	3.80	7.95	3.90	1.99 : 1	2.04 : 1
50	11.21	6.02	11.48	5.48	1.86 : 1	2.09 : 1
65	12.21	6.23	11.63	7.94	1.96 : 1	1.46 : 1
80	10.08	5.52	7.65	7.20	1.83 : 1	1.06 : 1
Inoculated plants						
35[b]	3.32	3.13	7.05	3.81	1.06 : 1	1.85 : 1
50	5.43	5.56	9.18	4.63	.98 : 1	1.98 : 1
65	5.03	5.78	5.63	4.68	.87 : 1	1.20 : 1
80	3.96	4.72	3.80	4.37	.84 : 1	.87 : 1

[a]Weights of forage are for last cutting. For additional data concerning conditions of these experiments see Table 3, page 50.

[b]While given as 35 percent, the moisture actually varied from 50 to 35 percent of the moisture-holding capacity. See text page 57.

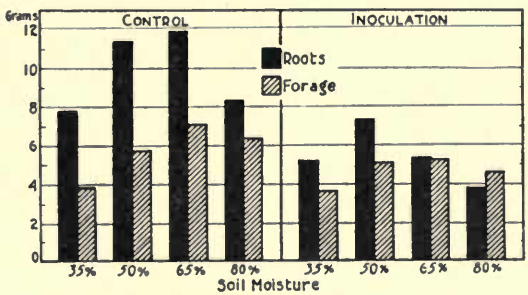

FIG. 11.—EFFECT OF SOIL MOISTURE ON OVEN-DRY WEIGHT OF ALFALFA ROOTS AND FORAGE

These weights are an average of the weights obtained in Experiments 4 and 5 (Table 5). The reduction in dry weight caused by inoculation with the wilt bacterium was caused in part by a reduction in stand (Table 3). The lower ratio of weight of roots to forage in the inoculated group, however, would not be affected by differences in stand.

The appearance of representative groups of the plants under the four moisture conditions of Experiment 5 is shown in Fig. 12, *A*. The average heights of the plants were 26.2, 34.7, 41.5, and 42.2 centimeters respectively at 35–, 50–, 65–, and 80–percent soil moistures.

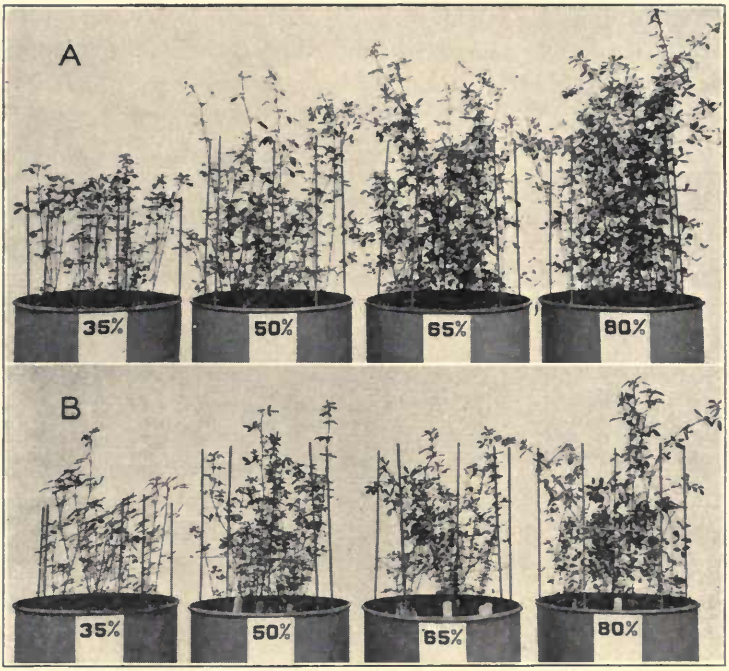

FIG. 12.—REPRESENTATIVE CANS OF ALFALFA PLANTS IN EXPERIMENT 5 GROWN UNDER FOUR DIFFERENT SOIL MOISTURE CONDITIONS

(*A*) Control, (*B*) inoculated with *Phytomonas insidiosum*. All plants were of the same age and ready for the final cutting. While the plants at 80-percent soil moisture (controls) appear to have made the best growth, those at 65-percent moisture were found to have the greatest dry weight (Table 7). The disease was least active at 35-percent soil moisture.

While the plants growing at 80-percent soil moisture were slightly the tallest and gave the general appearance of being the most thrifty, yet the dry weight of these plants fell considerably below that of the plants growing at 65-percent soil moisture. Richardson[21]* found that the lowest water requirement of alfalfa in his tests occurred at a soil moisture of 60-percent saturation, the requirement being higher at either higher or lower degrees of soil moisture. Perhaps this might be considered close to the optimum conditions for the growth of alfalfa so far as soil moisture is concerned.

The ratio of roots to forage in the control plants was higher at low moistures than at high moistures (Table 5). This situation was evident in Experiment 4 and very pronounced in Experiment 5. Richardson[21]* found a similar relationship in his experiments. It has sometimes been observed that alfalfa growing in moist soil does not overwinter so well as that on better drained soil. A lack of root development with consequent lack of food reserves may be one factor involved.

Influence of Soil Moisture on Development of Wilt

In examining alfalfa fields for the occurrence of wilt infection, it has often been observed that infected plants are most numerous along ditches or in the lower parts of the field. This condition probably is caused to a considerable extent by drainage water carrying the bacteria and concentrating them in these areas, tho the data obtained from the experiments under discussion show that the plants are also more subject to infection where the soil is moist.

The plants in the greenhouse experiments grown at 35-percent moisture showed a lower percentage of wilt infection than those grown at 50-percent moisture; the maximum infection occurred at a moisture of 65 to 80 percent (Table 3). On the whole, higher percentages of infection occurred in Experiment 4 than in Experiment 5 (Table 3). This situation is probably accounted for, at least in part, by the fact that the plants were much younger when inoculated with the wilt bacteria in Experiment 4 (about 6 inches tall) than they were when inoculated in Experiment 5 (one-fifth bloom). A similar condition has already been mentioned in the discussion of the experiments conducted under controlled soil temperatures (page 55). Natural infections under field conditions probably are rare in young plants, and so the results from Experiment 5 probably simulate more closely naturally occurring infections.

Altho the percentage of infected plants in Experiment 4 was considerably influenced by the soil moisture conditions, the percentage of killed plants was not influenced to the same extent. The plants remained alive longer at high soil moisture because in spite of decayed roots they were apparently able to obtain moisture longer. Had Experiment 4 been carried as far as Experiment 5, the relative proportion of dead plants probably would have changed somewhat.

A summary of the extent to which the three aspects of alfalfa wilt —root symptoms, foliage symptoms, and death—have taken place at the four moistures used is charted in Fig. 13.

The results from these experiments are in accord with the findings

of Peltier and Jensen.[19*] In summarizing their studies made under field conditions they state: "Stand reductions in Nebraska alfalfa fields, due to wilt irrespective of other factors, have been found to vary from most to least rapid in the following sequence: under irrigation, under subirrigation, on eastern uplands, and on the western tablelands." In other words, it appears that the severity of wilt varies with the abundance of soil moisture.

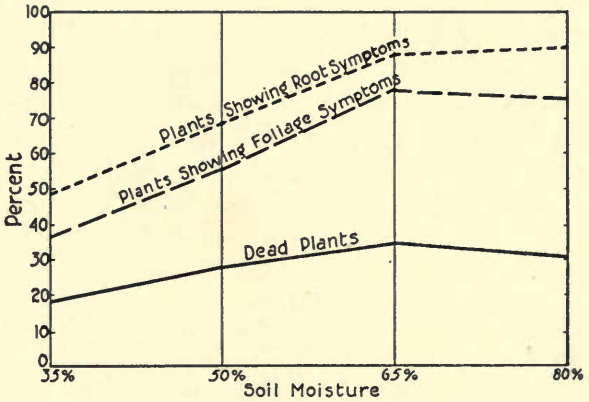

FIG. 13.—EFFECT OF SOIL MOISTURE ON THREE ASPECTS OF ALFALFA WILT

These three aspects of the disease—root symptoms, foliage symptoms, and dead plants—were least evident at 35-percent soil moisture, but the first two—root and foliage symptoms—mounted rapidly with increase in moisture up to 65 percent. The graph shows an average of the final results from Experiments 4 and 5.

From the data reported in Table 5 and Fig. 11 it is apparent that the ratios of roots to forage are considerably higher in the control plants than in the inoculated plants. Thus, as was observed in connection with the soil temperature studies, wilt-infected plants really are in much worse condition than one would judge from their above-ground appearance.

MODIFICATIONS IN ROOT STRUCTURE CAUSED BY VARIOUS ENVIRONMENTAL FACTORS

Certain histological studies were made as a part of these experiments in order to observe the growth response of the roots to changes in environment more closely than could be done in the general studies reported above. Numerous microscopic sections of roots from uninoculated and from wilt-inoculated plants were examined; their cell

structure was observed and the relative proportions of different types of tissue produced under different environmental conditions determined.

These experiments were not originally designed to study the effect of illumination on root structure. However, a cursory examination of root sections showed the most striking structural modifications to occur as the result of illumination, so that this phase of the subject is reported here, as well as the effect of temperature and moisture conditions.

Effect of Period of Illumination

The coincidence of the development of large vessels in the roots with tall tops under illumination has been observed repeatedly by the junior author in other unreported experiments with alfalfa in which artificial light was used. This relationship was again observed in the experiments reported in this bulletin. It was found that the small vessels characteristic of autumn are produced in short days even tho the stems produced in the summer remain attached, as in these experiments. Thus the length of the period of illumination affects not only the development of the shoot, as first described by Oakley and Westover,[18*] but the root structure as well.

The plants grown in the greenhouse until winter from seed sown in the spring (Experiments 1, 2, and 5) developed, before inoculation, zones of xylem having the very small vessels characteristic of autumn wood in plants growing in the field. This zone was abruptly terminated when illumination was supplied after inoculation.

The autumn wood formed a wide band in the roots of the plants in Experiment 1 and a narrower band in the roots in Experiments 2 and 5, while autumn wood was absent from the roots of the plants which were artificially illuminated from an early stage onward, as those in Experiments 3 and 4. The large vessels may push surrounding cells out of the position in which they were laid down by the cambium, producing cell arrangements characteristic of summer wood and very different from those in autumn wood; but inasmuch as these differences are not of demonstrated importance in the development of the disease in these experiments, they will not be described here.

That the cutting of the plants at inoculation had nothing to do with the character of subsequent tissue is indicated by the observed behavior of plants elsewhere. In a large bed of seedlings propagated in a greenhouse at Madison, Wisconsin, in the winter of 1929-30 rows of a strain of Provence alfalfa and of Grimm were grown side by side. On February 3 the plants of Provence had grown to a height of 6 inches while the stems of Grimm were hardly an inch high. A segment of the root

structure of representative plants of these varieties at this date is shown in Fig. 14, *A* and *B*. Shortly after this date lights used in an adjoining greenhouse illuminated these plants sufficiently to cause the Grimm plants to shoot to a height of 6 to 8 inches by February 21. The structure of a root of a small Grimm plant after the elongation

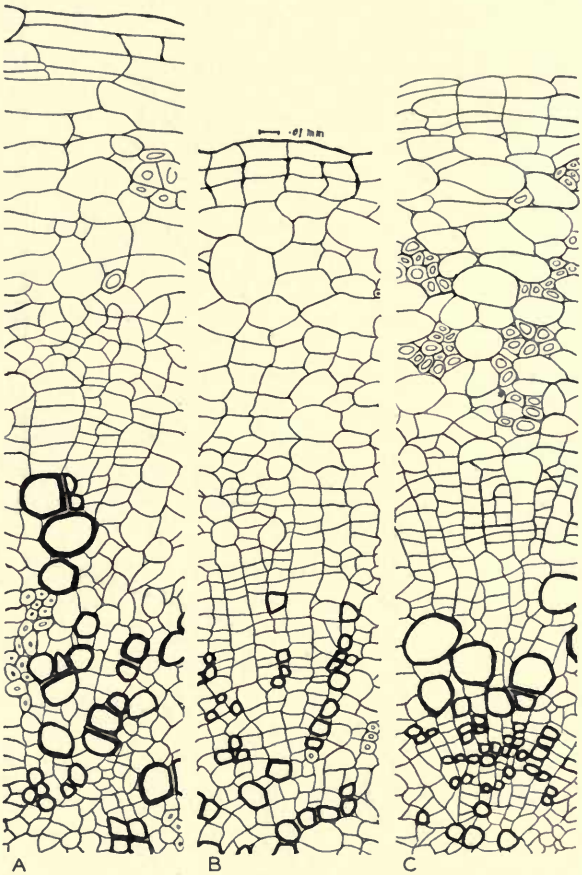

FIG. 14.—EFFECT OF PERIOD OF ILLUMINATION ON STRUCTURE OF ALFALFA ROOTS

A and *B* were drawn from representative roots of Provence and Grimm alfalfa respectively, taken February 3, 1930, the date on which artificial illumination was supplied and 84 days after seeding. At this time the Provence alfalfa had shoots 6 inches high while the stems of Grimm were scarcely an inch in height. The vessels in the roots of the tall Provence were wide while those of the short Grimm were narrow. With artificial illumination the Grimm stems elongated until on February 21 they stood 6 to 8 inches high. A cross-section of a small Grimm root at the latter date (*C*) shows that simultaneously with the elongation of the stem wide vessels were produced.

of the stem is shown in Fig. 14, *C*. From a comparison of these drawings it is apparent that while the Provence alfalfa, which was able to produce long internodes in the short days of February, had vessels of large diameter, the Grimm plants had vessels of comparatively small diameter so long as the internodes were short but they produced large vessels as soon as the internodes elongated under the influence of light.

Effect of Soil Temperature on Vascular Structure of Roots

Examination of sections of roots from these experiments has failed to discover striking differences in size of vessels or character of tissue produced, but it did reveal differences in the relative proportions of the structural elements that could be ascribed to the differences in

TABLE 6.—CROSS-SECTIONAL AREAS OF VASCULAR BUNDLES AND WOOD RAYS IN UPPER PART OF A NUMBER OF INDIVIDUAL ROOTS OF ALFALFA PLANTS GROWN AT CONSTANT SOIL TEMPERATURES

(Expressed in arbitrary units and in ratios of averages of these areas at each temperature)

Temperature	Experiment 2			Experiment 3		
	Area of xylem	Area of wood ray	Ratio of xylem to wood ray	Area of xylem	Area of wood ray	Ratio of xylem to wood ray
10° C.	83	81		51	36	
	74	61		42	34	
	63	66		39	16	
	58	45		24	11	1.62 : 1
	67	57				
	105	50	1.25 : 1			
15° C.	72	48		54	32	
	93	55		34	21	
	87	47		52	26	
	100	81		52	31	1.77 : 1
	72	86				
	79	37	1.42 : 1			
20° C.	113	92				
	61	61				
	106	78				
	112	70				
	63	51				
	68	47	1.31 : 1			
25° C.	118	93		71	76	
	105	65		112	55	
	69	43		62	63	
	105	66		65	45	1.31 : 1
	58	29				
	72	48	1.54 : 1			
30° C.	73	71		81	47	
	66	58		49	12	
	99	35		83	40	
	75	23		35	21	2.06 : 1
	49	27				
	135	50	1.88 : 1			

temperature at which the plants were grown. For instance, the roots grown at 30° C. appeared to be more woody, or to have a larger proportion of vascular tissue in comparison with ray tissue, than roots grown at intermediate and low temperatures.

This observation was tested by comparing the areas of vascular bundles and rays in cross-sections in the plants from Experiments 2 and 3. The comparison was made by drawing half the cross-section of each root, greatly enlarged by camera lucida, on uniform paper and marking the boundaries between rows and bundles. The areas representing rays were cut out and weights of paper corresponding to rays and bundles were compared. The weights and ratios are given in Table 6. Altho the relative amounts of ray and xylem in the individual plants at the same temperature differed greatly, and the number of plants at each temperature was too small to give accurate comparisons, nevertheless even in Experiment 2, in which the plants were grown but a part of the time at controlled temperatures, the average proportion of ray was highest at 10° C. and lowest at 30° C. The figures leave little doubt that the root produces a relatively high proportion of storage tissue when growing at a low temperature.

At the highest temperature the amount of phloem seemed to be proportionally smaller than the amount of xylem, or in other words the bark of the root seemed comparatively thin. Since no easily applied method of measurement was at hand whereby this difference might be tested, and since this and other suspected differences seemed to have little or no bearing on the behavior of disease in the plant, it was not studied further.

Effect of Soil Moisture on Vascular Structure of Roots

An examination of sections of roots from Experiments 4 and 5 for differences ascribable to differences in soil moisture disclosed that, in general, the plants grown at 50- and 65-percent moisture were very similar in structure. Those grown at the higher soil moisture, 80 percent, differed considerably from those grown at 50- and 65-percent moistures, while those grown at the lower soil moisture, 35 percent, showed less difference. Wide variations in individual roots grown at the same soil moisture were found.

Representative root structure in the dry and in the wet soils in Experiment 5 are shown by photomicrographs in Fig. 15. The roots of plants in this experiment at 80-percent soil moisture had relatively narrow rays and little parenchymatous storage tissue, and this contained no starch. Characteristic autumn growth was conspicuous, and conducting tissues and fibers were more abundant than at lower soil moistures. The larger size and greater abundance of vessels in the plants grown in wet soil appears to be in accord with results obtained by Wieler[26*] in experiments with several representative trees. The roots of the plants grown at about 35-percent soil moisture had wide

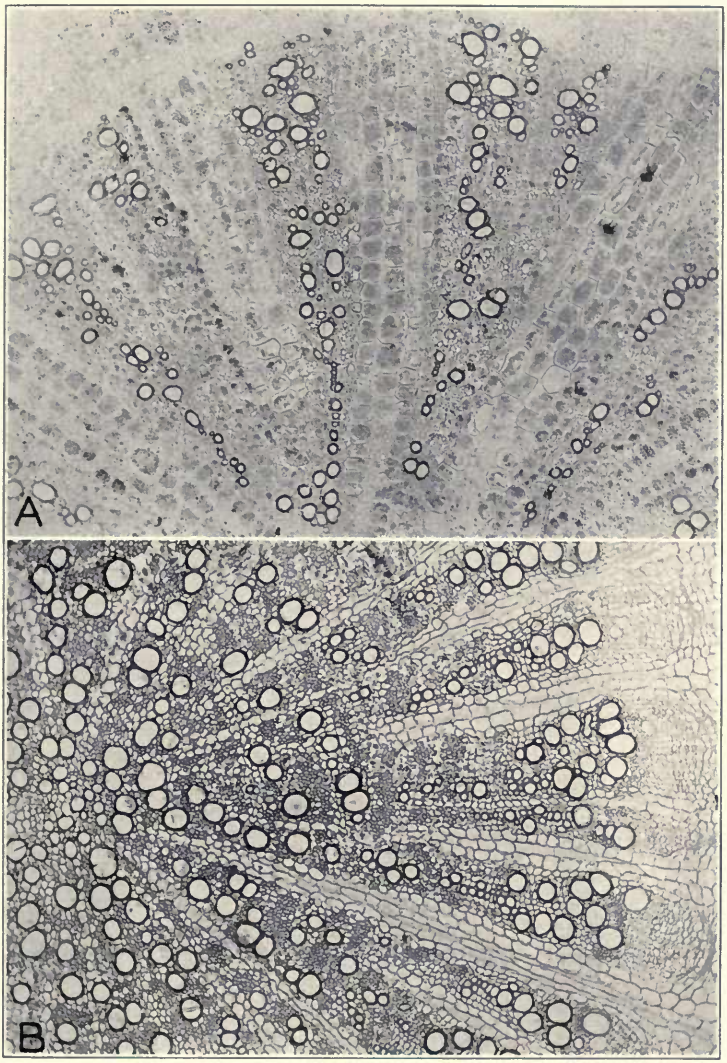

FIG. 15.—REPRESENTATIVE CROSS-SECTIONS OF THE UPPER PART OF TAPROOTS OF ALFALFA PLANTS GROWN AT LOW (*A*) AND AT HIGH (*B*) SOIL MOISTURES

The roots grown at 35-percent soil moisture (*A*) had wide rays well filled with starch; while those grown at 80-percent soil moisture (*B*) had relatively narrow rays, little or no starch, and a larger proportion of the vessels were of large diameter. The zone of autumn wood marked by vessels of uniformly small diameter was found in both. These roots are from Experiment 5.

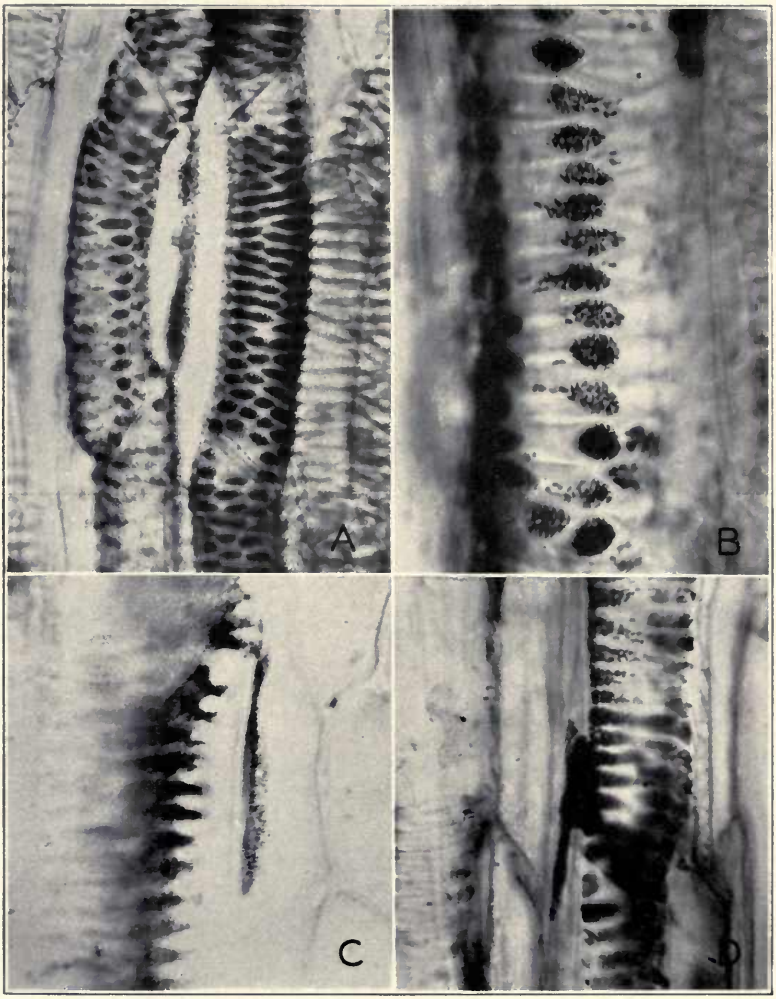

FIG. 16.—BACTERIA IN YOUNG VESSELS AND BETWEEN PARENCHYMATOUS CELLS ADJACENT TO THOSE VESSELS

(*A*) Bacteria in the pits of two young vessels and between two parenchymatous cells lying between these vessels. In the vessel at the left the bacteria have developed chiefly in three rows of pits, while they have developed in all pits in the vessel at the right. (*B*) Bacteria developing in a single row of pits in a large vessel. (*C*) Bacteria escaping from a pit in a vessel between contiguous parenchymatous cells. (*D*) Bacteria escaping from a single row of pits in a vessel between contiguous parenchymatous cells. The photomicrographs are from stained razor sections. *A* enlarged times 430, *B* times 1400, *C* times 1000, *D* times 700.

rays well filled with starch. Chemical data from the roots of plants grown in Experiment 5 are shown in Table 7. The starch content of roots grown at 80-percent soil moisture was very low compared with the starch content of roots grown under lower soil moisture conditions.

TABLE 7.—CHEMICAL DATA FROM ANALYSIS OF ALFALFA ROOTS OF PLANTS GROWN UNDER VARIOUS SOIL TEMPERATURE AND MOISTURE CONDITIONS, UNINOCULATED AND INOCULATED WITH *Phytomonas insidiosum*

Disease condition	Temperature or moisture at which plants were grown	Percentage of dry weight					
		Total sugars[a]	Dextrin and soluble starch[b]	Starch	Hemicellulose[c]	Crude fiber	Total nitrogen
Experiment No. 3							
Control	10° C.	12.04	1.32	18.64	12.45	17.23	1.80
	15	7.80	1.30	21.27	9.80	17.70	2.13
	20	8.43	2.00	16.86	7.39	20.73	2.55
	25	7.66	1.40	11.48	7.75	24.69	2.47
	30	7.85	.83	11.54	7.77	21.55	2.30
Inoculated	10° C.	13.02	.65	14.36	11.56	17.55	1.84
	15	8.17	1.64	21.01	9.28	19.42	2.19
	20	8.58	1.56	16.02	9.68	22.34	2.32
	25	6.85	.76	12.42	9.10	23.13	2.19
	30	6.95	.73	12.62	6.22	24.60	2.41
Experiment No. 5							
Control	35%	5.78	1.05	15.42	11.37	18.66	2.49
	50	7.34	.91	16.91	14.26	17.86	2.28
	65	7.06	.62	17.24	8.46	23.18	2.32
	80	6.90	.79	7.65	8.62	31.04	2.24
Inoculated	35%	5.37	.75	12.00	13.31	19.05	2.74
	50	6.00	1.13	17.07	10.54	21.62	2.34
	65	5.95	.66	13.45	7.56	29.35	2.24
	80	[d]	.23	4.99	9.25	34.56	2.41

[a]Calculated to glucose. [b]Calculated to dextrin. [c]Calculated to xylan. [d]No determination.

The strong contrast between root structures of plants grown at the lower and the upper extremes of soil moisture in Experiment 5 was not found in the younger plants in Experiment 4. The reason for the absence of this difference is not obvious. In this latter experiment starch seemed nearly as abundant in the roots growing at high soil moisture as in those growing at low soil moisture. Chemical analyses (Table 7) were made only of the roots from Experiment 5, as that experiment contained the oldest plants and the moisture control was probably more satisfactory than in Experiment 4, especially at the lowest moisture content, because less red-spider trouble was experienced and therefore less water spray was necessary.

Apparently soil moisture is capable of producing great alteration in root structure in alfalfa as well as in other plants, as shown by Wieler,[26*] even tho identical results were not obtained in the two experiments recorded here.

DEVELOPMENT OF WILT BACTERIA IN THE PLANT ROOTS

Movement of Wilt Bacteria in the Host Tissues

The study of the development of parasitic bacteria in plants under different environmental conditions was preceded by an examination of the relations of the parasite to the host tissues. The movement of the bacteria in the open vessels of the plant has been described and the course which the bacteria pursue in the parenchymatous tissue noted,[7]* but certain unpublished details of this migration thru the host, as they were observed in the present study, will be outlined here.

In a description of the development of the bacteria in the plant, Peltier and Jensen[19]* mention that the bacteria develop in pits of the vessels, but they do not describe further this seemingly important observation not previously recorded for any vascular parasite.

The relation of the bacteria to the pits was observed by the writers in razor sections of roots of infected plants after staining with Gram's stain and counterstaining with orange G. In recently invaded young vessels of summer wood, the bacteria were found in the pits in flat mat-like colonies adherent to the primary wall of the vessels between the inner thickenings, which were not always completely developed or lignified. Sometimes in very young vessels the bacteria were found in all of the pits, sometimes in but a few scattered pits, but often in rows of pits along one or more sides of the vessel (Fig. 16, *A*, *B*). When the bacteria were in rows of pits, these pits were found to be opposite either a contiguous vessel or the line of junction of two parenchymatous cells against the vessel wall. Seemingly the development of the bacteria in the vessel is influenced by the age of the vessel when first invaded. If the vessel is very young with incomplete thickenings and surrounded with young parenchymatous cells, the bacteria may develop in all of the pits; if older, in rows of pits as described; and if invaded when still more mature, very few pits will be found to contain bacterial colonies.

This distribution of the bacteria in pits is strikingly different from the distribution which occurs when bacteria are introduced into vessels by cutting stems or roots under a dilute bacterial suspension. In this latter case the bacteria are found adherent to the inner thickenings and massed where they have formed an obstruction, but the characteristic lodgment of the bacteria in the pits is absent.

Further evidence that the bacteria make their initial development in vessels adherent to the wall in pits was found from an examination

of roots of infected plants of sweet clover. In this plant the bacteria were often found clinging together in masses thru all the treatment required to stain sections, and in young vessels rounded bacterial masses were frequently found attached to the series of pits from which they were presumably extruded, very few being found scattered or in masses blocking the lumen (Fig. 17).

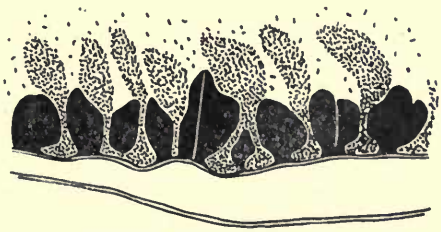

FIG. 17.—BACTERIA DEVELOPING IN SINGLE ROW OF PITS IN A LARGE VESSEL OF AN ALFALFA ROOT

Sometimes the bacteria remain attached in masses after they have been extruded from the pits, as shown in the semidiagrammatic drawing above.

Whether the bacteria multiply only in the pits in contact with or in close proximity to the vessel wall or whether further increase in number takes place in the lumen of the vessel could not be determined by observations of this character. However, inasmuch as the bacteria in older larger vessels rarely develop in sufficient abundance to fill the entire lumen, it does not seem necessary to assume that they develop elsewhere than in the pits. Development in pits, moreover, seems dependent not only on the age but on the character of the contiguous parenchymatous cells. In any case the age and character of the contiguous tissue cells condition the escape of the bacteria from the vessels into the parenchymatous tissue, a migration necessary to the rapid progress of the disease.

In autumn wood the bacteria may dissolve the middle lamella of the walls of all contiguous cells, thus separating the vascular elements (Fig. 18). Extensive solution of the middle lamella of the walls of cells surrounding vessels has never been observed in spring or summer wood. Here escape of the bacteria from vessels is limited to a few pits or groups of pits and appears to be effected by the dissolution of the thin primary wall of the vessel and the development of bacteria in the middle lamella of contiguous cells thru which they spread in a thin layer (Fig. 16, *C*, *D*). At the time of this escape the lumen of the vessel may not be completely filled with bacteria, and therefore little if any pressure from bacterial growth can be employed in break-

ing the vessel wall. The bacteria progress slowly thru the middle lamella of the cell walls of the vascular bundle where there are no intercellular spaces, and from many of these places of escape they fail to reach the ray tissue where intercellular spaces facilitate progress.

The increase of parasitic bacteria in the host plant when introduced by any method of inoculation is very slow at first. The history

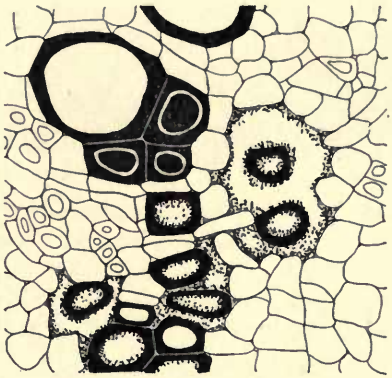

FIG. 18.—BACTERIAL POCKETS AROUND INVADED VESSELS OF AUTUMN WOOD FORMED BY THE DISSOLUTION OF THE MIDDLE LAMELLA OF PARENCHYMATOUS CELLS

This drawing was made from roots grown at 15° C. soil temperature. Bacteria have escaped out of the vessels and have dissolved the middle lamella of the surrounding parenchymatous cells.

of the development of the disease in a vigorous plant is complex, involving a consideration of the morphological and physiological condition of the various tissues as the bacteria enter them, the rate of growth of the roots and finally the altered morphology and physiology of wood laid down by the cambium when bacteria are abundant in the vessels which supply the plant with water. The experimental data obtained thus far do not reveal any critical temperature within which the disease ceases to develop in infected plants.

Effect of Soil Temperature

Examination of the effect of soil temperature on the development of parasitic bacteria in the roots of plants is a more complex matter than was realized when these experiments were undertaken. In the present study consideration must be given to the fact that the bacteria were introduced into the lower parts of the stems of plants which had been produced under the same uncontrolled temperature conditions (Experiment 1) and that the bacteria had to develop extensively

in this tissue before they could invade the new tissue produced under controlled temperature conditions. Moreover in Experiment 1 the bacteria were introduced into a wide band of autumn wood with very small vessels which presumably are relatively inefficient in drawing bacteria far into the roots, tho the cells surrounding these vessels are favorable for invasion. In Experiments 2 and 3 the bacteria were introduced into tissue produced at controlled temperatures (Table 3) and having large vessels as a result of extra illumination.

Because of the complexity of environmental effects upon the host plants, the small number of plants examined in each sample, and the considerable variation in size of the plants themselves, some of the effects of temperature upon the development of the bacteria in the plant may have escaped detection. The following observations, however, are of interest.

At all temperatures the progress of the bacteria thru the host tissues took place in essentially the same manner. At 10° C. the growth of the bacteria was clearly so slow in the roots in comparison with the growth of the plant that the diseased tissue was deeply buried and harmless to the plants as a whole at the conclusion of the experiment. At 15° C. the roots were apparently able in most plants to lay down new tissue on the outside of the vascular cylinder about as fast as the bacteria could invade the vessels from the inner position, and the plants were therefore able to develop very well for a long time in spite of the disease. At higher temperatures the bacteria had occasionally passed out into the phloem and were thus present in the parenchymatous tissues in much greater abundance than at lower temperatures.

At 25 and 30° C. the bacteria were relatively few in proportion to the amount of vascular plugging found, but the presence of bacteria in vessels was usually followed by a relative increase in the number and decrease in the size of vessels laid down by the cambium, so that the last portion of the bundle formed before the plant died consisted almost wholly of small vessels closely crowded.

Effect of Soil Moisture

The infected roots grown at different degrees of soil moisture could not be differentiated with respect to the manner in which the bacteria developed in the tissue. The occurrence of less infection in the roots grown in the driest soil was presumably due to the fact that the drier soil affected the plants in some manner that prevented the bacteria from developing effectually when introduced thru the cut stems. It is not possible, however, from the examination of roots of plants actually infected to get evidence relating to the reasons for this failure to infect.

CHEMICAL COMPOSITION OF ROOTS AS AFFECTED BY SOIL TEMPERATURE AND SOIL MOISTURE

The roots from the experiment in which the temperatures were controlled from the time of seeding (Experiment 3) and the roots from the second moisture experiment (Experiment 5) were used for chemical analysis. The crowns were separated from the roots and the roots dried at a temperature of 90° C. They were then finally ground in a Wiley mill until nearly all the substance passed thru a 60-mesh sieve; the material was then stored in bottles until used for analysis.

With the exception that all analyses were made from the dried samples, the procedure followed was very like that used by Graber *et al*,[4*] Albert,[1*] and Leukel.[13*]

Effect of Soil Temperature

While the quantity of roots produced at the different temperatures used in these experiments varied considerably, as shown in Table 2, the discussion of the chemical nature of the roots will be considered here on a percentage basis rather than on the basis of absolute quantity. The quantities of the various constituents can be easily computed by multiplying the weights given in Table 2 by the percentages given in Table 7 (see pages 49 and 67).

Altho the plants used in these analyses were grown under glass with artificial light to supplement daylight, yet in general the chemical composition of the roots correspond very closely to that reported by other investigators[1, 4, 13, 24*] for alfalfa plants grown under field conditions.

A striking fact shown in Table 7 is that the sugar percentage was considerably higher in roots produced at 10° C. than in those produced at the higher temperatures. Other investigators have observed that alfalfa roots are highest in sugars during the cold part of the season,[1, 4, 13, 24*] and similar conditions have been noted in some other plants, but in none of the experiments by the investigators just cited were the temperatures so controlled that growth at definite temperatures could be correlated with the analyses. In alfalfa a high sugar content is not only characteristic of plants in a semidormant winter condition, but it is shown in these experiments to occur also at a temperature in which active normal growth takes place. A rather abrupt change must occur somewhere between 10 and 15° C., for at the latter temperature the percentage of sugar was much lower than at 10° C. and practically the same as at still warmer temperatures.

Wilt infection apparently had some effect on sugar content in that

at the higher temperatures the sugar content was somewhat lower in the wilt-inoculated plants than in the control plants. In this connection it should be recalled that disease had only a slight effect at the low temperatures and a very severe effect at the higher temperatures, especially at 25 and 30° C. All the chemical analyses were made on samples representing the entire population of inoculated plants alive at the time the plants were taken out of the soil, regardless of the percentage of infected plants (Table 3, Fig. 9) or severity of infection (Table 2, Fig. 7).

Under the different temperatures the percentages of dextrins and soluble starches varied almost exactly with the growth curve (Fig. 6). These compounds probably are transition substances in metabolism and therefore are most abundant where growth is most active. The inoculated plants ranged lower in dextrins and soluble starches than the controls, especially at the higher temperatures, this also coincides with the differences in rate of growth.

The hemicelluloses varied inversely with crude fiber to a marked extent. The former were high at the low temperatures; the latter was high at the high temperatures. The transition from hemicelluloses to true cellulose apparently proceeds much more slowly at low temperatures than at higher ones. The wilt disease had no significant effect on the relative percentages of these two groups of compounds.

The percentage of nitrogen was lower at 10° C. than at higher temperatures in both the control plants and wilt-inoculated plants. Aside from this, no significant effect either from temperature or disease was noted in regard to nitrogen content.

Effect of Soil Moisture

Some decided differences in chemical composition occurred between plants grown at the different soil moistures used in the experiments reported in this bulletin. In general, this phase of plant physiology has as yet received little attention, and with respect to alfalfa no record of previous work on this subject seems to be available.

The sugar percentage was slightly higher at the intermediate moisture contents than at either the lowest or highest moisture used. In other words, sugars were slightly more abundant at moistures favorable for good alfalfa growth than at moistures less favorable. The plants inoculated with alfalfa wilt ranged somewhat lower in sugar content than the controls. Thus the effect of the wilt disease on the sugar content of the plants was the same here as in the experiment on soil temperature.

The results from the determinations of the percentages of dex-

trins and soluble starches were somewhat erratic. They did not correspond closely to the growth curve, as in the temperature series. High soil moisture had a decided effect on checking the relative amount of starch stored in the plant roots. The percentage of hemicelluloses varied inversely with the percentage of crude fiber in the soil moisture experiment as in the temperature experiment. The percentage of hemicelluloses was high at the two lower moistures, while that of crude fiber was high at the two higher soil moistures. Apparently an abundance of moisture promotes the change from hemicelluloses to true cellulose, thus making the roots more fibrous or woody. The roots from the inoculated plants were higher in fiber content than were the controls.

A slightly greater percentage of total nitrogen occurred at the 35-percent moisture than at higher moistures. Otherwise no consistent differences in nitrogen content could be noted.

GENERAL DISCUSSION OF RESULTS AND CONCLUSIONS

Bacterial wilt of alfalfa is primarily a disease of the perennial part of the alfalfa plant, that is, of the taproot and the crown. The bacteria enter the plant close to the soil surface and may make their most extensive and important development in the crown and upper part of the root. Under field conditions temperature fluctuates much more widely at the soil surface than at a greater depth where the several root diseases which have been found to be affected so decisively by soil temperature destroy or invade roots. In the experiments reported in this bulletin the bacteria were introduced into the plants above the soil level, where their early development was certainly in part at a temperature more nearly that of the crown than of the soil. Thus in the early stages of infection, at least, the soil temperatures in these experiments may have had a less decisive influence in the development of the disease than had the air temperatures. Especially is this likely to have been true of soil temperatures of 10 and 30° C., for at these soil temperatures the air temperatures ranged from 15 to 18° C. and 25 to 28° C., which temperatures are presumably more favorable for bacterial development.

The highest soil and air temperatures used in these experiments were a little higher than the mean July temperature ordinarily occurring in central Illinois,[17*] but the upper limit beyond which the disease does not develop has not been reached. Thruout the year the mean temperature of soil 1 to 3 inches beneath the surface is slightly higher than the air temperature.[17*] For short periods during the day soon after the

crop is cut, at which time the crowns are exposed to the direct rays of the sun in the field, the temperature of the crown no doubt runs up above the soil temperature and above the temperatures used in these experiments.

So far as the natural range of field temperatures in central Illinois is concerned, it may be inferred that the rate of development of wilt varies directly with temperature. However, the growth of normal alfalfa plants of the strain used did not follow this course, at least not in these experiments, but approached a normal curve with the high point near 20° C.

The longevity of plants after infection was rather short at the upper temperature (30° C.), deaths occurring in 45 days in Experiments 1 and 2, and in 30 days in Experiment 3. At 10° C. longevity was not determined, but it seems likely that all the inoculated plants would have continued to live for a much longer time if the experiments could have been continued; most of the plants probably would not have died at all as a direct result of the disease.

In analyzing the effects of varying degrees of soil moisture on the development of alfalfa wilt, it again is difficult to apply to field conditions results obtained by growing this naturally deep-rooted plant under controlled conditions in cans in the greenhouse. Some confidence in the validity of such an application is suggested, however, by the fact that the rate of wilt development in different parts of fields having different conditions of soil moisture has been observed to vary in much the same way as it varied in the experiments reported in this bulletin. The conclusion seems to be that wilt infection is least likely and the development of wilt is slowest at low moistures, while the disease is most active at medium to high moistures.

The extremes of soil moisture used in these experiments were about as great as alfalfa growth would allow. In the low-moisture series the low point, which was reached approximately once a month, was practically at the wilting coefficient.[a] Under field conditions in Illinois the upper two feet of soil (the depth of the cans used) seldom reaches a point as low as the wilting coefficient, and rarely if ever does it become this dry at the lower reaches of the roots. In some drier climates the upper soil layers may become drier than the soil used in these experiments, and thus the full effect of drouth upon the development of wilt under natural conditions may not have been obtained experimentally. The upper moisture content used may obtain in field soils during heavy

[a]The wilting coefficient refers to the soil moisture condition at which wilting of a plant begins.

rains and for some days after, especially where the water table is not far below the surface.

Finally, it is highly probable that temperature and moisture conditions have a more decisive influence on the entrance of bacteria into plant tissue thru wounds than they have on the subsequent development of the disease within the plant; and this influence of temperature and moisture is probably exerted in an entirely different manner on initial infection than on the later development of the infection. Only one method of wound inoculation with its subsequent course of disease development within the plant was studied in these experiments.

In the study of most diseases of roots the need for distinguishing the effects of different environmental conditions upon infection and disease development is not so important as it is with this disease, nor is experimental analysis so difficult. Further study of the effect of various environmental factors upon the relation of the parasite to the host is necessary before a fuller interpretation of the field development of the disease will be possible.

SUMMARY

Surveys of the prevalence of wilt in alfalfa fields in Illinois made in 1925 and again in 1930 show that this disease has been increasing in the frequency of its occurrence. It is estimated that in 1930 it was present in 65 percent of Illinois fields two years old and older.

A study of the effect of soil and air temperature and soil moisture upon the development of wilt in the alfalfa plant was made by comparing the effect of these environmental conditions upon infected and uninfected plants. The experiments, extending over three years, were conducted in the greenhouse under controlled conditions of soil temperature and soil moisture.

The best growth both of roots and of forage parts of healthy plants was obtained in the temperature experiments at a soil temperature of 20° C. and in the moisture experiments at a soil moisture content which was 65 percent of the moisture-holding capacity of the soil. The ratio of root growth to top growth was highest at the lowest temperature (10° C.) and at the lowest moisture (35 percent) used.

Within the range of 10 to 30° C. soil temperature the percentage of plants infected by inoculation with the wilt organism thru cut stems increased with increase in temperature in each of three experiments. The length of life of infected plants diminished with increase of temperature.

The weight of the roots of the plants infected with the wilt organism was diminished more than the weight of the tops by the disease. The diseased plants were thus in worse condition than one would judge from their aboveground appearance.

Within the range of 35 to 80 percent of the moisture-holding capacity of the soil, infection of plants by the method used in these experiments increased as the moisture content increased up to 65 percent but above this point there was no significant further increase in infection. The length of life of infected plants tended to be shorter under moisture conditions that proved most favorable for the controls.

The use of artificial illumination in the autumn to supplement the normal length of day was followed by the development of vessels of large diameter, like those produced in spring wood, regardless of the soil temperature or moisture.

Increased soil temperature, within the range used in these experiments, tended to be associated with an increase of the vascular tissue in the roots in relation to the other root tissues. At the lower temperatures there was an increase in the amount of storage tissue formed.

There were indications that, in proportion to the amount of ray, larger amounts of vascular tissue developed at 80-percent soil moisture than at lower moistures.

The wilt bacteria developed in essentially the same manner in the plants grown at the different soil temperatures (10 to 30° C.); that is, they multiplied in the pits of the vessels against the primary wall, especially where the vessel was contiguous to another vessel or to the line of contact of two contiguous parenchymatous cells.

Differences in soil moisture within the range maintained in these experiments (35 to 80 percent) did not produce noticeable differences in the relation of the bacteria to the plant.

Chemical analyses of the roots showed that the sugar content was considerably higher at 10° C. than at the higher temperatures. At 25 and 30° C. the sugar content was lower in the roots of inoculated plants than in the controls. The starch, hemicellulose, crude fiber, and nitrogen contents of the roots were influenced by temperature.

Differences in soil moisture affected the sugar, starch, hemicellulose, and crude fiber contents of the roots. Plants infected with alfalfa wilt showed a lower sugar content and a higher crude fiber content than did the plants not inoculated with wilt.

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