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Effect of Temperature and Moisture on Parasitization of *Heterodera schachtii* Eggs by *Acremonium strictum* and *Fusarium oxysporum*

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ABSTRACT

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Parasitization of *Heterodera schachtii* eggs by *Acremonium strictum* and *Fusarium oxysporum* occurred after 200 degree-days growth of the nematode at 24 C and increased with time to 58 and 46% by *A. strictum* and *F. oxysporum*, respectively. Parasitic activity of *A. strictum* was greater at 24 C than at 28 C, that of *F. oxysporum* was similar at 24 and 28 C and little parasitism was expressed by either fungus at 20 and 32 C. *A. strictum* was parasitic when soil moisture was near saturation, but not when it was held near wilting point levels. The reverse was true for *F. oxysporum*.

Additional key words: host-parasite interactions.

Acremonium strictum Gams and *Fusarium oxysporum* Schlect were isolated from *Heterodera schachtii* Schmidt eggs collected from sugar beet fields in California, and laboratory studies indicated that the fungi were active parasites of *H. schachtii* eggs (2). Both fungi invade the female nematode through natural openings with no destruction of the cuticle or disruption of subsequent development of the cyst wall.

This study was undertaken to determine the effects of temperature and soil moisture on parasitization of *H. schachtii* eggs by *A. strictum* and *F. oxysporum*.

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MATERIALS AND METHODS

Effect of temperature on radial colony growth in vitro. Petri plates (9 cm in diameter) of potato dextrose agar (PDA) were inoculated with 5-mm-diameter plugs of *A. strictum* isolate 1 and *F. oxysporum* isolate 1 (2). Five replicate plates of each fungus were incubated at 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 C and colony diameters were measured after 20 days. Both fungi grew at all temperatures, although colonies of *A. strictum* were severely contorted at temperatures above 30 C. Optimum temperature ranges were 21–27 C for *A. strictum* and 15–27 C for *F. oxysporum*.

Effect of soil temperature on parasitization of eggs. Egg parasitization within females developing on roots was determined by incorporating mycelium of each of the two fungi into autoclaved sandy soil as described previously (2) at a rate equivalent to 1.05 mg dry mycelium per gram of dry soil. The soil was placed in 350-ml styrofoam pots and sugarbeet seedlings, inoculated 5 days previously with 1,000 *H. schachtii* juveniles per seedling, were transplanted into the soil. A similar number of plants were transferred to autoclaved soil without the fungus. Pots were maintained in constant-temperature water baths in a greenhouse at 24 or 28 C. Thirty pots with *A. strictum*-infested soil, 30 with *F. oxysporum*-infested soil, and 30 pots with noninfested soil were maintained at each temperature. Six plants in each series were removed from each temperature bath after accumulating heat factors of 200, 250, 300, 350, and 400 degree-days at each temperature (14.3, 17.9, 21.4, 25.0, and 28.6 days at 24 C and 11.1, 13.9, 16.7, 19.4, and 22.2 days at 28 C). A basal threshold development temperature of 10 C based on preliminary degree-day data taken with the nematode population was used in this study. Nematodes were extracted from the plants and soil by using a modified Fenwick can as described elsewhere (2). About 15 cysts were picked from each sample and eggs were liberated, counted, and plated on WA to determine percent parasitization as previously described (2).

Mycelium of *A. strictum* or *F. oxysporum* was incorporated into autoclaved sandy loam soil as described previously at a rate equivalent to 1.0 mg dry mycelium per gram of dry soil. Autoclaved soil without fungi or infested with one of the fungi was placed in 350-ml styrofoam pots, and sugar beet seedlings inoculated 5 days previously with 1,000 *H. schachtii* juveniles per seedling were planted in the soil. Pots were transferred to constant-temperature water baths at 20, 24, 28, and 32 C in the greenhouse. Six plants in soil without fungi and six plants from each fungus treatment were removed from each bath after accumulating heat factors of 300 degree-days at each temperature. Nematodes and eggs were extracted and percent eggs parasitized was determined as described above.

Effect of soil moisture on parasitization of eggs. Mycelium of *A. strictum* or *F. oxysporum* was incorporated into autoclaved UC mix (1) at a rate equivalent to 1.2 mg dry mycelium per gram of dry soil. Autoclaved soil without fungi or containing one of the fungi was placed in 10-cm-diameter clay pots, and sugar beet seedlings inoculated 5 days previously with 1,000 *H. schachtii* juveniles per seedling were transferred to them. Pots were maintained in an environmental growth chamber at 28 C with 12-hr days.

Watering regimes were used to maintain the soil moisture at or near the wilting point (27% water [w/w] in 24 pots; eight pots per treatment) and at or near the saturation point (47% water [w/w]) in the other 24 pots. Plants were removed from the chamber after 12 wk, nematodes and eggs were extracted, and the percent parasitization was determined.

TABLE 1. Effects of temperature and time on the parasitization of *Heterodera schachtii* eggs by *Acremonium strictum* and *Fusarium oxysporum*

	Percent eggs parasitized[z] by:			
	A. strictum		*F. oxysporum*	
Degree-days	24 C	28 C	24 C	28 C
200	7.3 b	0.0 b	8.4 b	6.2 b
250	53.9 a	40.0 a	45.3 a	43.1 a
300	57.8 a	43.4 a	46.1 a	44.4 a
350	54.2 a	41.6 a	45.8 a	45.1 a
400	56.6 a	47.8 a	44.9 a	42.4 a

[z] Means are from six replicates each with 25 females or young cysts (average of 4,450 eggs per replicate for *A. strictum* and 3,790 per replicate for *F. oxysporum*). None of the uninoculated control eggs were parasitized. For *A. strictum*, an average of 63.4% and 36.7% of eggs from control and inoculated treatments contained second-stage larvae. For *F. oxysporum* the respective averages were 61.6% and 43.2%. Numbers in a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

TABLE 2. Effect of temperature on parasitization of *Heterodera schachtii* eggs[z] from females on sugar beet plants grown for 300 degree-days in soil infested with *Acremonium strictum* or *Fusarium oxysporum*

	A. strictum		*F. oxysporum*	
Temperature (C)	Eggs parasitized (%)	Eggs/female (no.)	Eggs parasitized (%)	Eggs/female (no.)
20	10.1 d	236.1 b (224.6) b	8.3 d	224.8 b (217.9) b
24	58.9 a	250.6 ab (230.9) b	47.4 b	248.6 ab (249.3) ab
28	42.1 b	161.1 bc (170.9) bc	40.7 b	187.4 bc (196.3) bc
32	30.6 c	124.6 cd (113.3) d	12.2 d	149.7 c (128.6) cd

[z] The data are means of six replicates with 10–15 females per replicate. Data in parentheses were from control plants in autoclaved soil. An average of 62.6% and 37.3% of eggs from control and inoculated treatments contained second-stage juveniles. Numbers followed by the same letters are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

RESULTS

Effect of soil temperature on parasitization of eggs. Egg laying by *H. schachtii* began after 200 degree-days at both 24 and 28 C. Parasitization by *A. strictum* began at 200 degree-days at 24 C and increased to 54% parasitized eggs by 250 degree-days, after which it remained constant (Table 1). At 28 C there was slightly less parasitization and it was first detected at 250 degree-days. Similar results were observed with *F. oxysporum* (Table 1). Production and development of *H. schachtii* eggs in soil was similar on plants exposed to 20, 24, 28, and 32 C for the same number of degree-days. Numbers of eggs produced by females in noninfested soil compared with fungus-infested soil differed little at each temperature (Table 2). Eggs were parasitized at all temperatures in soil containing *A. strictum* or *F. oxysporum*. Parasitism by *A. strictum* was lowest at 20 C and highest at 24 C. *F. oxysporum* was most active at 24 and 28 C with a significant reduction in percent eggs parasitized at the lowest and highest temperatures (Table 2). Based on size and color females in all treatments appeared to be at the same state of maturity. We found no parasitization or damage of the females by the fungi.

Effect of soil moisture on parasitization of eggs. *A. strictum* did not parasitize *H. schachtii* eggs in soil with moisture near the wilting point, but it parasitized 59.4% of eggs produced in moist soil (Table 3). Little parasitism by *F. oxysporum* occurred in moist soil; in dry soil 37.8% were parasitized. Egg production by the nematode did not vary significantly among treatments.

DISCUSSION

The effect of temperature on the development and survival of *H. schachtii* has been studied with California populations by Thomason and Fife (3). Five generations were produced in one

growing season in the Imperial Valley of California where sugar beets are planted in the fall, grown through the winter, and harvested the following April, May, or June. The first two generations were produced in 1,255 and 502 degree-days, respectively. Soil temperatures rapidly decreased from 24 to 8 C at 12.5 cm during the first generation and increased from 8 to 21 C during the second. Thereafter, generation time was much shorter, taking about 349 degree-days for the third, 396 for the fourth, and 319 for the fifth. These last generations were produced when soil temperatures increased from 21 to 34 C, temperatures apparently nearly optimal for reproduction and development. Results of our experiments show that the optimal temperature for radial growth of *A. strictum* in vitro was 21–27 C, and for *F. oxysporum* it was 15–27 C. Both fungi actively parasitized eggs at all temperatures tested (20–32 C).

Most parasitism of eggs by both fungi had occurred by the time 250 degree-days had accumulated at soil temperatures of 24 and 28 C. The failure of the fungi to parasitize additional eggs with increased time beyond 250 degree-days may be explained by the fact that eggs rapidly developed into second-stage juveniles, which are not readily parasitized (2). In control plots, 60% of the eggs in females were in this stage of development after 250 degree-days. Therefore, it appears that maximum egg parasitization occurs early in the development of young females rather than in mature cysts.

Soil temperatures recorded during California growing seasons are within the range of temperatures at which *A. strictum* and *F. oxysporum* are parasitically active. *H. schachtii* may produce five generations during the growing season of sugar beets (3). Early generations develop at low soil temperatures and the last few develop as soil temperatures reach 34 C. In our study, *H. schachtii* produced as many eggs at 20 C as at 24 or 28 C, but *A. strictum* and *F. oxysporum* did not grow as well at 20 C so their parasitism was reduced. At 32 C, the nematode generally produced fewer eggs than at the lower temperatures, but the parasitism of *A. strictum* was reduced relatively less. Thus, there was actually a greater proportionate reduction of the number of viable eggs at 32 C. It may be difficult to reduce *H. schachtii* populations when conditions are ideal for the nematode because its reproductive capacity is so great. However, conditions in the field are not always ideal for the nematodes, so the levels of parasitism we observed, though seemingly low, may be sufficient to reduce the number of viable eggs below economic threshold levels.

Previously, we observed that *A. strictum* was more active than *F. oxysporum* in some soils that were kept very moist (2). In this study we found that *F. oxysporum* was much more active than *A. strictum* in dry soil, but that in wet soil the reverse was true.

TABLE 3. Moisture effects on the parasitization of *Heterodera schachtii* eggs by *Acremonium strictum* and *Fusarium oxysporum*

	Soil moisture (%, w/w)			
	27%		47%	
Inoculation	Eggs parasitized (%)	Eggs w/ J_2[x,y] (%)	Eggs parasitized (%)	Eggs w/ J_2[x,y] (%)
A. strictum	0.0 b	55.7 ab	59.4 a	32.0 b
F. oxysporum	37.8 a	46.6 b	0.1 b	59.7 a
Uninoculated Control	0.0 b	60.6 a	0.0 b	58.9 a

[x] Means were calculated from eight replicates with 15 females or young cysts (average, 2,610 eggs) per replicate. Numbers in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple-range test.

[y] Eggs in the second juvenile (J_2) stage appear to be resistant to both fungi.

An important aspect of the ecology of soilborne fungi is their ability to survive adverse conditions such as extreme temperatures and moistures. There are no known survival structures of *A. strictum*. Its survival in soil during such adverse conditions is an interesting point of discussion. Eggs containing viable fragments of *A. strictum* were isolated from cysts that were presumably at least 10 yr old (2). It appears that the fungus-filled eggs gain the same protection from the cyst wall as viable eggs containing juveniles and are capable of remaining viable in soil for several years and provide resistant sources of inoculum of *A. strictum*. Results of our experiments show that temperature and moisture influence the host-parasite interactions of *H. schachtii* eggs and fungal parasites and that these two factors should be considered when manipulating the environment for enhanced biological control of *H. schachtii* by *A. strictum* and *F. oxysporum*.

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