Influence of Soil Temperature and Moisture on Survival and Growth of Strands of Phymatotrichum omnivorum

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ABSTRACT

Strands of *Phymatotrichum omnivorum* on roots of cotton plants killed by the fungus during the summers of 1968 and 1969 were not viable after midsummer of 1969 and 1970, respectively. Strands buried 25 cm deep in the rhizosphere of cotton plants growing in the field did not survive longer than 3 months. Strands introduced into nonsterile Gila silt loam (GSL) at 10 C at initial moisture levels of 12, 22, and 30% (equivalent to 15, one-third, and one-tenth atmospheres tension, respectively) were viable after 9 months when moisture levels had decreased to as low as 8% (oven-dry basis). No strands survived 6 months at 27 and 32 C at the three moisture levels.

Optimum strand formation occurred at 27 and 32 C in nonsterile GSL at 22 and 30% moisture levels. Strand formation was sparse at 16 and 35 C. No structures occurred at 10 or 40 C.

Strands placed in nonsterile GSL at 22% moisture, germinated at 10 to 32 C, but not at 35 C.

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Phymatotrichum omnivorum (Shear) Duggar causes a root rot of over 2,000 species of dicotyledonous plants. Sclerotia occur commonly in fields in Texas, and are known to survive for at least 5 years (8, 9, 16). The function of sclerotia as survival propagules in Texas seems clear, but failure to recover them from fields in Arizona, except on rare occasions (5, 6, 7), raises questions regarding survival of the fungus here.

Mycelial strands are also produced on roots of at least 20 species of plants killed by *P. omnivorum* in Arizona (17). Some studies indicate that association with live cotton roots is required for strand survival (14, 15, 16). Other evidence indicates that strands do not survive the winter (10).

No information is available on survival of strands in the irrigated mineral soils of Arizona. Therefore, we determined the contribution of strands to survival of *P. omnivorum* in Arizona and defined the influence of soil temperature and moisture on their survival.

MATERIALS AND METHODS.—*General procedures.*—A single isolate of *P. omnivorum* from cotton was maintained on potato-dextrose agar or No. 70 medium (3). Soil used was Gila silt loam from Marana, Ariz., with a long history of root rot caused by *P. omnivorum*. Soil characteristics were: pH 7.8, 46.7% sand, 36.5% silt, 16.8% clay, 0.95% organic matter, 305 ppm NO_3, 0.025% N (Kjeldahl), and 11.7, 22.0, and 29.5% moisture retention at 15, 1/3, and 1/10 atm tension, respectively. Soil was air-dried and sieved through an 8-mesh screen.

Strands were produced in Gila silt loam (GSL) using Dunlap's technique (2). They were washed twice in sterile distilled water, blotted, and placed on water agar containing antibiotics (17) to test for viability. After 5 days they were observed microscopically.

RESULTS.—*Survival and growth of strands.*—Roots from dead cotton plants were dug in the field at random depths from 10 to 45 cm on 28 occasions during 1968, 1969, and 1970. Roots were immediately placed in humid chambers and strands removed in the laboratory. Viability of strands was determined within 1 to 2 hr after collection. No living strands were found after the first 8 days of July in 1969 from roots of plants killed during the summer of 1968. Results were similar in 1970 (Table 1). Figure 1 illustrates typical growth of strands after 3 days on water agar.

Survival of strands was also investigated by the burial of glass fiber sacks containing 3-week-old strands (ca. 2 cm long) at 10- and 25-cm depths in the rhizosphere of 76-day-old cotton. Fifteen to 75 strands were recovered periodically at each depth from the sacks buried on 26 June. Approximately 50% of the individual strands survived after 2.5 and 3 months at depths of 10- and 25-cm, respectively. No strands were alive after 4 months at either depth.

An additional study was undertaken to clarify the influence of sclerotia on survival. Recovery of sclerotia was attempted from seven cotton fields with a long history of Phymatotrichum root rot. Soil was successively screened through two sieves having 2-mm and 841-μ openings. One viable sclerotium was recovered from the material retained from over 250 kg of samples collected from December 1969 through March 1970. The majority of samples were taken from depths of 10 to 95 cm where sclerotia were reported to be most prevalent (6).

TABLE 1. Germination of strands of *Phymatotrichum omnivorum* recovered from naturally infected cotton roots during 1968, 1969, and 1970[a]

Collection date	Total no. strands	% Alive
1968		
13 Sept.	84	7
17 Sept.	77	25
19 Sept.	262	44
26 Sept.	133	33
1 Oct.	71	20
22 Oct.	87	16
31 Oct.	103	14
19 Nov.	63	9
5 Dec.	60	32
12 Dec.	58	26
1969		
7 Jan.	93	27
4 Feb.	129	16
10 Feb.	56	27
17 Mar.	62	61
9 Apr.	52	27
6 June	52	6
7 July	71	0
18 July	51	0
12 Aug.	45	0
1970		
3 Mar.	77	16
9 Apr.	59	29
8 May	67	10
20 May	27	30
15 June	122	13
30 June	72	10
8 July	61	3
12 July	110	0
24 July	91	0

[a] Roots were immediately placed in humid chambers and strands removed in the laboratory for determinations of viability within 1 to 2 hr after collection.

Laboratory studies.—Naturally infected, recently killed cotton roots with strands were placed in 200 g of nonsterile GSL in 500-ml plastic containers. Strands produced in soil cultures (2) were placed in petri dishes containing 100 g of nonsterile GSL. All soils were adjusted to 12, 22, 30, or 45% moisture levels, after which the containers were sealed with masking tape and placed in incubators at 10, 27, and 32 C (± 1 C). Strands were recovered from each of three containers/treatment after 0.5, 3, 6, and 9 months.

Some strands, naturally produced on cotton roots, survived for 9 months at 10 C at all soil moisture levels (Table 2). No strands survived for 6 months at 27 and 32 C at any of the four moisture levels. Most

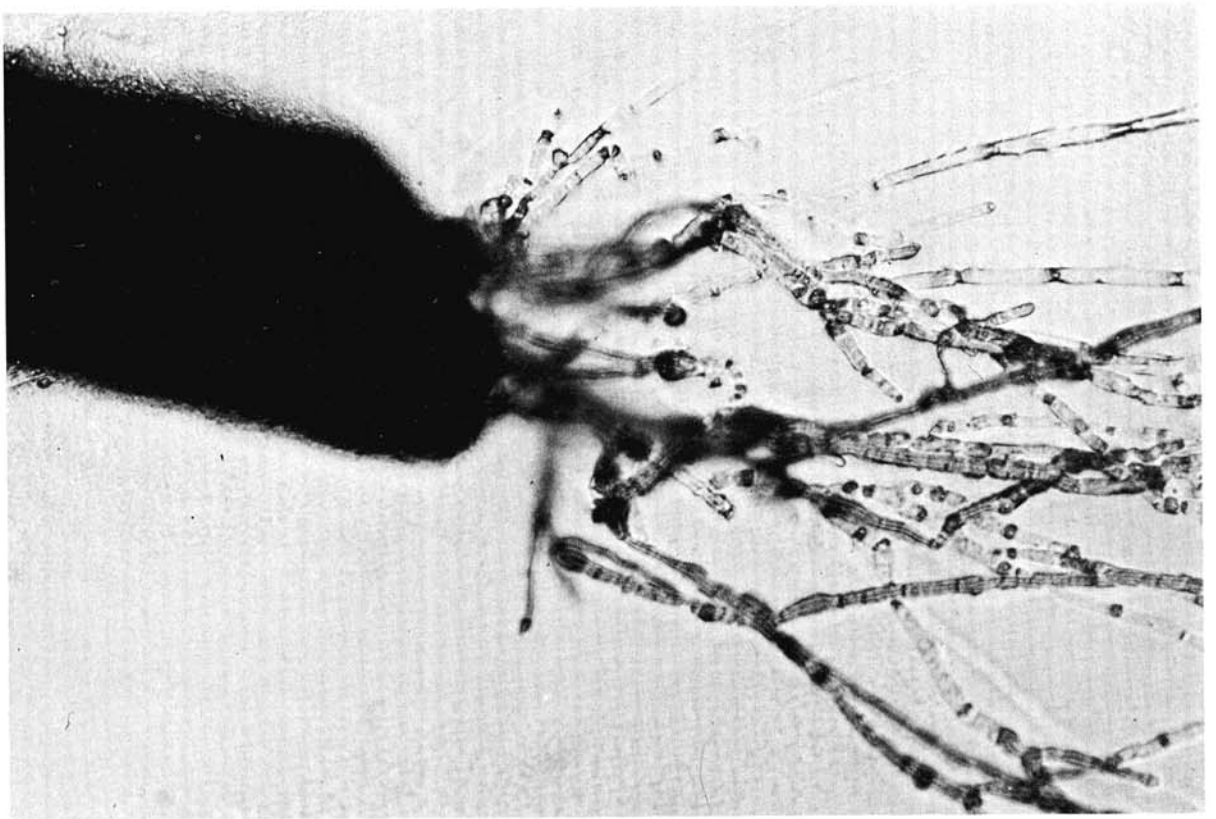

Fig. 1. Mycelial growth from strand of *Phymatotrichum omnivorum* after 3 days' incubation on water agar.

of the survival after 3 months at 27 and 32 C occurred at the 12% moisture level (Table 2).

New strands formed on the soil surface from the strands on the dead cotton roots in the soil during the first 2 weeks, but only at 27 and 32 C at the four moisture levels. After 2 weeks, 77% of 70 new strands recovered were alive; none was alive after 3, 6, and 9 months, although 130 and 121 strands were recovered at 27 and 32 C, respectively. Comparable soils had a greater moisture decrease at 27 and 32 C after 9 months than at 10 C. Alternatively, after 9 months, several values at 27 and 32 C exceeded those at 10 C when the four moisture levels in each of the columns are compared (Table 2).

After 9 months at 12, 22, 30, or 45% moisture levels at 10 C, 18, 61, 89, and 86% of laboratory-produced strands were alive. However, no strands survived the same period at 27 or 32 C. These results are similar to those obtained for the comparably treated cotton roots with naturally occurring strands. In contrast, 0, 8, 53, and 59% and 0, 5, 63, and 45% of the laboratory strands exposed to the same moisture levels survived 6 months at 27 and 32 C, respectively. These data indicate that at a given temperature, laboratory-produced strands survive best when exposed to higher initial moisture levels. However, survival of naturally produced strands does not appear to be influenced by different moisture levels after a given time interval (Table 2).

Formation of new strands on the soil surface was similar to that observed with naturally produced strands; i.e., formation occurred after 2 weeks, but only at 27 and 32 C at the four moisture levels. After 6 months, 50% of the 154 strands recovered were alive, but none was alive after 9 months, although 24 and 35 were recovered at 27 and 32 C, respectively.

Strand development was tested in nonsterile GSL at 12, 22, 30, 45, and 50% moisture levels at 10, 16, 27, 32, 35, and 40 C for 5 weeks. Five g of infested sorghum seed were placed in the center of petri dishes (150-mm diam) containing 150 g of nonsterile GSL. Each treatment consisted of at least four replications. Strand formation was best and similar at 27 and 32 C both at the 22 and 30% moisture levels. At these two moisture levels, sparse formation occurred at 16 and 35 C, but not at 10 or 40 C. At the 45% moisture level at 27 and 32 C, strand production was ca. 75% of that under optimum conditions, but no strands formed at the other four temperatures. No strands were formed at the 50% soil moisture level regardless of temperature.

Germination of mature strands produced in the laboratory was tested at 10, 16, 20, 27, 32, and 35 C (± 1 C) in nonsterile GSL initially having a 22%

TABLE 2. Survival of strands of *Phymatotrichum omnivorum* on cotton roots collected in the field and placed in nonsterile Gila silt loam at various temperatures and moisture levels in the laboratory

Recovery time (months)	% Soil moisture	10 C			27 C			32 C		
		Final soil moisture	Germination		Final soil moisture	Germination		Final soil moisture	Germination	
			Total no.	%[a]		Total no.	%		Total no.	%
0.5	12	10	33	34	9	34	41	8	36	39
	22	19	80	78	17	66	92	17	43	14
	30	24	55	69	22	54	6	21	45	20
	45	31	56	64	29	51	49	28	51	29
3.0	12	12	40	40	9	28	4	10	38	8
	22	17	71	59	16	35	0	13	31	0
	30	24	42	36	20	38	0	21	38	3
	45	27	56	57	27	38	0	24	24	0
6.0	12	11	49	14	9	45	0	9	16	0
	22	16	50	36	15	55	0	14	49	0
	30	24	33	45	21	17	0	20	20	0
	45	28	40	25	27	52	0	25	14	0
9.0	12	8	59	5	7	67	0	5	52	0
	22	17	72	3	11	47	0	9	22	0
	30	20	62	4	15	25	0	16	63	0
	45	32	58	3	22	40	0	18	44	0

[a] Percentages are based on an average of three replications; survival was determined by removing strands from roots, washing in sterile distilled water, blotting, and placing on water agar containing antibiotics.

moisture level. Petri dishes (90-mm diam) containing GSL and cultures of strands were preincubated separately at 10 C for 12 hr. Strands from cultures were then placed on the nonsterile GSL. Each treatment consisted of three petri dishes with 10 strands/dish. Strands were examined after 1 and 2 days using a stereomicroscope. After 5 days, strands were removed and examined microscopically. Strands germinated best and grew most rapidly at 27 and 32 C. At 16 and 20 C, strand growth was noticeable after the 2nd day. Strand growth after 5 days was evident only microscopically at 10 C, but not at 35 C.

DISCUSSION.—In cultivated soils of southern Arizona, temperatures at 20-cm depths range from ca. 5 to 15 C during the winter and 23 to 32 C through the summer (4). Moisture levels of these cultivated soils seldom, if ever, fall outside 12 to 45% at depths where strands of *P. omnivorum* are most prevalent on infected cotton roots. It is also noteworthy that these soils are lower in organic matter than Texas soils, where most of the field research has been done.

Our data support the hypothesis that a given population of strands is not a means of long term survival in cultivated soils in Arizona. This is in contrast to evidence from Texas, where strands have survived for several years (12, 13). However, strands may account for survival under certain cropping conditions.

Temperature has a critical influence on strand survival. Low soil temperatures are the key to survival. During the winter and at 10 C in the laboratory, strand survival was best regardless of the moisture level. The effect of temperature is further emphasized by the lack of recovery of live strands from the field after midsummer, although 368 were collected.

Alternatively, higher soil temperatures are detrimental to survival, in part due to the promotion of faster growth by strands. Strands from soil cultures and strands on cotton roots failed to survive at the four moisture levels at 27 and 32 C after 9 months; however, new strands formed on the soil surface during the first 2 weeks. Furthermore, strands buried at 25 cm in the cotton rhizosphere did not survive longer than 3 months during June, July, and August.

The general decline in survival at 10 C over 9 months (Table 2) appears to be a result of low metabolic activity of the strands. This is supported by the observation that strands germinated at 10 C, but could only be detected microscopically after 5 days. In addition, formation of strands did not occur at 10 C in any of the experiments. However, formation did occur at 16 to 35 C but not at 40 C.

We concur with Dunlap (2), but differ with Chavez et al. (1) that optimum formation of strands and sclerotia occurs at 22 and 30% moisture levels. This suggests that type of soil, nutrient source, and other variables must be closely scrutinized when such experiments are performed.

Survival could be facilitated indirectly by strands through yearly infection of susceptible dicotyledons in rotation. We observed that cotton plants were infected in 1969 and 1970, whereas live strands could still be recovered from dead cotton roots of the previous crop. Strands subsequently formed on roots of the new crop. This mechanism could account for the perpetuation of the fungus indefinitely.

An alternative to the above concept is that new strands form each growing season on roots of other plants. Strands have been reported to form on monocotyledonous roots in nature (11, 12), suggesting that monocotyledonous crops in rotation

and naturally occurring monocotyledons may serve as a link for survival.

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