



THE RELATION OF SOIL
TEMPERATURE AND SOIL MOISTURE
TO THE INFECTION OF ONIONS BY
PHOMA TERRESTRIS HANSEN

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THE RELATION OF SOIL TEMPERATURE AND SOIL MOISTURE TO
THE INFECTION OF ONIONS BY PHOMA TERRESTRIS HANSEN

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THE RELATION OF SOIL TEMPERATURE AND SOIL MOISTURE TO
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INTRODUCTION

The onion is one of the leading truck crops grown on muck soils in Michigan. That it ranks highly as a cash crop is shown by comparing it with a few of the other widely grown truck crops of the state. During the years 1939 and 1940 its farm value was exceeded only by strawberries and celery, and its value was nearly double that of cabbage. During the past decade plantings in this state have increased from approximately 8,900 acres in 1931 to 10,800 acres in 1940. The state has advanced from fourth to third position among the states in total acreage and production, being surpassed only by Texas and New York. The total production for 1940, based on statistics obtained from the U.S.D.A. Crop Marketing Board (12), was approximately 1,836,000 hundred pound sacks valued at \$2,203,000.00.

In Michigan onion growing is practically restricted to muck soils. This has led to the concentration of the major onion growing sections in southwestern and southcentral parts of the state where there are extensive muck-lands and where the markets are near at hand. The industry is concentrated mostly in Allegan, Newaygo, Calhoun, Ionia, Ingham, and Jackson counties, with smaller acreages in Ottawa, Kent, Barry, Eaton, Van Buren, Washtenaw, and Lapeer counties. In Allegan, Barry, and Eaton counties, especially, and to a less extent in some of the other counties, the land has been used continuously for onion production many years. This continuous use of land for growing one crop year after year is especially conducive to the development of disease. Reports from Allegan, Barry, and Eaton counties indicate that the total acreage for the counties has not changed much during the last few years, but on the other

hand, the total yield has been steadily decreasing. In Newaygo and Calhoun counties the opposite condition is true. Onion production is fairly new in those two counties so that yields per acre are considerably higher there than in Allegan county. Continuous cropping of the soil, year after year, has stimulated the propagation of soil organisms parasitic on the roots of onions. Consequently pink root of onion, which is strictly a root disease, has become a serious menace to the onion crop of Michigan. Because of the great importance of the onion industry in this state and the potential destructiveness of this disease to the onion crop, a long range program of control has been started and the work reported herein is a part.

THE DISEASE

History

The early history of onion pink root is somewhat complicated because several distinct organisms have been suspected as causal agents. The disease was first mentioned in 1917 by Taubenhaus and Johnson (9), who reported it in Texas. In 1921 Taubenhaus and Mally (10) published an extensive paper on its etiology and control. They believed the causal agent was a Fusarium which they named Fusarium mallyi Taub. Later Sideris (7,8) reported several species of Fusarium which he considered capable of causing the disease. In 1926 Hansen (5), by immersing firm onion roots showing early pink root symptoms in 1-500 mercuric chloride for three minutes, obtained subsequently on isolation a Phoma only. Hansen (4,5) was unable to reproduce the disease with cultures of Fusarium mallyi, Fusarium corymbiferum Sideris, and other fusarial species. On the other hand, he readily produced the disease with the species of Phoma he had isolated and which he named Phoma terrestris.

Importance And Distribution

Pink root of onion has become of major importance in nearly all of the onion growing sections in the United States. Definite data are not available for the onion growing districts of Michigan, but it can be stated that the effects of the disease are reflected in a progressive decrease in yields for many years.

During the harvesting season of 1940 every commercial onion field in Eaton County was visited to determine the extent of pink root disease in that county and to correlate this information with experimental results obtained in the greenhouse. The normal annual yield for onions in Michigan on noninfested fields which have been properly fertilized is approximately 500-1,000 crates per acre. The estimated average yield for Eaton County in 1940 was 165 crates per acre with variations from 50-700 crates per acre. Except for one or two fields damaged by a hail storm, the reduction in yield was nearly all due to pink root, running as high as 90 per cent in some instances. The disease is even more prevalent and destructive in the Gun Marsh area of Allegan County, one of the oldest and largest onion growing sections in the state.

Taubenhaus and Mally (10), as early as 1921, estimated losses in Texas fields from 35 per cent to an occasional complete crop failure in badly diseased fields. Hansen (4) noted in California that where onions are grown in the same field for two or three seasons the crop frequently is so diminished that no attempt is made to harvest it.

The disease is very wide spread. It has been reported in nearly all of the large commercial onion growing sections in the United States. In addition it has been reported from Bermuda (4), Canada (6), and South Africa (2). Hansen (4) isolated P. terrestris from cowpeas,

Lima beans, and potatoes. Thornberry and Anderson (11) reported infection of the small lateral roots of tomato with P. terrestris which resulted in a stunted condition of the plants. In addition they cited some unpublished information supplied to them by Dr. E. I. Melhus on the host range of the fungus. According to this report the following plants are susceptible: barley, cane, cantaloupe, carrot, cauliflower, corn, cucumber, millet, muskmelon, oats, pea, pepper, soybean, spinach, squash, tomato, and wheat. This indicates that the fungus is not confined to members of the genus *Allium* and that care should be taken in the selection of plants to be used in crop rotations on lands used for onion production.

Symptomatology

The first evidence of pink root infection in an onion plant is a gradual loss of turgidity in the older leaves. These leaves soon become soft and flaccid, droop, and finally die back from the tips. Plants in the later stages of development are so weakened by the disease that they bend over at the neck and appear to have reached maturity. If the infection is severe death may result to the whole plant. These symptoms are similar to those which result from drought and other unfavorable conditions. An examination of the roots of infected plants reveals the presence of a pinkish discoloration. In severe infections all, or nearly all, of the roots rot off or become so disintegrated that they wither and turn a deep red to purple color. New roots are produced to replace the dead ones. Roots recently infected are turgid but usually have a yellow or yellow-brown or even a slight pink color. The top symptoms are due to disintegration of infected roots and the consequent impossibility of the few remaining roots to supply the growing plant with enough salts and water to maintain normal

processes of development. The final result is that the available food is exhausted in the production of new roots and bulbs of marketable size are not produced.

PRESENT INVESTIGATIONS

In testing commercial onion strains and hybrids for relative resistance to disease it is exceedingly important to know the optimum conditions for the growth and development of both the host and the pathogen. There are certain limits within which most organisms are capable of initiating and producing disease. These limits are determined by the ability of the organism to tolerate various environmental factors among which temperature, moisture, and pH are very important. Phoma terrestris is strictly a soil inhabiting organism and the environmental factors of the greatest importance to it are those of the soil. Previous reports (4, 10) have indicated that pink root is associated with high soil temperatures. There are no data available to indicate the relation of soil moisture to the infection of onion roots. Hansen (4) planted onion bulbs in inoculated soil and grew them in constant temperature chambers with no illumination. He reported the greatest amount of infection at 25° C. Field observations reveal that the disease does its greatest damage at the time of rapid bulbing in August. In Michigan the latter part of July and the first two or three weeks in August are generally hot and dry. It is then the fungus does the greatest amount of damage.

In the present investigations the environmental factors influencing the mycelial development of P. terrestris have been studied. Concomitantly, the soil factors which influence the growth and development of the onion were studied under controlled conditions in the greenhouse,

as were those which influence the development of the disease. The object was to determine the relationship of soil temperature and soil moisture to infection of onions by the pink root fungus.

Materials and Methods

Soil Temperature and Soil Moisture Studies

The soil temperature and soil moisture studies were made through the use of soil tanks. The tanks consisted of several individual water baths each with a separate, thermostatically controlled heating unit. Fluctuations in temperature did not exceed $\pm 0.25^{\circ}\text{C}$.

The effects of widely fluctuating air temperatures were eliminated by carrying on the experiments during the fall, winter, and early spring. Artificial light was provided during the short days to lengthen the day and so simulate the light conditions of the natural growing season.

Downing's Yellow Globe onion was used in all the experiments. The onions were either grown in sterilized sand and transplanted to the soil cans made especially for the soil tanks, or the seed was sown in the cans and the seedlings thinned to the required number.

The moisture content of the soil was maintained at a uniform level throughout each experiment. This was done first by determining the water-holding capacity of the soil (the amount of water the soil will retain in opposition to gravity when free drainage is provided). The soil was then divided into several portions, depending upon the number of saturation gradients to be used in the experiment, and each portion was adjusted to the desired moisture content by either adding more water or by air-drying. After the soil moisture was adjusted a weighed quantity of soil was placed in each can. Onion transplants were then set in

the cans and the soil covered with one inch of ground cork to prevent undue surface evaporation. At regular intervals each can was restored to its original weight by the addition of distilled water.

Environmental Relations

Influence of Hydrogen-ion Concentration and Temperature on The Growth of Phoma Terrestris

Hydrogen-ion Studies

Several synthetic agar media were tried out to determine the one best suited for growth studies in this experiment. Of the five synthetic media selected for trials, Elliot's agar proved superior.

The fungus was grown at various temperatures in incubators where the temperature fluctuations did not exceed $\pm 0.25^{\circ}$ C. The temperatures ranged from 10° to 34° C. By the use of the colorimetric method, the agar was adjusted to a pH of 4, 5, 6, 7, and 8 respectively. There were four replicates for each pH and at each temperature. Records were taken on the diameter growth of the mycelia after the plates had been incubated 12 days.

Phoma terrestris grew well over a wide range of hydrogen-ion concentrations. The optimum concentration for growth was between pH 5 and pH 6. The growth at pH 4 and pH 8 was slightly less than the optimum at all temperatures. Similar results were obtained by Davis and Henderson (1), who found the optimum hydrogen-ion concentration for the growth of P. terrestris to be from pH 5.4 to pH 5.8 with a growth range from pH 3.8 to pH 7.6. This is well within the pH range of soil used for onion culture.

Temperature Studies

The influence of differential temperatures upon the growth of P. terrestris was determined in experiments prior to those on the relation of soil temperature to the infection of onion roots. The information obtained from such studies does not indicate the actual soil temperatures at which the onion plant is susceptible, but it does indicate those which may be significant.

Three kinds of media were used in this experiment. They included cornmeal agar, potato-dextrose agar, and Elliot's agar. The agar was adjusted to pH 5.8 and exactly 30 cc. poured into each plate. The plates were inoculated by the agar plug method with an isolate of P. terrestris that had proved highly pathogenic in previous pathogenicity tests.

After remaining at room temperature for 24 hours the inoculated plates were placed in the incubators at 10°, 14°, 18°, 20°, 24°, 26°, 28°, 30°, and 32° C. Twelve plates, four for each medium, were placed in each incubator. At the end of 10 days the plates were removed and the average radial growth for each medium determined for each temperature. Table 1 gives the average radial growth of the mycelia of P. terrestris on artificial media.

TABLE 1. Growth of Phoma terrestris at various temperatures.
Incubation period 11 days.

Temp. ° C.	Average radial growth in mms. of 4 mycelia on:		
	Elliot's agar	Cornmeal agar	Potato-dextrose agar
10	2.5	2.5	2.0
14	8.0	9.0	9.0
18	16.0	15.0	15.0
20	20.0	21.0	22.0
22	22.5	25.0	26.0
24	27.5	30.0	28.5
26	32.0	33.0	31.5
28	33.0	35.0	33.0
30.	32.0	31.0	31.5
32	20.5	14.5	15.0

The maximum radial enlargement of mycelia occurred at 28° C. on all media. It will be noted that there was a sharp decrease in growth at 32° C. and that the growth was as good or even better at 18° C. than at 32° C. The growth of P. terrestris on cornmeal agar is illustrated in Plate I. In a previous experiment on growth and pH where the range extended to 34° C. there was no growth at 34° C. This establishes the maximum temperature for this fungus between 32° and 34° C. The minimum temperature for growth was not determined, but at 10° C. mycelial development was practically stopped. The minimum temperature can then be placed slightly below 10° C. Hansen (4) found that 26° C. was the optimum

temperature for the growth of the fungus, while Davis and Henderson (1) obtained the best growth at 28° C.

The Relation of Soil Temperature and Soil Moisture to the Growth Of Onion

Onions make the most rapid growth and best development during cool and moist seasons. Pink root does not become apparent until the plants have been exposed to the high temperatures of mid-summer. To aid in analyzing the data on the relation of soil temperature and soil moisture to the disease, some knowledge of the effects of these factors upon the development of the host is desirable. This information was obtained by growing the plants under controlled conditions in noninfested soil.

In a preliminary experiment it was found that 50 per cent saturation was too dry and that 85 per cent saturation of the soil was too wet for good onion growth. Many of the plants at 32° C. were killed because of the high soil temperature. The best root development took place in soil held at 18° C., but the plants did not bulb very well at this temperature. In subsequent experiments these lower and higher soil temperatures and soil moistures were eliminated. Instead the plants were grown in soil held at 20°, 22°, 24°, 26°, 28°, and 30° C., with moisture contents of 60, 70, and 80 per cent of its water-holding capacity.

The muck used in this experiment was obtained from an onion field near Kalamazoo, Michigan. Its texture was improved by adding one part of sand to three parts of muck. All the soil was thoroughly steamed and stored in a covered metal drum for a month before it was used. Before the onions were seeded a 3-12-12 fertilizer (600 lbs. per acre) and manganese sulphate (200 lbs. per acre) were thoroughly mixed with the soil. The results of this experiment are given in Table 2.

TABLE 2. The relation of soil temperature and soil moisture to the development of onion. Seed sown directly in cans, and plants grown for 68 days.

Per cent saturation of soil	Temp. Soil °C.	No. of plants	Dry weight of tops - gms.	
			Total	Average per plant
60	20	25	1.730	.069
	22	26	1.449	.056
	24	21	1.155	.055
	26	25	1.073	.043
	28	26	.673	.026
	30	22	.402	.018
70	20	25	2.066	.083
	22	25	2.120	.085
	24	25	1.529	.061
	26	25	1.282	.051
	28	25	1.208	.048
	30	23	0.692	.030
80	20	24	2.880	.120
	22	25	2.189	.088
	24	25	2.244	.090
	26	25	1.478	.059
	28	25	1.433	.057
	30	23	.850	.037

The results of this experiment clearly show that soil temperature and soil moisture exert considerable influence on the top development of onions. This is especially noticeable when a comparison is made of the average dry weights of the tops per plant as given in Table 2. The indications are that the lower soil moistures have a tendency to limit the soil temperature range in which good onion growth takes place. In the 60 per cent saturated soil good growth took place only at 20° C. Above this temperature plant development was retarded considerably and became progressively poorer as the soil temperatures increased. In the 70 per cent saturated soil growth was good at 20° and 22° C., and not

until the soil temperatures reached 24° C. were there any indications of growth retardation. The best top development took place in the 80 per cent saturated soil. At this moisture level the plants made excellent top growth at soil temperatures of 20° , 22° , and 24° C.; but at 26° C. there was a definite decrease. The effects of these soil temperatures and soil moistures on the top development of onions are illustrated in Plate III.

Root development was better in the lower than in the higher soil moistures. There was a progressive decrease in the extensiveness of the root system from the 60 to the 80 per cent saturated soil. In the 60 and 70 per cent saturated soil the roots were well developed, showed much secondary branching, and had penetrated throughout the soil. In the 80 per cent saturated soil, on the other hand, root development was inhibited. Free water was present in large enough quantities to prevent soil aeration. Because of this the roots were not nearly so well developed, showed very little secondary branching, and did not penetrate very deeply into the soil. This, however, did not retard the development of the plant. It was in the 80 per cent saturated soil that the best top growth took place.

From $18-24^{\circ}$ C., inclusive, soil moisture apparently has more influence on root development than soil temperature. At these soil temperatures there was very little difference in the root system of plants growing at a constant soil moisture level. Above 24° C., however, soil temperature appeared to have as much effect upon root development as soil moisture. The root systems became progressively poorer as the soil temperatures increased from 24° through 30° C.

The plants initiated bulb development at all soil temperatures and all soil moistures, the poorest bulbing taking place in the 60 per cent and the best in the 80 per cent saturated soil. The difference between the various soil moistures, however, was very slight for any soil temperature. At soil temperatures of 18° and 30° C., bulbing was very poor as compared to that at other temperatures. At 18° C. the growth went into the development of roots instead of bulbs. At 30° C. the high soil temperature alone probably inhibited bulbing. Optimum bulbing took place at 20°, 22°, and 24° C. in the 80 per cent saturated soil.

The Relation of Soil Temperature and Soil Moisture to the Disease

The effects of soil temperatures and soil moistures were studied in conjunction in all experiments. This appeared justifiable since these environmental factors are probably the most important as far as the infection of susceptible onion roots are concerned. It was possible by the use of soil tanks to obtain information on the degree of interaction between these environmental factors, in addition to studying the effects of each one separately. This interaction between two closely associated environmental factors is likely to set up a set of conditions which may be of considerable importance in governing the degree of infection and the rate at which the fungus will destroy the tissues after infection has taken place.

In all of the tests the average dry weight per plant was used as a basis for determining the amount of pink root infection. In preliminary tests it was observed that the amount of top development was closely correlated with the degree of pink root infection. The greatest amount of pink root infection was accompanied by the greatest amount of stunting, consideration being taken, of course, of the effect of these

same environmental factors upon the development of the host. In addition to the dry weight of the plants, data were obtained on the extent of infection, the number of plants killed, the relative number of diseased and healthy roots, and other data which seemed pertinent at the time for drawing specific conclusions in the final analysis.

Previous tests indicated that 26° C. was the optimum soil temperature for the development of pink root in naturally infested soil. In those tests there were no visible signs of infection at 18° C. and very few at 20° and 22° C. Most of the plants at 32° C. died from the effects of soil temperatures alone. The plants growing in the 50 and 60 per cent saturated soils showed the least pink root infection, while those in the 70 and 85 per cent saturated soils showed the greatest pink root infection. The 50 per cent saturated soil, however, was too dry for the growth of onions and the 85 per cent too wet for the development of a healthy root system. Consequently, in the experiments to be described these extremes of soil temperature and soil moisture were eliminated.

Infection of Onion Transplants in Naturally Infested Soil

The muck used in this experiment was obtained from an onion field near Orangeville, Michigan, in which pink root was very destructive in 1939. Its texture was improved by adding one part of sand to three parts of infested soil. This soil was divided into three lots, each of which was adjusted to 60, 70, and 80 per cent of its water-holding capacity respectively. The soil temperatures were maintained at 20°, 22°, 24°, 26°, 28°, and 30° C. At the time of planting a 5-9-18 fertilizer (1000 lbs. per acre) and manganese sulphate (200 lbs. per acre) were added to the soil. The plants were harvested 47 days after planting. Table 3

shows the effect of soil temperature and soil moisture upon the development of pink root in these transplants growing in naturally infested soil.

TABLE 3. The relation of soil temperature and soil moisture to the development of pink root in onion transplants grown 47 days in naturally infested soil.

% Sat. of Soil	Soil Temp. ° C.	No. Plants	Green wt. of tops & bulbs - gms.	Dry wt. of tops & bulbs - gms.	Ave. dry wt. of tops & bulbs per plant - gms.
60	20	16	58.70	4.16	0.26
	22	16	69.97	4.80	0.30
	24	16	91.90	6.16	0.38
	26	16	76.92	5.15	0.32
	28	16	75.20	5.39	0.34
	30	16	79.42	5.63	0.35
70	20	16	91.08	5.48	0.34
	22	16	82.77	5.63	0.35
	24	16	98.49	6.45	0.40
	26	16	68.10	4.44	0.28
	28	16	80.81	5.54	0.35
	30	16	74.72	5.21	0.33
80	20	16	76.92	4.97	0.31
	22	16	105.80	6.74	0.42
	24	16	110.70	6.97	0.44
	26	16	75.01	4.73	0.30
	28	16	100.55	6.59	0.41
	30	16	92.04	6.57	0.41

All the plants in the 60 per cent saturated series showed a very uniform growth at all temperatures. At 20° C. there was slight infection and at all the other temperatures the roots were moderately infected. The tops showed die-back symptoms at all temperatures. In all probability this was due to the dry soil and not directly due to pink root. There was slightly more infection at 26° C. than at any other temperature.

In the 70 per cent saturated series the severity of infection increased considerably over the 60 per cent series. There was no visible evidence of infection in the roots at 20° C., only moderate infection at 20°, 24°, and 30° C., but severe infection at 26° and 28° C.

The plants in the 90 per cent saturated soils were not as severely damaged at the lower temperatures as were those in the other two series. At 20° and 22° C. there were no visible signs of infection in the root system. At 24°, 26°, and 30° C. only slight infection was noticeable, but at 28° C. the infection was severe. All the plants above 24° C. showed typical die-back symptoms.

At all soil moistures pink root developed more rapidly and was more severe at 26° C. than at any other soil temperature. The severity of infection increased at this temperature from the lower to the higher soil moistures. In the 80 per cent saturated soil severe infection appeared to be restricted to 26° C., while at the lower soil moistures severe infection occurred over a wider range of temperatures.

Infection of Onions Seeded in Naturally Infested Soil

The materials and methods used in this experiment were identical with those used in studying the infection of onion transplants except that the onions were seeded directly in naturally infested soil and grown for a period of 69 days. The relation of soil temperature and soil moisture to the infection of onions grown from seed in naturally infested pink root soil is shown in Table 4.

The results of this experiment corroborate those on onion transplant infection and indicate that 26° C. is the optimum soil temperature for the development of pink root in naturally infested soil. At this temperature 100 per cent of the onions were infected in the 70 per cent saturated soil,

$\Delta \theta = 0$, $\Delta \phi = 0$, $\Delta \psi = 0$, $\Delta \chi = 0$, $\Delta \eta = 0$, $\Delta \zeta = 0$, $\Delta \xi = 0$, $\Delta \gamma = 0$, $\Delta \beta = 0$, $\Delta \alpha = 0$.

TABLE 4. The relation of soil temperature and soil moisture to the development of pink root in onions grown from seed in field infested soil for a period of 69 days.

Per cent Soil sat.	Soil Temp. °C.	Total No. plants	Per cent of plants infected	Dry wt. of		Dry wt. of roots - gms.		Per cent of	
				tops - gms.		Healthy	Diseased	Total wt. per plant healthy & diseased	total dry wt. of roots diseased
				Total	Ave. per plant	Total	Ave.		
70	20	17*	18	2.77	0.16	0.714	.042	.048	.033
	22	17	41	2.91	0.17	0.750	.044	.064	.004
	24	22	64	3.35	0.17	0.930	.042	.090	.004
	26	19	100	1.88	0.10	0.156	.008	.096	.005
	28	22	100	4.11	0.19	0.450	.070	.129	.006
	30	22	100	3.23	0.15	0.387	.018	.122	.006
80	20	12	55	1.73	0.14	0.359	.030	.010	.001
	22	20	40	2.73	0.19	1.000	.050	.066	.003
	24	18	55	3.93	0.22	0.990	.055	.074	.004
	26	22	91	3.43	0.16	0.517	.023	.119	.005
	28	22	91	4.31	0.20	0.499	.023	.128	.006
	30	16	94	3.05	0.19	0.366	.024	.085	.005

* The variation in the number of plants was due to development of smut in the seedling stage.

All the smutted plants were removed.

TABLE 4. The relation of soil temperature and soil moisture to the development of pink root in onions grown from seed in field infested soil for a period of 69 days.

Per cent Soil sat.	Soil Temp. °C.	Total No. plants	Per cent of plants infected	Dry wt. of		Dry wt. of roots - gms.		Per cent of	
				tops - gms.		Healthy	Diseased	Total wt. per plant healthy & diseased	total dry wt. of roots diseased
				Total	Ave. per plant	Total	Ave.		
70	20	17*	13	2.77	0.16	0.714	.042	.048	.023
	22	17	41	2.91	0.17	0.750	.044	.064	.004
	24	22	64	2.35	0.17	0.930	.042	.090	.004
	26	19	100	1.88	0.10	0.156	.008	.096	.005
	28	22	100	4.11	0.19	0.450	.020	.129	.006
	30	22	100	3.23	0.15	0.337	.013	.122	.006
80	20	12	25	1.73	0.14	0.359	.030	.010	.001
	22	20	40	2.78	0.19	1.000	.050	.066	.003
	24	18	55	3.93	0.22	0.990	.055	.074	.004
	26	22	91	3.42	0.16	0.517	.023	.119	.005
	28	22	91	4.31	0.20	0.499	.023	.126	.006
	30	16	94	3.05	0.13	0.336	.024	.083	.005

* The variation in the number of plants was due to development of smut in the seedling stage.

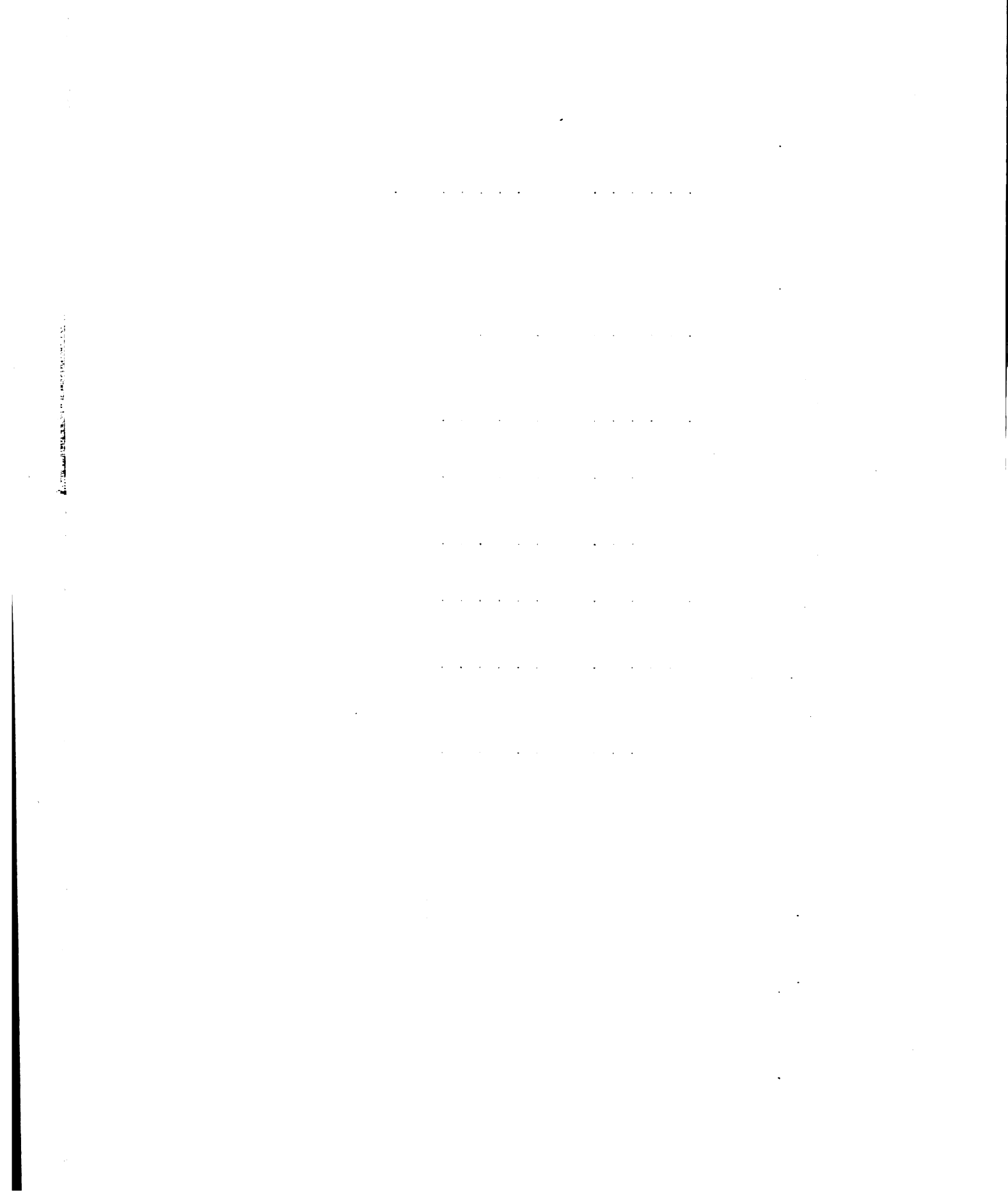
All the smutted plants were removed.

TABLE 4. The relation of soil temperature and soil moisture to the development of pink root in onions
Grown from seed in field infested soil for a period of 69 days.

Per cent Soil sat.	Soil Temp. °C.	Total No. plants	Per cent of plants infected	Dry wt. of		Dry wt. of roots - gms.		Per cent of	
				tops - gms.		Healthy	Diseased	Total wt. per plant healthy & diseased	total dry wt. of roots diseased
				Total	Ave. per plant	Total	Ave.		
70	20	17*	13	2.77	0.16	0.714	.043	.043	6.0
	22	17	41	2.91	0.17	0.750	.044	.064	7.9
	24	22	64	3.35	0.17	0.930	.042	.090	8.8
	26	19	100	1.88	0.10	0.156	.008	.096	27.9
	28	22	100	4.11	0.19	0.450	.020	.129	22.4
	30	22	100	3.23	0.15	0.537	.013	.122	23.8
80	20	12	25	1.73	0.14	0.359	.030	.010	2.6
	22	20	40	2.73	0.19	1.000	.050	.066	6.2
	24	18	55	3.93	0.22	0.990	.055	.074	6.9
	26	22	91	3.47	0.16	0.517	.023	.119	18.7
	28	22	91	4.31	0.20	0.499	.025	.126	20.4
	30	16	94	3.05	0.13	0.356	.024	.033	17.8

* The variation in the number of plants was due to development of smut in the seedling stage.

All the smutted plants were removed.



and more than 90 per cent were infected in the 80 per cent saturated soil. In contrast to this only 55 to 60 per cent of the plants were infected at 24° C. At 24° and 29° C. the average dry weight per plant was from 25 to 45 per cent greater than at 26° C.

A further examination of Table 4 reveals the relative amount of root infection. At 20° C. less than 6 per cent of the total dry weight of the roots was diseased, at 22° C. less than 8 per cent, and at 24° C. less than 9 per cent. At 26°, 29°, and 30° C., however, more than 17 per cent of the roots were diseased in the 80 per cent saturated soil and more than 22 per cent in the 70 per cent saturated soil.

The effect of pink root infection on onion roots is illustrated in Plate 2. This clearly shows the reduced number of roots on the plants grown in soil maintained at 26° and 28° C.

In this experiment infection was most severe in the plants growing in the 70 per cent saturated soil. In the previous experiment using onion transplants infection appeared to be slightly more severe in the 80 per cent than in the 70 per cent saturated soil, although the differences were slight. This was the only major difference in the results obtained in this and the previous experiment.

Infection of Onion in Artificially Infested Soil

The muck used in this experiment was obtained from an apparently pink root-free onion field near Kalamazoo, Michigan. It was steamed 8-10 hours and stored for a month before it was used. Oat cultures of a very pathogenic isolate of P. terrestris were used for soil infestation and the onions were seeded directly in this soil. The plants were harvested 68 days after seeding. The results of this experiment are given in Table 5.

1. *Chlorophyll a* and *b* contents were determined by the method of Lichtenthaler (1987).

TABLE 5. The relation of soil temperature and soil moisture to the infection of onions seeded directly in artificially infested soil and grown for a period of 68 days.

Temp. °C	No. of plants		Average dry wt. of tops per plant - gms.		Per cent of plants dead in infested soil
	Infested soil	Noninfested soil	Infested soil	Noninfested soil	
60 per cent saturated soil					
20	24	25	.058	.069	0
22	23	26	.039	.056	0
24	24	21	.056	.055	0
26	25	25	.017	.043	12
28	26	26	.008	.026	38
30	16	22	.008	.019	50
70 per cent saturated soil					
20	25	25	.086	.033	0
22	26	25	.079	.085	0
24	25	25	.049	.061	0
26	25	25	.021	.051	12
28	25	25	.013	.048	35
30	24	23	.005	.030	73
80 per cent saturated soil					
20	23	24	.103	.120	0
22	25	25	.105	.088	0
24	25	25	.076	.090	0
26	25	25	.022	.059	10
28	26	25	.014	.057	44
30	27	23	.000	.037	100

The fungus was most destructive in soil held at 30° C. At this temperature the largest number of plants were killed by pink root. At and below 24° C. there were no plants killed by the disease. At 26° C., 10-12 per cent of the plants were destroyed by the fungus; at 28° C., 35-44 per cent were killed; and at 30° C., 50 - 100 per cent were killed.

The plants growing in the 80 per cent saturated soil were more severely damaged than those at 70 per cent, and the plants at 60 per cent saturation were the least damaged. Indications were, however, that the amount of soil moisture influenced the critical temperature at which infection became serious. In the 60 per cent saturated soil the effects of the fungus were evident in the plants growing at 22° C. In the 70 per cent saturated series the effects did not become noticeable until the soil temperatures reached 24° C. In the 80 per cent saturated series the effects of the fungus infection did not become evident until the soil temperature was 26° C. There were indications of infection at 24° C., but these were very slight as compared to those in the 70 per cent saturated series at this same temperature. The effects of pink root infection were visible 45 days after the onions were seeded at all soil temperatures above 24° C. At this time all of the plants growing in the soil maintained at 30° C. and 80 per cent saturation had succumbed to the disease. The effects of these soil moistures and soil temperatures on the development of the disease are clearly illustrated in Plate IV.

TABLE 6. Analysis of variance of pink root infection in onions grown at soil temperatures of 24° and 26° C.

Source of variation	Degrees of Freedom	Sums of squares	Mean square	F value
Between temperatures (T)	1	1.81	1.81	78.7*
Between infested and noninfested soil (I)	1	1.51	1.51	65.6*
Between soil moistures (%)	2	1.39	0.70	31.6*
T x I	1	0.47	0.47	20.4*
T x %	2	0.59	0.30	12.8*
I x %	2	0.04	0.02	0.9
T x I x %	2	0.32	0.023	
Within	12			
Total	23	6.13		

* Statistically significant at 5% level of F.

Table 6 is an analysis of variance showing the significance of the various factors which entered into this experiment. The analysis is limited to the 24° and 26° C. soil temperatures. These soil temperatures were used because the experiments indicated they were the critical temperatures for infection. This analysis shows there is a significant difference between the results obtained at the two soil temperatures. The differences between the plants growing in the infested and noninfested soils are also significant. The difference between the infested and noninfested soils at 26° C. was so great that there is no doubt about its significance. On the other hand, the difference between these two factors was not as great at 24° C., but a test of significance of the means at this temperature indicated that the difference in weights of the plants is significant. In other words, the fungus does enough damage at this temperature to be important.

The analysis also shows that the interaction between the two soil temperatures (24° and 26° C.) and between the infested and noninfested soil is significant. This is not true for the interaction between the infested and noninfested soil and the various soil moistures.

DISCUSSION

The results obtained in the experiments just described indicate considerable difference in severity of infection of plants growing in naturally infested soil and artificially infested soil. In the naturally infested soil the plants were considerably stunted in growth at 26°, 28°, and 30° C.; but there were no plants killed directly by the fungus. On the other hand, several plants were killed at these temperatures in the artificially infested soil. It is possible that there would have been some mortality at these temperatures in the naturally infested soil had

the experiment been continued for a longer period. This was evident from an examination of the root system. The roots on many of the plants grown at a soil temperature of 26° C. were completely disintegrated at the time the plants were harvested.

It is very difficult to find an explanation for the differences in optimum soil temperatures for the infection of onions in naturally and artificially infested soil. In steamed soil, artificially infested, the optimum was 30° C., and in naturally infested field soil it was 26° C. The studies on the relation of temperature to the growth of Phoma terrestris indicated that the differences in the growth rate of the mycelia of the fungus were very slight at 26°, 28°, and 30° C. On two kinds of media the average radial growth was the same at 26° and 30° C. This would indicate that plants growing at soil temperatures of 26° and 30° C. should be equally susceptible to the disease providing other conditions are the same. There was, however, a difference in the growth and development of the onion at these two soil temperatures. The average dry weight of the onions growing in steamed soil was considerably less at 30° than at 26° C. It is possible that onion plants growing under the more unfavorable conditions (30° C.) are more susceptible to pink root than those growing under conditions more favorable for growth (26° C.). If this is so, then 30° C. would be the optimum soil temperature for the development of pink root in onion. This was true for the plants growing in the artificially infested steamed soil. The results obtained in the naturally infested soil do not comply with this hypothesis. In that case the competition among the soil microorganisms, which was practically eliminated in the artificially infested soil by steaming, may have been capable of limiting the activity of P. terrestris at the higher soil temperatures.

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The effect of the fungus on plant growth was much more evident at the lower soil temperatures in the artificially infested soil than in the naturally infested soil. In the naturally infested soil the effects of the fungus were not evident below 26° C., while in the artificially infested soil they were visible at 22° C. This noticeable effect at the lower temperatures in the artificially infested soil may be because of the presence of P. terrestris in the soil without a great deal of competition from the soil microflora, the soil microflora having previously been reduced in number by thoroughly steaming the soil.

The effects of P. terrestris upon onion development are summarized in Figure 1. This chart shows graphically the relation of soil temperature and soil moisture to the growth of onions in noninfested soil, and also the relation of these factors to infection in artificially infested soil. The one thing that is especially noticeable on this chart is the sudden increase in the amount of pink root infection at 26° C. This is further illustrated by a comparison of the plants shown in Plate III and IV. These two plates are directly comparable.

This sharp line of demarcation in the amount of pink root infection at soil temperatures between 24° and 26° C. can be partially explained on the basis of different optimum temperatures for the fungus and for the host. The onion plant grows well at or below 24° C. It is hindered in its development by higher soil temperatures. Phoma terrestris made its best growth at 26°, 28°, and 30° C. (Plate I). Below 26° C. its growth rate was retarded by the lower temperatures. The difference between the optimum temperature for the growth of the fungus and the host was from 6-8° C., depending upon the soil conditions under which the plants were growing. It is highly probable that onion plants growing under favorable environmental conditions are less susceptible to disease than those

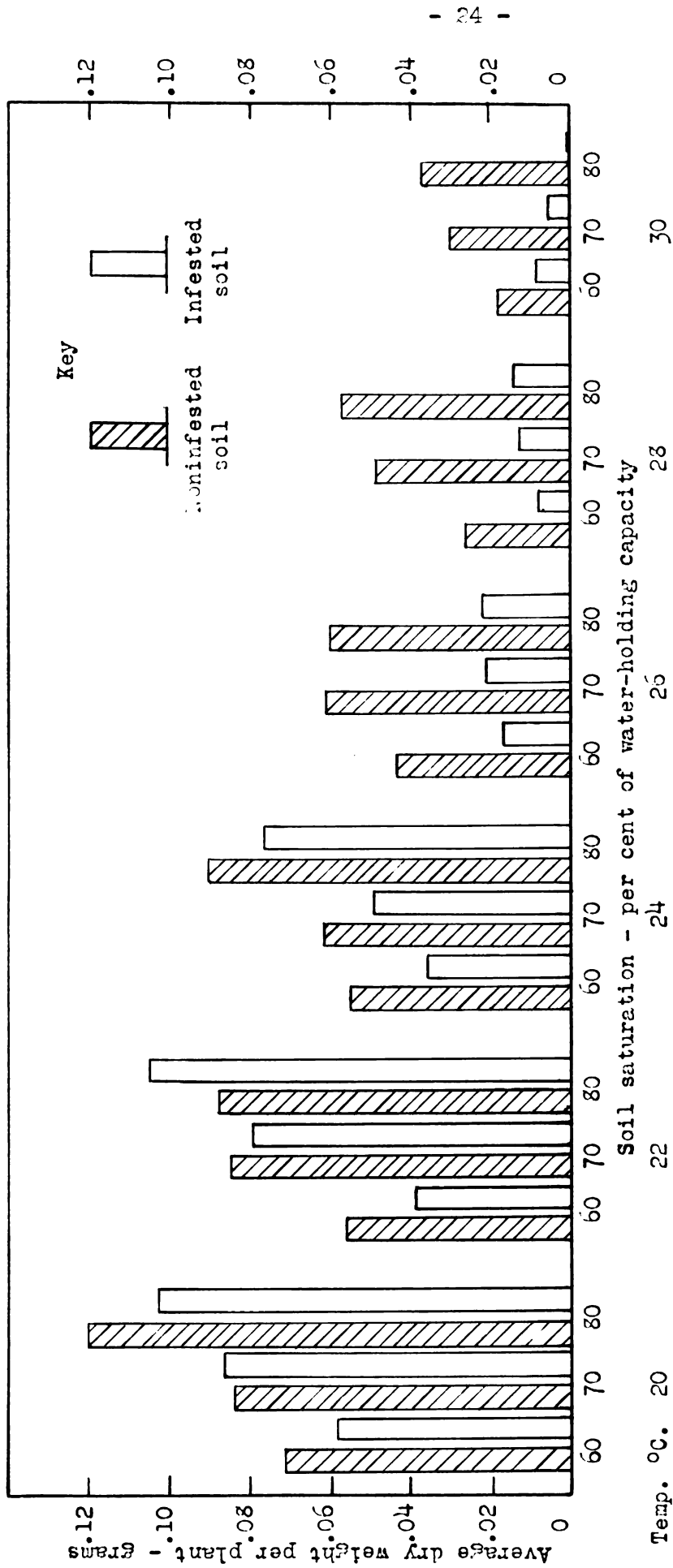


FIGURE 1. The relation of soil temperature and soil moisture to the growth of onion in noninfested soil and to the infection of onion by *Phoma terrestris* in artificially infested soil. Onions grown from seed for 68 days.

growing under unfavorable conditions. It was shown in the experiments on environmental relations that P. terrestris was more infectious when growing under optimum conditions for its development than when growing under unfavorable conditions. Therefore, a logical explanation for this phenomenon can be based on the interaction between two different optimum temperatures.

It is evident from the results obtained in the experiments on the relation of environment to the disease that pink root does not become serious until the soil temperatures approach 26° C. This would indicate that pink root may become epidemic in any region where the causal fungus is present in the soil, provided the soil temperatures have remained favorable for infection over an extended period. In Michigan this occurs nearly every year because hot, dry weather is experienced during the later part of July and the early part of August when the late onion crop is setting bulbs.

The summer of 1940 was no exception. Pink root was epidemic in the older onion growing areas and caused serious losses. At Kalamazoo, Michigan, the minimum temperatures from July 18 to August 18 rarely fell below 15.5° C., and the lowest maximum temperature for any day during this same period was not below 26.6° C. Because of the unseasonably high temperatures, few onion fields infested with the pink root fungus escaped injury from the disease.

The condition of two large adjacent fields near Eaton Rapids, illustrates the seriousness of pink root during an epidemic year. The fields were similar except that pink root was found in only one. Both were adequately fertilized. The field free from the disease was still green at the end of the first week in September. At that time the tops were rolled down to hasten maturity of the plants, a common commercial practice. The bulbs were large and were held firmly in the ground by

a healthy root system. At harvest time this field yielded approximately 700 crates to the acre, and if the onions had been allowed to mature naturally the yield would probably have been much greater. On the other hand, the crop on the diseased field had ripened prematurely by the last week in August. The bulbs were small and it was practically impossible to find a healthy root on them. This field yielded approximately 300 crates of onions to the acre. Figuring the value of onions per crate at \$1.00, the loss per acre was at least \$300.00.

SUMMARY

Evidence has been presented to show that P. terrestris grows well at temperatures from 26° to 30° C. with an optimum at 28° C. The maximum temperature was between 32° and 34° C., and the minimum was slightly below 10° C.

The radial growth of the mycelium of P. terrestris on culture plates was not greatly influenced by different hydrogen-ion concentrations. The fungus grew well within the range of pH 4 and pH 8, with the optimum between pH 5 and pH 6.

The Downing Yellow Globe onion made its best growth and development at soil temperatures from 18° to 24° C. in 70 and 80 per cent saturated soil.

Onion plants grown directly from seed in naturally infested soil developed pink root most rapidly at 26° C. when the soil moisture content was maintained at 70 per cent of its water-holding capacity. Onion transplants did not show a comparable amount of infection until the soil moisture was brought up to 80 per cent of its water-holding capacity. In steamed soil infested with P. terrestris the optimum soil

temperature for infection of the onion roots was 30° C., and the optimum soil moisture content was at 80 per cent of its water-holding capacity.

The marked increase in the amount of infection in plants growing at a soil temperature of 26° C. can be partially explained on the basis of different optimum temperatures for the growth of the fungus and of the host.

The disease is likely to reach epidemic proportions in any field where the fungus is already established, if the soil temperatures approach 26° C. and remain there for an extended period of time.

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PLATE I.

The relation of temperature to the growth of Phoma terrestris.

Phoma terrestris grown on cornmeal agar for 11 days at 10°, 14°, 18°, 20°, 22°, 24°, 26°, 28°, 30°, and 32° C. The maximum growth occurred at 28° C.

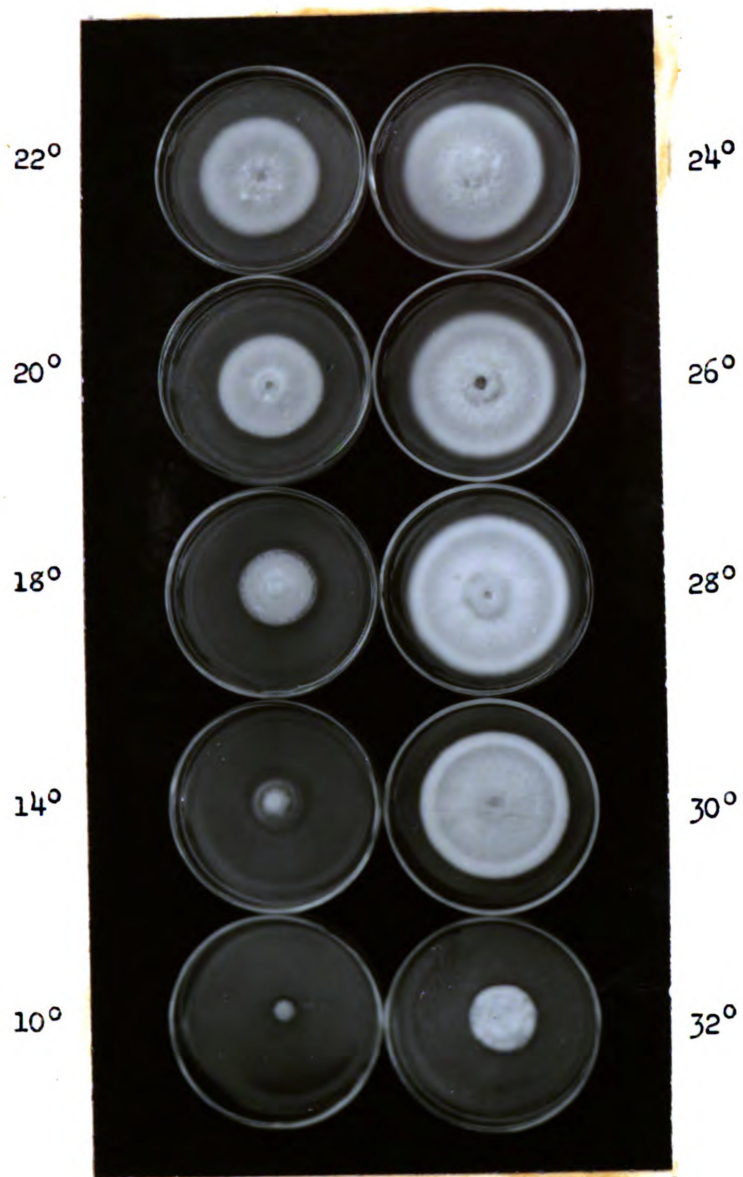


PLATE I

PLATE II.

The relation of soil temperature and soil moisture to the infection of onion roots by P. terrestris.

The roots are from plants grown from seed in naturally infested field soil for 69 days at 20°, 22°, 24°, 26°, 28°, and 30° C. with moisture contents of 60, 70, and 80 per cent of its water-holding capacity. Each moisture series shows one root system from each of the soil temperatures. Note the reduced root systems at 26° and 28° C. In this soil the optimum temperature for infection on onion roots was 26° C.

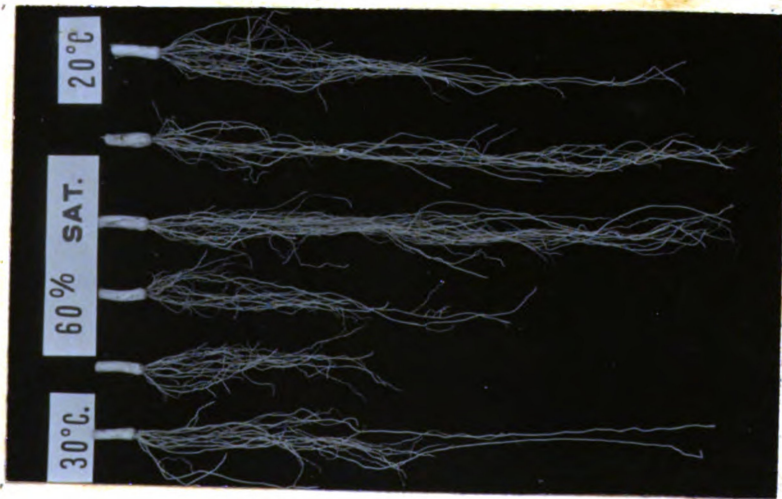
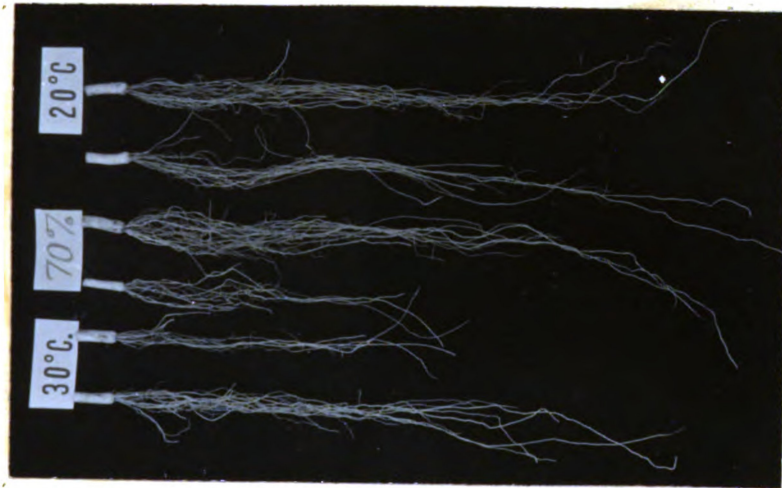
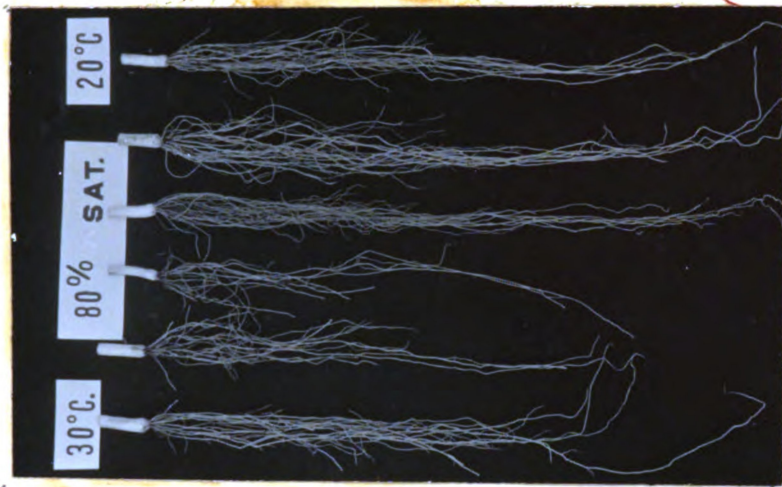


PLATE II

PLATE III.

The relation of soil temperature and soil moisture to the growth and development of the onion.

The plants were seeded directly in the soil cans and grown for 68 days. The soil was thoroughly steamed a month before using and was well fertilized prior to seeding. The plants were grown at the same soil temperatures and soil moistures as described for Plate II. The best growth occurred in the soil maintained at 80 per cent saturation and at temperatures from 20° - 24° C., inclusive.

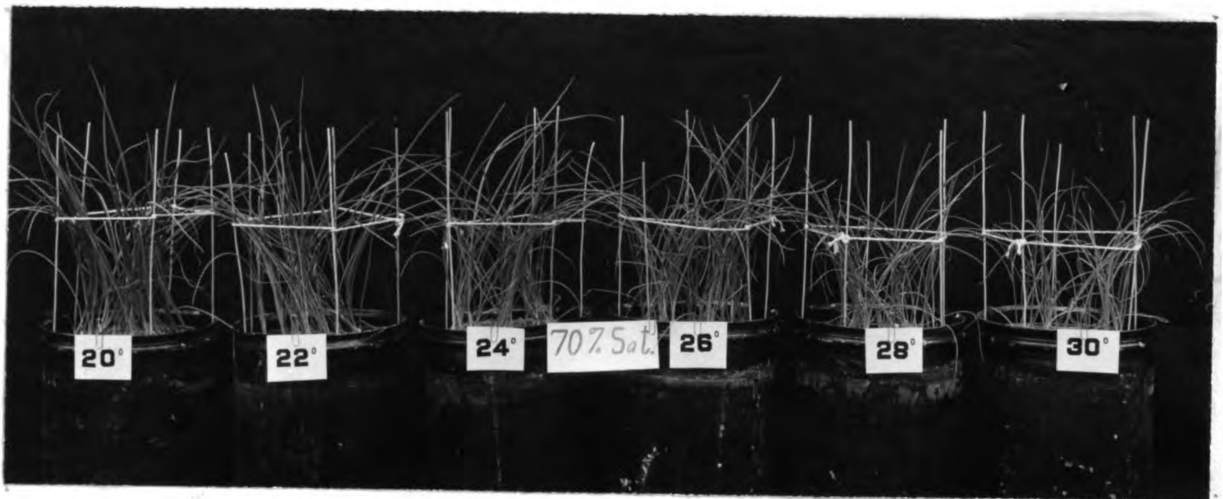


PLATE III

PLATE IV.

The relation of soil temperature and soil moisture to the development of pink root in onions grown in artificially infested soil.

The plants were grown directly from seed in soil infested with oat cultures of a pathogenic isolate of P. terrestris. The plants were grown under the same environmental conditions as those shown in Plate III. The only difference between the two sets is that the soil in one (Plate III) was not infested with the fungus. Heavy infection occurred at soil temperatures from 26° to 30° C., inclusive, with the heaviest infection at 30° C.

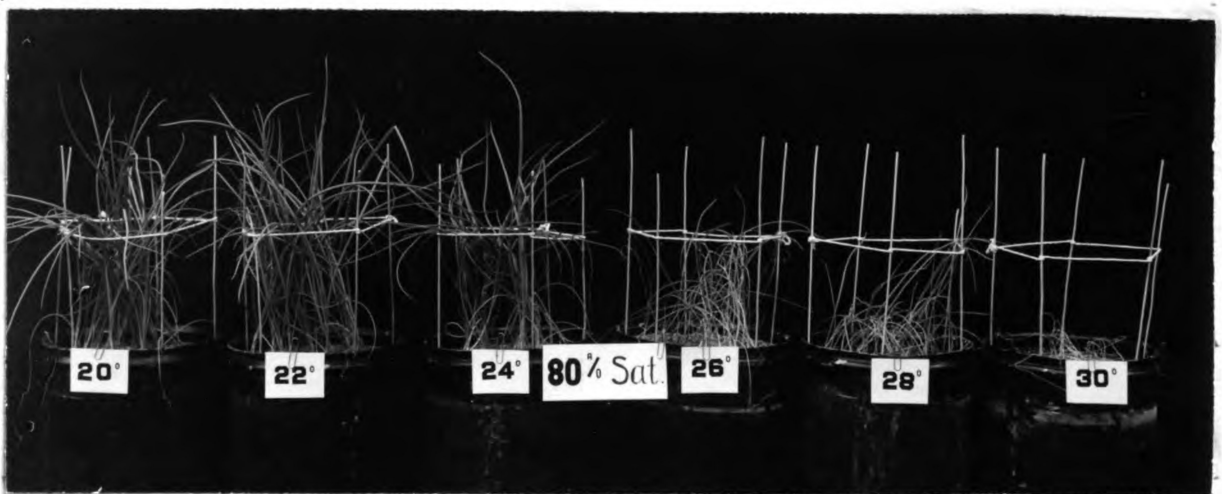
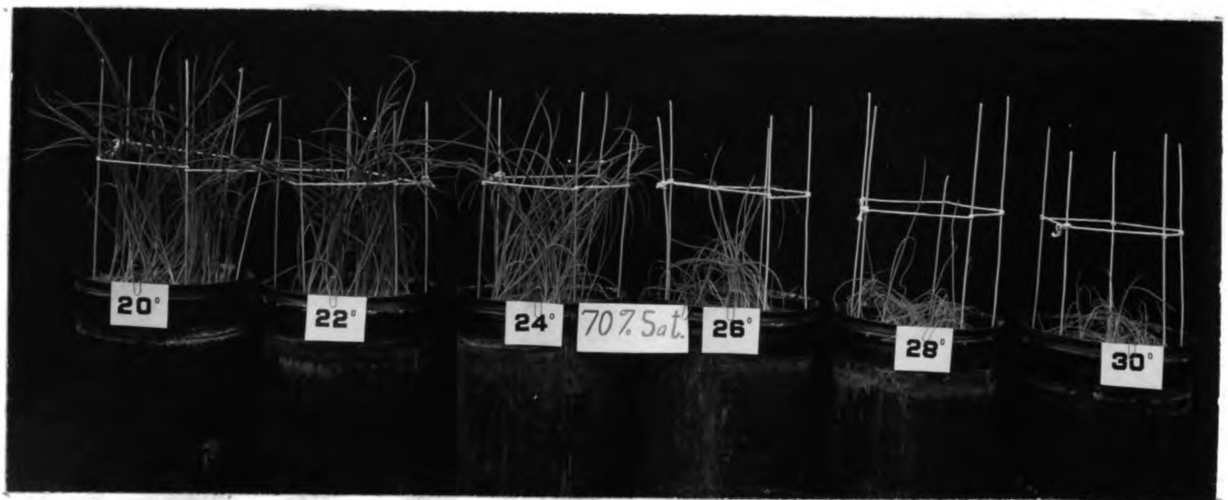
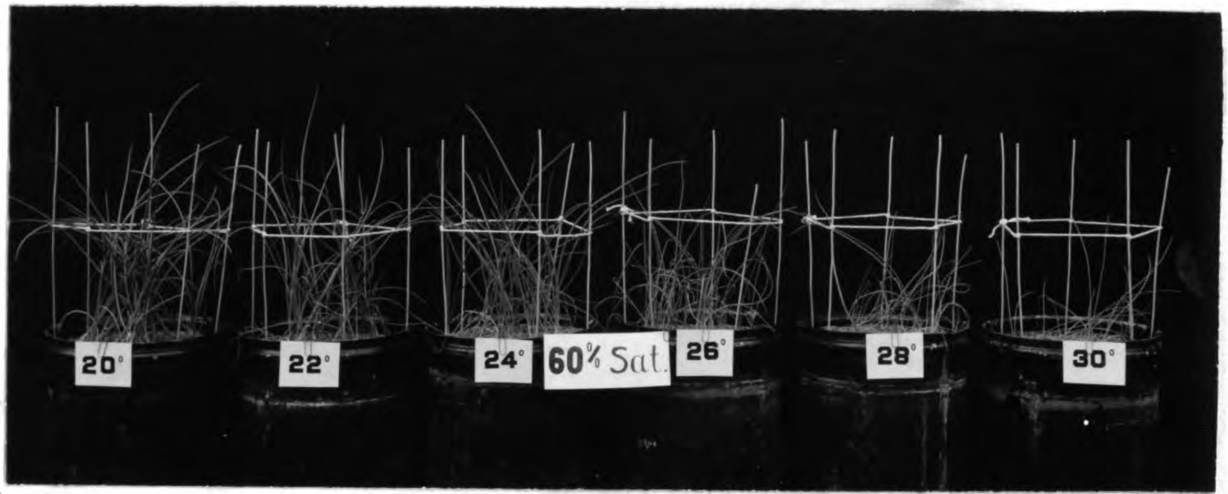


PLATE IV

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