Influence of Temperature on Phymatotrichum Sclerotial Formation and Disease Development

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ABSTRACT

Phymatotrichum omnivorum cultured in sterilized soil containing grain sorghum formed sclerotia at temperatures of 15 to 35 C. Sclerotial production reached a plateau after 8 weeks at 30 C and after 11 weeks at 20 C. When sclerotia were used as inoculum to infest soil, *Phymatotrichum* root rot developed on cotton more rapidly at 27 C than at 22 C. No disease developed at 12 or 17 C.

More sclerotia were recovered from artificially infested soils held at 12 C than at 17, 22, or 27 C. At 27 C, these sclerotia germinated and caused root rot of cotton. Phytopathology 61:728-730.

Phymatotrichum root rot of cotton is widespread throughout the calcareous soils of southwestern United States and northern Mexico. Its distribution within this region is believed to be limited by soil moisture and soil temperature (2, 3, 4, 7, 10). Development of *Phymatotrichum* root rot is enhanced by soil temperatures near 28 C, which is near the optimum temperature for growth of *Phymatotrichum omnivorum* (Shear) Duggar. Presley & Bird (8) stated, "the root rot pathogen requires soil temperatures above 80° F for growth".

Taubenhaus & Dana (12) compiled weather records for a 5-year period and observed that *Phymatotrichum* root rot of cotton was most severe when air temperatures were near 27 C. Disease development declined rapidly as air temperatures dropped. Chavez et al. (1) believed failure to obtain *Phymatotrichum* root rot under greenhouse conditions in Arizona could be attributed to high air temperature, which sometimes exceeded 45 C. The disease is prevalent in Texas throughout areas in which the mean annual temperature is above 15 C, and with a frost-free period of at least 200 days. It does not occur to any extent in those regions with a lower mean temperature (4).

The fungus produces sclerotia which serve to perpetuate the organism in the absence of a susceptible host. The minimum, optimum, and maximum soil temperatures for sclerotial production by *P. omnivorum* were reported to be 18, 29, and 36 C, respectively (5). Different results were reported by Rogers (9, 10), who found during an 80-day test that the maximum number of sclerotia were produced at 18 C. He found that a few sclerotia were also produced at 11 C. In tests of shorter duration, optimum temperature for sclerotial formation was about the same as for mycelial growth of the fungus (27 C).

It is our observation that *Phymatotrichum* root rot occurs earlier in the season on perennial crops than it does on annual crops, which indicates that temperature may not be the limiting factor for disease development.

Experiments were conducted to establish the temperature limits for sclerotial production in soil, and to define the optimum soil temperature for disease development with sclerotia as the primary source of inoculum.

MATERIALS AND METHODS.—*Temperature range for sclerotial production.*—Sclerotia were produced in sterile soil culture, using a procedure described previously (6). Wide-mouthed pint jars containing screened Houston black clay (100 g), sorghum seed (10 g), and water (45 ml) were autoclaved at 20 psi steam, 131 C for 30 min. After cooling, the sorghum seed were infested with a small agar disc containing mycelium of *P. omnivorum*. Thirty-six jars were placed in each of seven incubators held at seven different temperatures ranging from 10 to 35 C at 5-degree intervals, and at 28 C. Three jars were removed from each incubator at weekly intervals for 12 weeks. The sclerotia were separated from the soil by wet-sieving. The soil was passed through a 16-mesh (U.S. Standard Sieve Series No. 18). A considerable quantity of snail shell fragments from the soil was retained on the sieve with the sclerotia. The total oven-dry wt of sclerotia and shell fragments was determined; then water was added to float the sclerotia away from the snail shell fragments. Dry wt of the residual material was determined by oven-drying at 100 C for 24 hr. Dry wt of the sclerotia was taken as the difference between total wt and wt of residual material.

Temperature influence on disease development and sclerotial survival.—Air-dried sclerotia, produced as described above, were mixed uniformly into sieved, Houston black clay (3.5 kg) at the rate of 1 sclerotium/g of soil. The infested soil was placed into 112 rectangular, galvanized containers (4.5 × 5 × 7 inches). Twenty-eight of the containers were placed into each of four constant temperature, water-bath tanks. Soil temperatures of 12, 17, 22, and 27 C were maintained for 83 days, at which time the temperatures of the 12 and 17 C tanks were elevated to 27 C for an additional 35 days.

Five cottonseeds (Lankart 611) were planted in each

TABLE 1. Dry wt of *Phymatotrichum* sclerotia formed in soil cultures at various temperatures over a 12-week period[a]

Temp	Weeks											
	1	2	3	4	5	6	7	8	9	10	11	12
C	*dry wt, g*											
10	0.0 a[b]	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
15	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.2 a	0.1 a	0.0 a	0.5 a	0.1 a	1.0 a	3.3 b
20	0.0 a	0.0 a	1.0 b	2.6 b	3.2 b	4.4 b	6.0 b	6.9 b	8.7 c	8.7 b	9.6 c	9.8 e
25	0.0 a	1.2 b	2.8 c	4.1 c	5.5 cd	7.0 cd	8.8 cd	8.5 c	8.7 c	9.4 b	9.0 c	9.1 d
28	0.0 a	2.0 c	2.8 c	4.7 c	6.1 d	7.2 cd	8.1 c	9.1 c	9.6 c	9.3 b	10.1 c	9.1 d
30	0.0 a	2.1 c	3.7 c	4.1 c	6.0 d	7.5 d	8.9 d	9.5 c	10.5 c	10.5 b	10.1 c	11.1 f
35	0.0 a	0.1 a	1.0 b	2.5 b	4.8 c	6.6 c	7.0 b	5.7 b	4.3 b	6.4 b	6.5 b	4.6 c

[a] Means of three replications.
[b] Means followed by a letter in common in columns for each sampling period do not differ at the 5% probability level, according to Duncan's multiple range test.

container. After emergence, the stand was thinned to two plants/container. Fluorescent lighting was provided to give 12 hr of light/day. Soil water was adjusted gravimetrically to 40% of the initial soil wt, and subsequent additions of water were made so that the water content never dropped below 30% of the initial water held.

Daily records were made of disease development after the first symptoms of *Phymatotrichum* root rot appeared. During the course of the temperature test, three soil samples were collected from each container and examined for numbers of viable sclerotia present. Twenty-five g of soil were removed from each container with a soil-probe at 0, 35, and 78 days following incorporation of sclerotia into the soil. The sclerotia were recovered from the soil by wet-sieving, surface-sterilized for 2 min with 0.0525% NaOCl, and plated on 1.5% water agar containing 200 ppm streptomycin sulfate. Microscopic observations were made of each sclerotium within 24-48 hr to determine viability.

RESULTS.—*Temperature range for sclerotial production.—Phymatotrichum omnivorum* produced sclerotia at 12 to 35 C, with maximum production between 25 and 30 C (Table 1). There was slight mycelial growth, but mature sclerotia were not recovered at 10 C. Sclerotial production was very slow at 15 C, with an average of 19 mg dry wt/chamber after 3 weeks. Significant differences between 10 and 15 C were noted after 12 weeks, when 3.5 g dry wt were recorded for the higher temperature.

More soil moisture was lost from the culture chambers kept at the three highest temperatures, which may have influenced the growth plateaus noted at about 8 weeks. No attempt was made to separate the effects of soil moisture loss from temperature on rate of sclerotial production.

The potential sclerotial yield probably was never reached at the lower incubation temperatures during the course of this study. The soil was not dry in the lowest three incubation temperatures, and the sorghum-seed substrate was still firm at the time of harvest.

Influence of soil temperature on disease development.—The importance of high soil temperatures for the development of *Phymatotrichum* root rot of cotton is shown in Fig. 1. The disease developed most rapidly at 27 C, which is near the opt temperature for growth of the pathogen (10). Under greenhouse conditions, it generally takes 3 to 4 weeks before cotton plants begin dying from *Phymatotrichum* root rot when sclerotia are used as inoculum in soil maintained at 27 C. At 22 C, it was nearly 8 weeks before any cotton plants succumbed. Root rot did not develop after 12 weeks at 12 or 17 C; however, elevation of soil temperature to 27 C resulted in disease development within 10 days. Eight plants emerged at 12 C, and 16 at 27 C. When the soil was elevated to 27 C, 5 of 8, and 12 of 16 plants were killed by *Phymatotrichum* root rot within 5 weeks, which indicated that the sclerotia had survived in sufficient numbers to cause root rot.

Numbers of sclerotia recovered from artificially infested soil are presented in Table 2. The maximum number recovered was 429 out of a theoretical 675 in the combined samples at zero time. The data show that more sclerotia survived at 12 C than at the other temperatures, and the viability of sclerotia recovered during this test was high at all temperatures.

DISCUSSION.—*Phymatotrichum omnivorum* formed

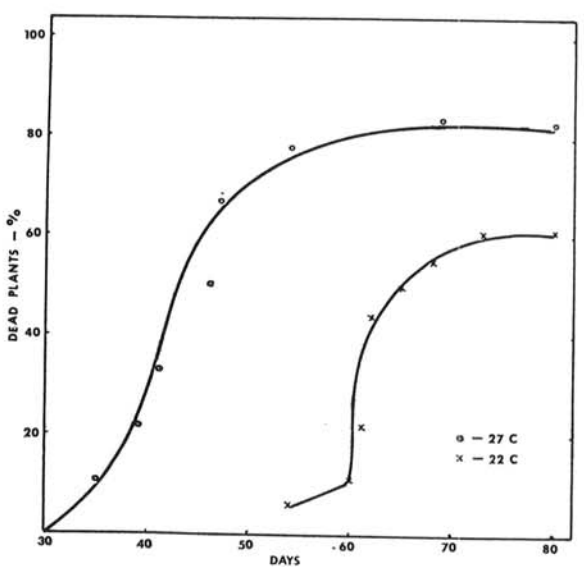

Fig. 1. The rate cotton plants are killed by *Phymatotrichum omnivorum* at two soil temperatures. The soils were artificially infested with one sclerotium/g soil.

TABLE 2. Effect of time and temperature on recovery and viability of *Phymatotrichum* sclerotia from soil artificially infested with one sclerotium/g soil

Days	No. sclerotia recovered and % viability							
	Tank temperature							
	12 C		17 C		22 C		27 C	
	no.	%	*no.*	%	*no.*	%	*no.*	%
0	429[a]	92	347	60	271	82	357	90
35	243	76	201	76	67	88	66	80
78	207	82	61	95	56	75	36	92

[a] Total number of sclerotia found in 675 g oven-dry soil.

sclerotia in soil culture over the 20-C range from 15 to 35 C. Soil temperatures should not be a limiting factor for sclerotial production in the *Phymatotrichum*-infested soils of Texas.

Rogers (10), who found that the greatest abundance of sclerotia occurred at 18 C in an 80-day test, used pieces of freshly infected cotton roots as a source of inoculum to infest the soils in his culture jars, and it is possible that sterile conditions were not maintained. *Phymatotrichum omnivorum* does not compete with other soil microbes well, and in contaminated culture jars the microbial activity would probably be enhanced at temperatures from 25 to 30 C. This could have accounted for the lower yields of sclerotia at these temperatures. Also, Rogers believed that sclerotial formation occurred in the fall (11). He stated, "It has been found that sclerotia formation takes place mainly in the fall, primarily for the reason that light-colored ones are sometimes found more frequently at that time". We observed that sclerotial color was associated with temperature. Sclerotia produced at 28-35 C were darker than those formed at 12 and 20 C. If some other environmental factor does not become limiting, *P. omnivorum* should be able to produce sclerotia throughout the year.

The time of *Phymatotrichum* root rot development is strongly influenced by soil temperature. We have shown that the disease developed at soil temperatures down to 22 C, but it developed much faster when the soil temperature was near 27 C, which is opt for growth of the fungus (10). It merely requires more time to kill the host at the lower soil temperature. This probably explains why perennial hosts are killed earlier in the year than annuals. The fungus overwinters as strands on the surface of perennial roots, and as soil temperatures rise, the fungus resumes growth and destroys the roots. Alfalfa plants usually begin dying in April or May at the Blackland Research Center, Temple, Tex., whereas cotton plants usually begin dying in mid-June. Presumably, the inoculum for annual plants comes from deep-seated sclerotia, and it takes some time for the fungus to grow to the surface of the soil after the sclerotia germinate.

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