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In Vitro Parasitism of *Rotylenchus robustus* by Isolates of *Hirsutella rhossiliensis*[1]

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Abstract: We tested the hypothesis that isolates of *Hirsutella rhossiliensis* from host nematodes in the family Hoplolaimidae (*Rotylenchus robustus* and *Hoplolaimus galeatus*) would be more virulent to *R. robustus* than would isolates from host nematodes not in the Hoplolaimidae (*Heterodera schachtii* and *Criconemella xenoplax*). Nematodes were touched to 10–20 spores of different isolates and incubated at 20 C in 4.5 mM KCl; the percentage of nematodes colonized (filled with hyphae) was determined after 2, 5, 10, 20, and 30 days. The hypothesis was rejected because isolates from *H. schachtii* and *C. xenoplax* were equivalent or better at parasitizing *R. robustus* than were isolates from *R. robustus* and *H. galeatus*. In addition, the *R. robustus* and *H. galeatus* isolates were as pathogenic to *C. curvata* as they were to *R. robustus*, but produced fewer spores per colonized nematode (*H. schachtii*) than did the other isolates.

Key words: biological control, endoparasite, fungus, *Hirsutella rhossiliensis*, host specificity, parasitism, *Rotylenchus robustus*, virulence.

In a recent study, pathogenicity and morphology were shown to be similar among 19 of 25 isolates of the nematophagous fungus *Hirsutella rhossiliensis* (5). The 19 isolates, obtained from different hosts and geographical locations, readily parasitized *Heterodera schachtii* in soil and *H. schachtii, Meloidogyne javanica,* and *Steinernema glaseri* on agar. Although the other six isolates also attacked these nematodes, they parasitized few *H. schachtii* in soil, grew slowly on agar and within hosts, and produced larger spores than did the other 19 isolates.

The six isolates of *H. rhossiliensis* were also unique because all of them were isolated from nematode hosts in the family Hoplolaimidae. Five were originally isolated from *Rotylenchus robustus* in San Mateo County, California, and one was isolated from *Hoplolaimus galeatus* in Adams County, Pennsylvania (5). The objectives of this study were to determine whether isolates of *H. rhossiliensis* obtained from hosts in the Hoplolaimidae are more virulent to *R. robustus* than are isolates from other hosts, and whether the Hoplolaimid isolates are more virulent to *R. robustus* than to *Criconemella curvata.*

MATERIALS AND METHODS

Fungal isolates: We selected representative *H. rhossiliensis* isolates from the 25 that were previously studied (5). Isolates 88 and 92 were from *R. robustus,* isolate 41 was from *H. galeatus,* isolates 73 and 76 were from *H. schachtii,* isolates A3a and 53 were from *C. xenoplax,* and isolate 63 was from a soil mite. Accession numbers and other information on these isolates have been published (5). Isolates were subcultured on quarter-strength cornmeal agar (CMA). Six plugs (0.5-cm-d) from the margin of 2-week-old cultures were transferred to a petri dish (9-cm-d) containing fresh CMA. Dishes were incubated at 20 C for 2 weeks to allow the isolates to grow and sporulate; we refer to these as CMA cultures.

Specificity experiment: Soil was collected from a daisy field in San Mateo County, California, where isolates 88 and 92 were originally collected (5). *Rotylenchus robustus* and *C. curvata* were extracted from the soil by wet sieving (25-μm-d pore) and centrifugal flotation (3). Spore-free specimens of *R. robustus* and *C. curvata* were touched to 10–20 spores of *H. rhossiliensis* (1). Fungal isolates and nematodes varied among the three trials of this experiment. In trial 1,

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five females of *R. robustus* were touched to spores of each isolate; control nematodes were not touched to spores. Nematodes were transferred into each of six replicate petri dishes containing 3 ml of 4.5 mM KCl at 20 C and were nondestructively observed at ×100–140 magnification after 2, 5, 10, 20, and 30 days; all trials were terminated after 30 days because control nematodes were in poor condition. The percentage of nematodes colonized by *H. rhossiliensis* (filled with hyphae) was calculated for each observation time.

Trial 2 was similar to trial 1 except that both *R. robustus* (females) and *C. curvata* (females and fourth-stage juveniles) were touched to spores. Five nematodes were transferred into each of three replicate dishes for each combination of isolate and nematode. *Criconemella curvata* was included because it is not in the family Hoplolaimidae, and high numbers were extracted from field soil along with *R. robustus.*

Trial 3 also was similar to trial 1, except that healthy juveniles rather than adults of *R. robustus* were touched to spores. Five nematodes were transferred into each of five replicate dishes for each combination of isolate and nematode.

Trials were completely randomized within moisture chambers (plastic boxes containing moistened paper towels). Data were analyzed using the general linear models procedure of SAS (4) for each observation time within each trial. Significance was determined at $\alpha \leq 0.05$, In trial 1, we compared parasitism by the two Hoplolaimid isolates vs. the two other isolates; the analysis for trial 2 was similar except that separate tests were done for the two target nematodes, and data from isolate A3a were excluded from the analysis, as explained in the Results. In trial 3, parasitism by one isolate from *R. robustus* vs. one from *C. xenoplax* was compared.

Sporulation experiment: Cysts of *Heterodera schachtii* from sugarbeet (*Beta vulgaris* L. 'SSNB-2') pot cultures were incubated on Baermann funnels, and healthy second-stage juveniles (J2) were collected every 2 hours (2). Nematodes were stored at 10 C and used within 24 hours. To obtain *H. rhossiliensis*-colonized *H. schachtii,* approximately 400 healthy J2 in 0.5 ml of 4.5 mM KCl were added to CMA cultures of five

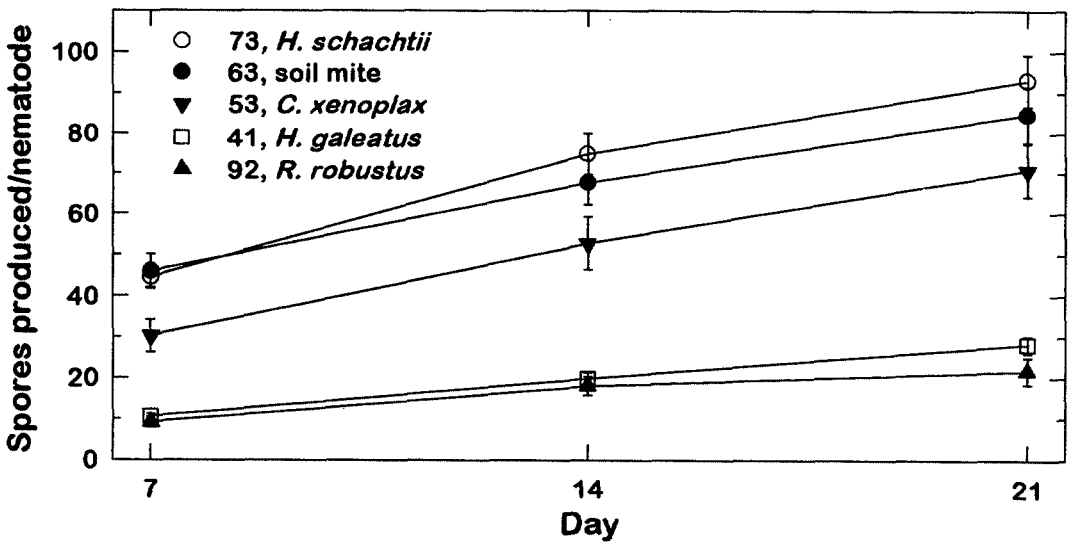

FIG. 1. Sporulation of selected isolates of *Hirsutella rhossiliensis* from *Heterodera schachtii* juveniles (J2). Fungal-colonized J2 were placed in a moist chamber at 20 C and observed weekly for 3 weeks. Isolate number and original host or source are shown. Each value is the mean ± SE from 30 replications (pooled data from trials 1–3), with one nematode per replication.

isolates (Fig. 1). Isolates used in this experiment differed from those in the specificity experiment. However, isolates 41, 88, and 92 (from Hoplolaimid nematodes) are morphologically and pathogenically similar as are isolates 76, A3a, 73, 63, and 53 (not from Hoplolaimid nematodes) (5). Dishes were uncovered for 30 minutes to allow excess water to evaporate, and then were covered and incubated 30 minutes to allow nematodes to move across the culture and acquire spores. Nematodes were washed from the agar with 4 ml of 4.5 mM KCl and collected in 3.5-cm-d petri dishes. The dishes were incubated at 20 C for 3 days (isolates 53, 63, and 73), 4 days (isolate 41), or 10 days (isolate 92). Incubation times varied because the *R. robustus* and *H. galeatus* isolates required more time to colonize nematodes than did the other isolates (5). Colonized nematodes (10 per isolate, each with one to three adherent spores) were incubated in moisture chambers at 20 C, and sporulation was quantified at ×100–140 magnification after 7, 14, and 21 days (2). The experiment was repeated twice (trials 1–3).

Each trial was completely randomized within moisture chambers. The SAS general linear models procedure (4) was used to perform analysis of covariance (linear regression with trial as a covariate). The effect of trial was not significant (α = 0.05), and data were pooled from all three trials.

Results and Discussion

Within any trial and at any observation time, the Hoplolaimid isolates never colo-

Table 1. Percentage of nematodes colonized by *Hirsutella rhossiliensis* in vitro as affected by fungal isolate, target host, and time.[a]

Isolate	Source[b]	Target[c]	% Colonized nematodes after 2–30 days				
			2 days	5 days	10 days	20 days	30 days
				Trial 1			
41	Hg	Rr	0 ± 0	0 ± 0	0 ± 0	13 ± 8	17 ± 8
88	Rr	Rr	0 ± 0	0 ± 0	18 ± 10	32 ± 9	44 ± 15
76	Hs	Rr	0 ± 0	26 ± 10	40 ± 13	40 ± 11	40 ± 13
A3a	Cx	Rr	0 ± 0	24 ± 16	28 ± 16	33 ± 18	33 ± 18
Control		Rr	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
				Trial 2			
41	Hg	Rr	0 ± 0	12 ± 6	30 ± 10	36 ± 16	54 ± 18
88	Rr	Rr	0 ± 0	7 ± 7	20 ± 11	33 ± 18	33 ± 18
76	Hs	Rr	0 ± 0	11 ± 6	27 ± 13	50 ± 29	50 ± 29
A3a	Cx	Rr	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Control		Rr	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
41	Hg	Cc	0 ± 0	0 ± 0	20 ± 1	44 ± 18	44 ± 18
88	Rr	Cc	0 ± 0	0 ± 0	0 ± 0	27 ± 7	27 ± 7
76	Hs	Cc	0 ± 0	49 ± 17	100 ± 0	100 ± 0	100 ± 0
A3a	Cx	Cc	0 ± 0	82 ± 10	100 ± 0	100 ± 0	100 ± 0
Control		Cc	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
				Trial 3			
88	Rr	Rr-J	0 ± 0	3 ± 3	26 ± 8	26 ± 8	36 ± 10
A3a	Cx	Rr-J	36 ± 13	47 ± 13	60 ± 12	65 ± 13	67 ± 12
Control		Rr-J	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

[a] Target nematodes were touched to 10–20 spores of *H. rhossiliensis* and placed in 3 ml of 4.5 mM KCl at 20 C. Nematodes were observed at ×100-140 magnification after 2, 5, 10, 20, and 30 days, and the percentage of nematodes filled with hyphae (colonized) was calculated. Values represent means ± SE from six, three, and five replicates (five nematodes per replicate) in trials 1, 2, and 3, respectively.

[b] Hosts from which isolates were obtained. Hg = *Hoplolaimus galeatus*, Rr = *Rotylenchus robustus*, Hs = *Heterodera schachtii*, and Cx = *Criconemella xenoplax*.

[c] Target nematodes. Rr = *R. robustus* females, Cc = *Criconemella curvata* females and fourth-stage juveniles, and Rr-J = *R. robustus* juveniles.

nized a greater ($\alpha = 0.05$) percentage of the target nematodes than did the other isolates (Table 1). In fact, colonization of *R. robustus* often was less with the Hoplolaimid isolates than with the other isolates. One exception was isolate A3a in trial 2, which failed to parasitize *R. robustus* for unknown reasons. Isolates from *R. robustus* and *H. galeatus* also parasitized *R. robustus* and *C. curvata* at similar rates and to about the same degree (Table 1, trial 2). To simplify presentation, only means and standard errors are presented.

Colonization of *R. robustus* seldom exceeded 50%, regardless of fungal isolate (Table 1, trials 1–3). In contrast, isolates 76 and A3a rapidly colonized 100% of the *C. curvata* in trial 2. These results suggest that *R. robustus* may be relatively resistant to the fungal isolates used in this study.

With respect to sporulation, isolates from *H. schachtii,* a soil mite, and *C. xenoplax* produced more spores per colonized *H. schachtii* that did isolates from *R. robustus* or *H. galeatus* on all observations dates (Fig. 1). This result seems reasonable: Given a finite resource, the number of propagules produced from that resource should be inversely related to the size of the propagules, and spores produced by Hoplolaimid isolates are larger than those from other isolates (5). The sporulation data also may explain, at least in part, the low levels of transmission of *R. robustus* and *H. galeatus* isolates to *H. schachtii* (5). Sporulation was not quantified beyond 21 days because the nematode substrate appeared to be depleted; only cuticle and empty assimilative hyphae remained in all cases.

The *H. rhossiliensis* isolates from nematodes in the family Hoplolaimidae differ from other isolates; they produce larger spores, grow more slowly in culture, and exhibit low pathogenicity toward *H. schachtii, M. javanica,* and *S. glaseri* (5). We had hypothesized that the Hoplolaimid isolates were adapted to parasitism of Hoplolaimid nematodes. The data in this study do not support that hypothesis.

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