Sclerotial Inoculum Density of Phymatotrichum omnivorum and Development of Phymatotrichum Root Rot in Cotton

Stuart D. Lyda and Earl Burnett

Associate Professor, Department of Plant Sciences, Texas A&M University, College Station 77843; and Research Soil Scientist, USDA, ARS, Blackland Conservation Research Center, Temple, Texas 76501, respectively.

The authors gratefully acknowledge the assistance of Hazel Hunn.

A contribution of the Texas Agricultural Experiment Station (TA-8069) and the Soil and Water Conservation Research Division, USDA. Supported in part by Contract Agreement 12-14-100-9339(41) from the Soil and Water Conservation Research Division, USDA.

Accepted for publication 30 November 1969.

ABSTRACT

Sclerotia of *Phymatotrichum omnivorum* were produced in sterile soil culture and recovered by wet sieving. They were air-dried and added to screened Houston Black clay to establish known inoculum densities. Maximum disease in cotton occurred with 125 to 625 sclerotia/kg of dry soil when the soil was maintained at 28 C. When the sclerotial inoculum was uniformly distributed throughout the soil, increasing densities to 3,125 and 15,625/kg of dry soil gave progressively less disease than 625/kg. Disease incidence was ascertained by number of dead plants. There was a delay in the rate of disease development when these same levels of sclerotia were centrally positioned within the soil; however, the final percentage of dead plants was the same as for 125 or 625 sclerotia/kg soil. Sufficient numbers of sclerotia survived air-drying and screening of the soil from the highest level of sclerotial infestation in the inoculum density tests to cause 100% plant kill when the soil was replanted to cotton. Phytopathology 60:729-731.

Since Pammel's (10) report that root rot of cotton is caused by a soil-inhabiting fungus, there have been many attempts to experimentally produce the disease under controlled conditions. Taubenhaus & Killough (14) were perhaps the first to carry out Koch's postulates when they cultured *Phymatotrichum omnivorum* on sterilized stems of mulberry and roots of cotton and used these to infest steam-sterilized soil. Cotton planted in this soil developed *Phymatotrichum* root rot, and the fungus was successfully recovered from 14 of 25 plants examined.

There have been several other reports of artificial inducement of the disease by the use of colonized substrates. Eaton & Rigler (4) inoculated sterilized milo seeds with *P. omnivorum*. They placed known numbers of colonized seeds at uniform depths about 1 inch from the tap root of the cotton plant, and used the number of days from soil infestation to death of the plant as a measure of resistance. Chavez et al. (2) used this same technique to induce the disease under greenhouse conditions in Arizona.

At the time of Taubenhaus & Killough's report (14), no information was available on a survival stage of the fungus. In 1929, King & Loomis (6) discovered a sclerotial stage of *P. omnivorum* that was produced in glass tubes filled with moist sterile sand. Also in 1929, Neal (9) found sclerotia in infested soil at Greenville and San Antonio, Texas. Many investigations since have considered the influence of sclerotia in perpetuating the disease. We believe that sclerotia occur in every locale where the disease occurs.

Rogers (12) found sclerotia at depths to 96 inches at the Blackland Conservation Research Center, Temple, Texas, although more than 80% of the sclerotia were in the upper 30 inches. Populations approaching 2 million sclerotia/acre were found in the 24- to 30-inch zone under a continuous cotton cropping system. King & Hope (5) found sclerotia 90 inches deep in some of the heavily infested soils at Sacaton, Arizona.

Though sclerotia have been found in most infested soils where *Phymatotrichum* root rot occurs, no information is available on the inoculum density required to give maximum disease. This paper presents data on several tests designed to provide this information.

Materials and Methods.—Air-dried Houston Black clay (HBC) was sieved through a 12-mesh wire screen to remove aggregated soil particles and small roots before addition of sclerotia of *Phymatotrichum omnivorum* (Shear) Duggar. Sclerotia were produced in sterile soil culture using a modification of the method described by Dunlap (3). Screened HBC (100 g), sorghum seed (10 g), and water (45 ml) were placed in wide-mouthed glass pint jars. The sorghum seed was placed on top of the soil, water was poured over the seed, and the jars were capped with a regular lid vented with a ¾-inch hole. The hole was stoppered with cotton, and the jars were autoclaved for 30 min at 20 psi steam, 131 C. After cooling, the sorghum seed was infested with a small agar disc containing mycelium of *P. omnivorum*.

The sclerotia were recovered by wet sieving between 30 and 40 days after inoculating the sorghum seed substrate. The soil was passed through a 16-mesh screen (U.S. Standard Sieve Series No. 18). Since there is a large difference in sclerotial size, they were given one additional separation. After air-drying, the sclerotia were screened through a 7-mesh screen (U.S. Standard Sieve Series No. 7). Only the sclerotia that passed through a 7-mesh screen and were retained on a 16-mesh screen were used for these tests. When added to the soil, they had a moisture content of 75.4% on a dry wt basis, and weighed 1.0 ± 0.11 mg each.

Infestation levels of 1, 5, 25, 125, and approximately 625, 3,125, and 15,625 sclerotia/kg dry soil were established by sclerotial counts for the first four levels, and on a wt basis for the last three levels. The sclerotia

were added to screened HBC at the desired density, and 2 kg soil were placed in each of 112 galvanized metal containers (4.5 × 5 × 7-inch depth). Under natural conditions, sclerotia frequently are found in compact masses within the soil profile, as well as individually and in chains. During the inoculum density studies, duplicate tests were conducted in which the sclerotia were (i) uniformly distributed throughout the soil and (ii) centrally positioned in the soil. There were seven replications for each treatment. Immediately after adding the soil to the containers, the moisture was adjusted to 35% of the soil wt, and five Lankart 611 cottonseeds were planted in each container. After emergence, the plants were thinned to two/container. All containers were placed in a controlled-temperature water tank set at 28 C. Disease records were taken daily after the first evidence of symptoms.

Two separate tests were conducted, and at the conclusion of the second test, the soil that had been adjusted to 15,625 sclerotia/kg was air-dried and passed through a 12-mesh screen, permitting passage of sclerotia but not of aggregated soil and small roots. Many sclerotia were still visible in the screened soil. One-half the soil was reinfested with 625 sclerotia/kg, the optimum level for disease development. The remaining one-half of the soil was not reinfested, serving as a check on viability of the previously introduced sclerotia. A concurrent test was conducted using fresh soil infested with 625 sclerotia/kg by uniform distribution and central placement.

RESULTS.—Plants began to succumb to *Phymatotrichum* root rot approximately 3 weeks after planting. The rate of disease development for three sclerotial levels is shown in Fig. 1-2. When the sclerotia were uniformly distributed throughout the soil mass, the optimum inoculum density was between 125 and 625 sclerotia/kg soil (Table 1). In the two tests, the two highest inoculum levels failed to give 100% plant kill when the sclerotia were uniformly distributed throughout the soil. When the sclerotia were placed in a central position within the soil mass, the optimum inoculum level was lower and extended over a greater range. There was a delay in disease development in soils infested with 3,125 and 15,625 sclerotia/kg soil when they were centrally positioned. The final percentage of dead plants was the same as for the soil infested with 25, 125, and 625 propagules/kg. At the conclusion of the test, soil was removed from each container and examined. Many sclerotia could be seen in the soil where they had been centrally positioned, and apparently many of them had not germinated. This suggests that all sclerotia do not germinate at the same time. A summary of the 2 tests is presented in Table 1.

Sufficient numbers of sclerotia survived the drying and screening of soil from the second test to cause 100% plant kill when the soil was replanted with cotton. Even after the soil had remained dry for nearly 1 month, there was enough viable inoculum to kill all the plants (Table 2). Our original supposition of microorganisms destroying the inoculum at the high levels was erroneous since the infested soil from the previous test without any new inoculum supported maximum disease development.

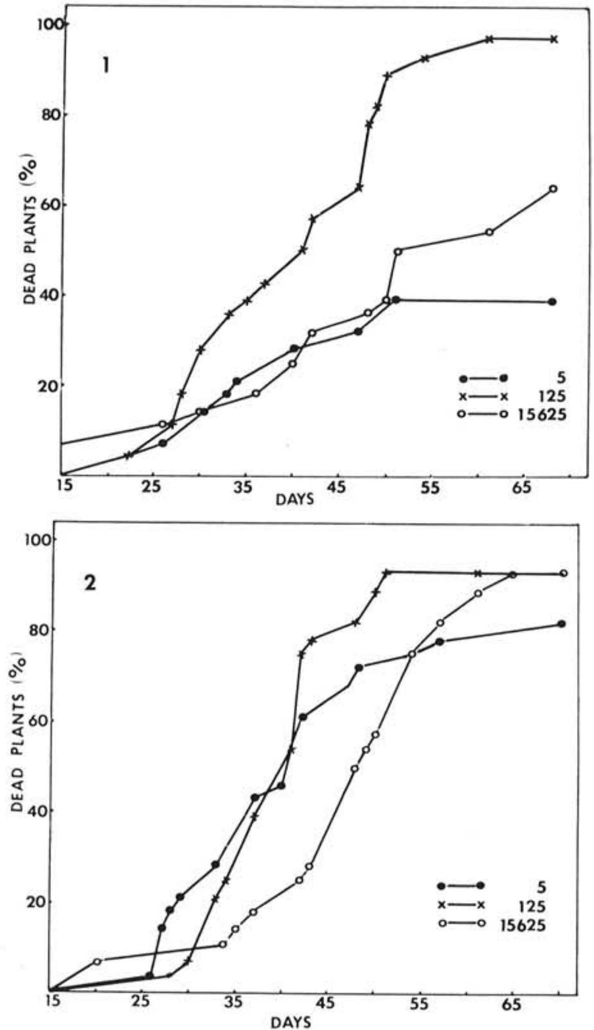

Fig. 1-2. The influence of inoculum density on rate of *Phymatotrichum* root rot development in cotton. **1)** Sclerotia were uniformly distributed throughout soil. **2)** Sclerotia were centrally positioned within the soil. Numbers in lower right corner of graphs refer to sclerotia/kg soil.

DISCUSSION.—Earlier investigators of *Phymatotrichum* root rot of cotton expressed concern over the inconsistencies obtained in experimentally inducing the disease in greenhouse and field studies. Many of their problems might be attributed to an inadequate control of soil temperatures and an inadequate inoculum density or inoculum type.

Baker & McClintock (1) suggested that populations of many soil-borne pathogens vary between 250 and 3,000 propagules/g soil; however, most of the organisms used in their mathematical model produced chlamydospores or microsclerotia as the primary survival unit. The optimum inoculum density for *P. omnivorum* at its optimum temperature for growth and pathogenicity (28 C) is considerably lower (125 to

TABLE 1. Influence of inoculum density on development of *Phymatotrichum* root rot of cotton in soil temperature tanks at 28 C

Sclerotia/kg soil	Uniformly distributed inoculum		Centrally positioned inoculum	
	Total plants	Diseased plants	Total plants	Diseased plants
no.	*no.*	%	*no.*	%
Test I, 68 days				
0	14	0	14	0
1	14	14	14	14
5	14	57	14	64
25	14	57	14	86
125	14	100	14	86
625	14	100	14	86
3,125	13	92	14	100
15,625	14	57	14	93
Test II, 70 days				
0	14	0	14	0
1	14	0	14	0
5	14	21	14	79
25	14	43	14	100
125	14	93	14	93
625	14	100	14	93
3,125	14	86	14	100
15,625	14	71	14	93

TABLE 2. Development of *Phymatotrichum* root rot of cotton in soil that had been heavily infested with sclerotia of *Phymatotrichum omnivorum* and then rescreened and reinfested with a lower level of sclerotia

Treatment	Plants	
	Healthy	Diseased
	no.	%
Noninfested soil from previous test, without added sclerotia	28	0
Noninfested soil from previous test, plus 625 new sclerotia/kg	4	86
Infested soil from previous test, without added sclerotia	0	100
Infested soil from previous test, plus 625 new sclerotia/kg	1	96
New soil, without added sclerotia	28	0
New soil, plus 625 new sclerotia/kg, uniformly distributed	6	79
New soil, plus 625 new sclerotia/kg, centrally positioned	2	93

625 sclerotia/kg soil). That lower numbers of *Phymatotrichum* sclerotia are required can probably be accounted for on the basis of total energy reserve within each propagule, since the sclerotia are relatively large and multicellular. Upon germination, each sclerotium, because of its size and food reserves, would have the potential for more extensive growth as compared to smaller chlamydospores or microsclerotia.

Presley (11) found that all cells of *Phymatotrichum* sclerotia are capable of germination. Thus, there could be many hundreds of hyphae emanating from each propagule, and the chances of the pathogen finding a suitable substrate would be increased. We believe that as the taproot of a susceptible host grows through the network of mycelium, the fungus surrounds the root periphery and ascends the root until it has nearly reached the soil surface. A girdle forms around the root just below the soil surface with concomitant destruction of the plant's vascular system.

Sclerotium rolfsii is another soil-borne fungus that produces sclerotia of a size similar to *P. omnivorum*. In naturally infested soil from sugar beet fields, Leach & Davey (8) found over 250 million viable sclerotia of *S. rolfsii* in a soil mass of 1 acre 8 inches deep. This would be a population of about 228 propagules/kg soil (assuming that a cubic foot of soil weighs 83 lb.), which is within the range found to be optimum for maximum disease development with *P. omnivorum*.

It has been reported that sclerotia of *P. omnivorum* do not survive if the soil is dried (7, 13). However, the data from this study show that sclerotial inoculum is capable of surviving air-drying, as the soil that had been infested previously and air-dried without addition of new sclerotia had 100% dead plants.

LITERATURE CITED

1. BAKER, R., & D. L. MCCLINTOCK. 1965. Populations of pathogens in soil. Phytopathology 55:495.
2. CHAVEZ, H. B., T. H. MCINTOSH, & A. M. BOYLE. 1967. Greenhouse infection of cotton by *Phymatotrichum omnivorum*. Plant Dis. Reptr. 51:926-927.
3. DUNLAP, A. A. 1941. A convenient soil-culture method for obtaining sclerotia of the cotton root-rot fungus. Amer. J. Bot. 28:945-947.
4. EATON, F. M., & N. E. RIGLER. 1941. Physiology of the cotton plant and susceptibility to root-rot. Fifty-third Annu. Rep. Texas Agr. Exp. Sta. p. 67-68.
5. KING, C. J., & C. HOPE. 1932. Distribution of the cotton root-rot fungus in soil and in plant tissues in relation to control by disinfectants. J. Agr. Res. 45:725-740.
6. KING, C. J., & H. F. LOOMIS. 1929. Further studies of cotton root-rot in Arizona with a description of a sclerotium stage of the fungus. J. Agr. Res. 39:641-676.
7. KING, C. J., & H. F. LOOMIS. 1931. Studies on sclerotia and mycelial strands of the cotton root-rot fungus. J. Agr. Res. 42:827-840.
8. LEACH, L. D., & A. E. DAVEY. 1938. Determining the sclerotial population of *Sclerotium rolfsii* by soil analysis and predicting losses of sugar beets on the basis of these analyses. J. Agr. Res. 56:619-631.
9. NEAL, D. C. 1929. The occurrence of viable cotton root-rot sclerotia in nature. Science 70:409-410.
10. PAMMEL, L. H. 1888. Root-rot of cotton or "cotton blight". Texas Agr. Exp. Sta. Bull. 4:3-18.
11. PRESLEY, J. T. 1939. Unusual features in the behavior of sclerotia of *Phymatotrichum omnivorum*. Phytopathology 29:498-502.
12. ROGERS, C. H. 1942. Cotton root-rot studies with special reference to sclerotia, cover crops, rotations, tillage, seeding rates, soil fungicides, and effects of seed quality. Texas Agr. Exp. Sta. Bull. 614:1-45.
13. TAUBENHAUS, J. J., & W. N. EZEKIEL. 1936. Longevity of sclerotia of *Phymatotrichum omnivorum* in moist soil in the laboratory. Amer. J. Bot. 23:10-12.
14. TAUBENHAUS, J. J., & D. T. KILLOUGH. 1923. Texas root-rot and methods of its control. Texas Agr. Exp. Sta. Bull. 307:1-98.