THE RELATION OF MOISTURE AND TEMPERATURE TO GROWTH OF THE COTTON ROOT ROT FUNGUS[1]

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INTRODUCTION

It is a matter of general observation by farmers and research workers that the spread of the cotton root rot fungus (*Phymatotrichum omnivorum* (Shear) Duggar) on cotton and other susceptible crops, and their resulting death, varies during the growing season to a rather large extent with available soil moisture. The disease spreads rapidly in hot weather following sufficient rainfall—that is, rains of about an inch or more. Taubenhaus and Dana (*8*)[2] analyzed air-temperature, rainfall, and air-humidity data as correlated with the percent of cotton plants killed by root rot during the years 1923, 1924, 1925, and 1926. They concluded that of the three factors, humidity has no direct influence; rainfall is outstanding in its importance; temperature for the crop-producing portion of the season is favorable to the development of the disease, and toward the end of the growing season lowered temperatures reduce root rot in severity in spite of favorable moisture. Taubenhaus and Ezekiel (*9*) reported a slow spread of the fungus from root to root throughout the winter to February 20 in two different fields near College Station, Tex.

To obtain a more direct relationship of moisture and temperature, especially the latter, to growth of the root-rot fungus, experiments were conducted under controlled conditions during the winters of 1931–32, 1932–33, and 1933–34. This paper gives the results of these studies.

METHODS

MOISTURE STUDIES

To determine the effect of moisture on the growth of the fungus and production of sclerotia, half-gallon size fruit jars were filled with soil of varying moisture content and inoculated with pieces of freshly infected cotton roots. The surface soil of Houston black clay was air-dried in the greenhouse for a few days, being turned at intervals to secure uniform drying. The moisture content of the air-dried soil was usually around 8 percent, oven-dry basis. In these experiments the cultures were set up so that soil moistures of 15, 20, 25, 30, 35, 40, and 45 percent, on the oven-dry basis, were obtained. These are equivalent to 21, 28, 35, 42, 49, 55, and 62 percent, respectively, of the maximum water-holding capacity as determined by the Hilgard method. Since water was mixed in an open container with the soil, a small amount was added in excess to take care of evaporation during the mixing process and while the soil was being transferred to jars,

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[2] Italic numbers in parentheses refer to Literature Cited, p. 708.

Samples were taken from each batch of soil to check the moisture content. All soil was rubbed through a quarter-inch mesh sieve while it was being mixed. After half a jar was filled with soil, five pieces of infected root inoculum were stuck vertically in the jar, and the remainder of the soil was added. In the case of higher moisture percentages, the soil moisture was made to about 25 percent by hand, and the remainder of the water added directly to the jar containing the soil.

Fungus growth was measured by methods described under Growth Measurements.

TEMPERATURE STUDIES

For making the temperature studies a water bath was constructed so that a series of nine different temperatures could be obtained at the same time. Sectional views of the apparatus are shown in figure 1. This equipment was constructed by making a single box of 20-gage galvanized iron, 126 inches long, and adding partitions of the same material so that nine compartments were formed, each 14 by 14 inches.

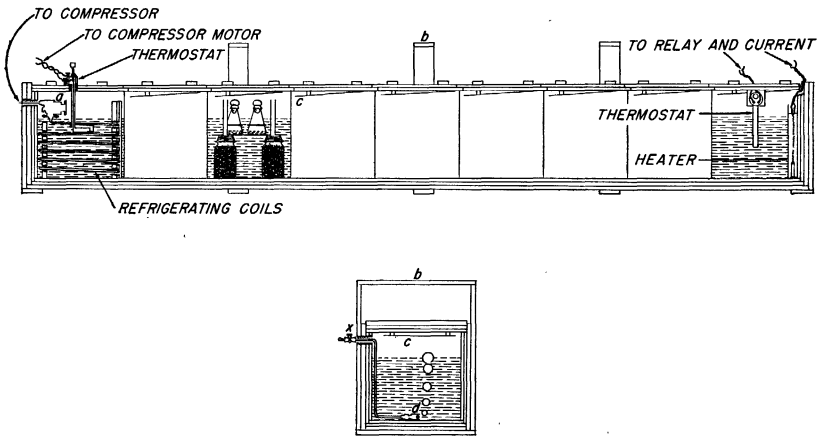

FIGURE 1.—Sectional views of water-bath apparatus used in making temperature studies.

Around this two layers of ½-inch Celotex were added and boxed in with 1-inch thick shiplap. Small slats were nailed on the bottom, around the sides, and across the top to support the box off the floor and to hold the shiplap against the Celotex (fig. 1, *b*). Covers for each compartment were made of two pieces of ½-inch Celotex held together by small slats. A piece of sheet iron was fastened at an angle on the under side of each cover so that water of condensation drained off to one side (fig. 1, *c*). Refrigerating coils were placed in one end of the box and connected with an ordinary household refrigerant compressor and motor assembly. Beginning with the expansion valve (fig. 1, *a*) coiling was begun at the top so that the coldest part of the coils was at the top of the water bath. Refrigeration was controlled by a bimetallic thermostat. In the opposite end high temperatures were obtained by housing the element from an electric oven in a copper box and connecting with the current through a relay of the type used on electric ovens and a pencil-type bimetallic ther-

mostat. In this manner a series of different temperatures resulted across the nine compartments that varied with the change of the two thermostats. Agitation was obtained in each water bath by compressed air. An air line, made of copper tubing connected with an automatic air compressor, provided air for each compartment through an inlet of the same size tubing connected with heavy-walled rubber hose and controlled by a pinch clamp (fig. 1, *x*). A small boxlike piece of sheet iron (fig. 1, *d*) was soldered to the end of each inlet at the bottom of its respective compartment and tilted at a slight angle downward. In this way a large amount of air accumulated in the small box before a bubble escaped. As a result of this, the bubbles were large and adjustments were made so that they escaped at the rate of about one each 5 seconds. Pressure in the air line was kept at 15 pounds by a regulator to which a filter and gage were attached. During the course of each experiment the entire apparatus, shaded with opaque paper, was housed in the greenhouse thermostatically controlled at 65° F. The temperature in each compartment seldom varied more than 1° C. The apparatus as set up in the greenhouse is shown in figure 2.

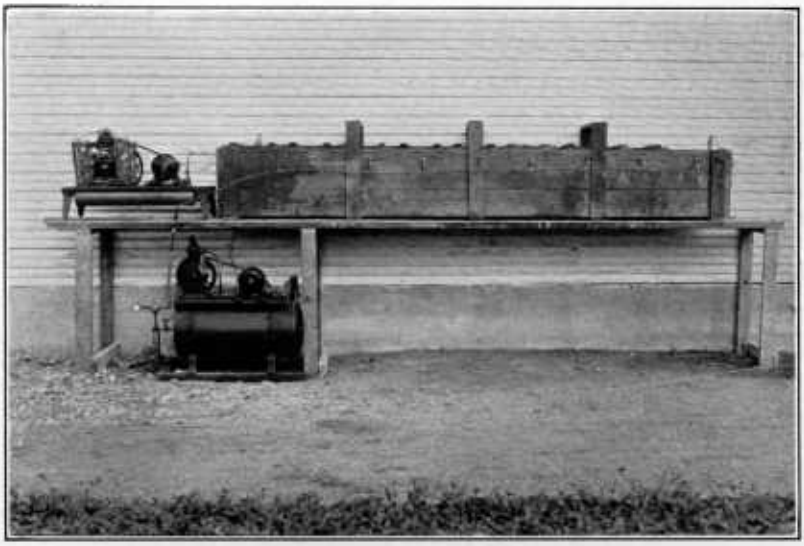

FIGURE 2.—Apparatus used for making temperature studies as set up in the greenhouse.

Growth responses were obtained in soil cultures in jars and in pure cultures on liquid media at the same time. Holes were punctured in the jar lids, and a 6-inch tube was fastened thereto so that it extended above the water line. A piece of hardware cloth was set on top of the jars, and the pure cultures in Erlenmeyer flasks were set on top of the hardware cloth. Each flask was weighted down with a lead ring. The jar cultures were made similar to those described under the moisture tests, and dry weights were obtained from liquid cultures made by using a medium constituted as follows: Water 1,000 cc, ammonium nitrate 1.2 g, potassium phosphate (secondary) 1.4 g, magnesium sul-

phate 0.8 g, potassium chloride 0.15 g, sucrose 30g, starch 30 g. Fifty cubic centimeters of this medium, which was made to pH 6.8, was used per flask and inoculated with small cubes of agar about 2 mm thick, containing young mycelial growth. Five jars and six flasks were used in each compartment for each experiment. At times *Rhizopus* or other fungi grew through and produced spores on the bottom of the cotton plugs. Such contamination was prevented by flaming the plugs and covering with sterilized sheet dental rubber.

GROWTH MEASUREMENTS

Whether *Phymatotrichum omnivorum* is growing superficially on culture media or along roots, compound mycelial strands are formed over the entire surface. These structures have been described previously (*1*, *2*, *6*). Toward maturity they assume a buff or brown color and may extend through the soil as much as 8 or 10 inches in either direction. The number and length of these strands as shown on the walls of the glass jars was used as one measurement of growth response. This applied only to the soil cultures in both moisture and temperature studies. Sclerotia are formed at intervals in strands by the compounding and compacting of hyphae. They are of great importance in making studies of cotton root rot because of their ability to remain in a viable state for many years and the fact that they may be formed in the soil at depths up to 4 feet or more. The number of sclerotia produced was used as another measurement of growth response in the soil-moisture and temperature cultures. Sclerotia were separated from the soil by methods previously described (*3*). In the pure cultures on liquid media growth was measured by obtaining dry weights of fungus, including the mat growing directly in and on the media and the strand mycelium and sclerotia growing on the flask walls.

RESULTS

SOIL-MOISTURE STUDIES

Production of strands and sclerotia in four different soil-moisture experiments are given in table 1. In the data the number and length of strands represent the average per jar in each individual test, set up in triplicate or quadruplicate for each different moisture percentage. These particular tests were conducted at different times during a 3-year period. The variation from test to test might therefore be caused by any one or all of three or four different factors, primarily differences in temperature from season to season and some differences in inoculum for the different years, or differences within the same year in age of plants, since in all cases cotton roots were used as the source of inoculum. Strands were produced in abundance in even the comparatively dry soil, diminishing rather sharply at and above 35-percent moisture content.

Sclerotia were produced somewhat sparingly even in those soils with optimum moisture for strand production. The optimum for both strand and sclerotial production seems to be from 25 to 30 percent. The number and length of strands is not always indicative of the growth in any culture. These strands branch rather freely in a dichotomous manner. Sometimes a single strand will cover two sides or more of a half-gallon jar. Over a number of cultures, however, the

number and length of strands is indicative of the growth response under any given set of conditions. Sclerotia and strands in the drier cultures had a downy covering of hyphae of the acicular type. In the soils having higher moisture percentages, especially those nearing soil saturation, the strands were smoother and did not show much of the acicular-hyphae covering.

TABLE 1.—*Production of strands and sclerotia in soil cultures with quantities of water varying from 8 to 40 percent* [1]

Duration of experiment (days)		15 percent	20 percent	25 percent	30 percent	35 percent
5	Strands—number	1.0	3.0	4.3	0.7	1.3
	Strand length—centimeters	.8	.3	1.0	.3	.7
	Sclerotia—number	.0	.0	.0	.0	.0
24	Strands—do	9.8	5.8	12.0	8.5	2.5
	Strand length—centimeters	6.4	4.1	6.3	4.8	1.6
	Sclerotia—number	11.0	4.0	2.0	.0	.0
72	Strands—do	12.3	10.3	7.3	2.3	.3
	Strand length—centimeters	8.3	7.3	8.8	14.3	.7
	Sclerotia—number	5.0	.0	10.0	91.0	.0
120	Strands—do	1.7	6.0	8.7	4.7	6.3
	Strand length—centimeters	1.4	5.4	6.2	6.1	9.8
	Sclerotia—number	.0	.0	5.0	.0	.0

[1] No growth at 8-percent, air-dry, nor at 40-percent moisture.

TEMPERATURE STUDIES

The average weights of mycelial growth per 250-cc flask from four different temperature experiments are shown in tables 2 and 3. In the last two experiments (table 3) the heat-control thermostat was changed from 50° to 45° C.

TABLE 2.—*Average dry weight of root-rot fungus per 250-cc flask after 10 and 20 days growth at different temperatures from 3° to 50° C.* [1]

Growth period (days)	Dry weight when grown at—			
	19°	24°	29°	34°
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
10	7	99	139	127
20	45	403	506	404

[1] No growth at 3°, 12°, 39°, 44°, or 50°C.

TABLE 3.—*Average dry weight of root-rot fungus per 250-cc flask after 10 and 60 days growth at different temperatures from 3° to 45° C.* [1]

Growth period (days)	Dry weight when grown at—					
	11°	18°	23°	27°	32°	37°
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
10	0	6	27	170	218	4
60	30	336	492	830	390	15

[1] No growth at 3°, 42°, or 45° C.

The optimum range for fungus growth is from approximately 23° to 34° C., the middle of this range being 27° to 29°, as shown by the experimental results. In the first experiment an average of 506 mg of fungus was obtained after a 20-day growth period at 29°. In the last experiment at the end of 60 days an average of 830 mg was obtained at 27°, these figures representing the maximum growth in each experiment. At all times in the 3° bath there was a rim of ice on the coils that almost reached the center of the compartment. Therefore the flasks in this compartment were surrounded by ice at least part of the time. Hyphae in the flasks in this coldest chamber grew about 2 mm during the first 2 days but stopped thereafter, and the tips seemed to curl backward in a somewhat hooked manner. Although there was no growth under such low-temperature conditions, the fungus readily resumed growth when transferred to warmer baths at 27° or 29° even at the end of the longest period of 60 days. At the higher temperatures the effect was directly opposite. At 39° or above no growth was obtained, and when cultures were transferred to 27° or 29° baths there was no resumption of growth, even at the end of the shortest experimental period of 10 days. This indicates that all fungus growth had been killed.

Microscopic examination of mycelial growth showed no general morphological differences. There was one striking difference in appearance. At the lower temperatures up to 19° C. the mycelium remained almost white, never getting darker than a light amber color. With increase in temperature the entire mycelium showed a darker color regardless of the amount of growth. The same thing held true in the case of the soil cultures at different temperatures. Both strands and sclerotia were formed with the light or whitish color that the mycelium usually exhibits in the younger stages of growth, retaining this light color throughout the experimental period. Throughout the optimum-growth range the fungus exhibited characteristics typical of those observed under field or ordinary laboratory conditions—that is, at first there was a whitish web which gradually increased in size and became darker until the brown or buff color of maturity was attained. At the higher temperatures, at and above 34°, the mycelium was first formed with the dark color of maturity and retained this or became darker throughout each growth period Color, therefore, is not necessarily an indicator of age of sclerotia, as is generally thought to be the case. In field areas almost all light-colored sclerotia are found in the late fall or early winter after cool weather has begun.

The number of sclerotia produced at different temperatures after 30-, 60-, and 80-day growth periods are shown in table 4. Each of these different periods represents a separate experiment, all the jars in each chamber being analyzed for sclerotia for each separate experiment. Where sclerotia are formed under such conditions they are usually produced within the first 30 days and certainly none after 80 days, because the inoculum or substratum has been depleted and decomposition has set in. Optimum temperature for sclerotial formation is somewhat the same as that for general mycelial growth, although in the 80-day experiment the greatest abundance of sclerotia was produced at 18° C. The greatest amount of strand formation and sclerotial production was obtained at 27°.

TABLE 4.—*Production of root-rot sclerotia in soil cultures at different temperatures from 3° to 45°C. after 30, 60, and 80 days* [1]

Growth period (days)	Number of sclerotia when grown at—					
	11°	18°	23°	27°	32°	37°
30	0.0	0.0	95.0	207.0	29.0	0.0
60	.0	.0	.0	209.0	8.0	52.0
80	66.2	293.8	131.6	147.2	12.0	8.8

[1] No growth at 3°, 42°, or 45° C.

DISCUSSION

As pointed out by Taubenhaus and Dana (*8*) and as commonly observed, moisture is the main limiting factor for root-rot spread during the growing season. The temperature is sufficiently high from May through October for ready growth of *Phymatotrichum omnivorum*. Usually by the first of May the ground is sufficiently warm for rapid spread of the fungus, if host material is present in a fairly dense stand. After rains in July, August, and September there is usually a rapid spread of root rot in cottonfields as evidenced by the wilting and speedy death of the cotton plants. In years of low soil moisture throughout the growing season root rot is held in check. In such years there may be a general kill of 25 percent, whereas in years of intermittent rainfall throughout the growing season the amount of cotton killed may be as high as 50 percent. Early or midseason killing of cotton plants results in low yields (*5*).

These moisture and temperature experiments and general field observations indicate that *Phymatotrichum omnivorum* thrives over a rather wide range of moisture and temperature conditions. The fungus is, however, readily affected by high temperatures and low moisture conditions. This accounts for the fact that no mycelial strands and sclerotia are found in the upper 6 inches of soil except in periods of warm wet weather. At such times the white mycelium may be found or seen growing as a collar around the stalks of cotton or other host plant just at the ground line. It is also during these periods that the mycelium grows to the surface and produces the downy conidial mats which may vary from the size of a dime to 18 inches or more in diameter. These structures are formed in the shade of low-growing field crops or sometimes under bushes or trees where the soil is not subject to the drying effect of the sun's rays and remains moist for a few days. Sclerotia have been found thus far to remain in a viable condition for 8 years in the soil (*4, p. 204; 7*). If these bodies are left exposed in the open, they lose their viability within a very short period, usually less than an hour, depending on air temperature and humidity. There is a decided difference in this respect between the sclerotia of *Phymatotrichum omnivorum* and those produced by certain other fungi. The mycelial strands also rapidly lose their viability under the same conditions.

Taubenhaus and Ezekiel (*9*) found that there was a spread of root rot along the cotton roots throughout the winter near College Station, Tex. This district is near the lower end of the blackland section, the section where cotton root rot is worst, but it is quite probable that

such spread occurs in all parts of the blackland. Soil temperature records taken at the Temple Substation at depths of from 2 to 48 inches show that at the 18-inch depth or deeper the temperatures are never too cold for growth of *Phymatotrichum omnivorum*. There is no doubt, however, that growth is slowed down greatly during the winter. Host material is the main limiting factor in the spread of the fungus. Active root rot has not been found earlier than April on winter annual weeds. The growth reported by Taubenhaus and Ezekiel was on old cotton plants growing in the field from the previous season. It is necessary that cotton plants become fairly well established before attacks by the fungus occur, since no seedlings have been found dying from the cotton root rot disease. This is partially owing to the fact that there is little or no mycelial growth in the upper layers of the soil.

SUMMARY

Mycelial strands of the cotton root rot fungus (*Phymatotrichum omnivorum* (Shear) Duggar) grew in Houston black clay soil in which the soil moisture varied from 15 to 35 percent, oven-dry basis. The optimum moisture content was around 25 percent, which is equivalent to 35 percent of the maximum water-holding capacity of the soil. Sclerotia of *P. omnivorum* were produced at a soil moisture content of from 15 to 30 percent. Neither sclerotia nor mycelial strands grew at or below 8 percent or above 35 percent soil moisture.

Both mycelial strands and sclerotia were produced in constant-temperature water baths at temperatures of from 11° to 37° C., the optimum for both being approximately 27°.

At the lowest experimental temperature of 3°, there was no active growth, but apparently the fungus was not injured. Temperatures of 39° and above resulted in the death of the fungus.

At the lower temperatures both the sclerotia and mycelium formed were of a whitish-amber color. At the minimum temperature at which growth occurred this light color was retained during the entire experimental period.

At higher temperatures both sclerotia and mycelium were formed with the usual dark color of maturity, as found in specimens taken from field soil.

Both mycelial strands and sclerotia were rapidly killed by exposure to high temperatures or to drying, such as takes place under ordinary field conditions in the summer.

Temperatures throughout the crop-growing season are favorable for growth and spread of *Phymatotrichum omnivorum*. During this period, moisture is the limiting factor, intermittent rains of sufficient quantities causing rapid spread of the fungus, a large proportion of the cotton plants being killed by the end of the season.

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