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Influence of *Rhizoctonia solani* on Egg Hatching and Infectivity of *Rotylenchulus reniformis*[1]

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Abstract: The effects of culture filtrates of *Rhizoctonia solani* and root exudates of *R. solani*-infected cotton (*Gossypium hirsutum*) seedlings on hatching of eggs and infectivity of females of *Rotylenchulus reniformis* were evaluated in an attempt to account for the enhanced nematode reproduction observed in the presence of this fungus. Crude filtrates of *R. solani* cultures growing over sterile, deionized distilled water did not affect egg hatching. Exudates from roots of cotton seedlings increased hatching of *R. reniformis* eggs over that observed in water controls. Exudates from cotton seedling roots not infected or infected with *R. solani* did not differ in their effect on egg hatching. However, infection of cotton seedlings by reniform females was increased in the presence of *R. solani,* resulting in the augmented egg production and juvenile population densities in soil observed in greenhouse studies.

Key words: cotton, culture filtrate, egg hatching, *Gossypium hirsutum,* infectivity, nematode, reniform nematode, *Rhizoctonia solani,* root exudate, *Rotylenchulus reniformis.*

Numerous reports detail fungus-related enhancement of nematode reproduction. For example, the presence of *Verticillium dahliae* resulted in increased reproduction of the lesion nematode, *Pratylenchus* spp., in eggplant (*Solanum melongena*) (13), tomato (*Lycopersicon esculentum*) (15), and peppermint (*Mentha piperita*) (5,6). Enhanced reproduction of the lesion nematode *Pratylenchus penetrans* was also observed to occur in alfalfa (*Medicago sativa*) in the presence of *Fusarium oxysporum* (3,4). In Louisiana, several plant-pathogenic fungi have been reported to augment nematode reproduction (12,16,17,24). Our greenhouse studies documented that the cotton seedling blight fungus *Rhizoctonia solani* enhanced the reproduction of the reniform nematode, *Rotylenchulus reniformis* (20). Such effects on reniform nematode reproduction may be direct because of the production of fungal metabolites or indirect because of fungal-mediated alterations in cotton seedling physiology. Laboratory investigations described herein attempted to test these hypotheses.

Materials and Methods

A single egg mass culture of *R. reniformis* was established on tomato cv. Rutgers in the greenhouse. The nematode was isolated from cotton (*Gossypium hirsutum*) roots collected from Morehouse Parish, Louisiana. Unless otherwise specified, eggs of *R. reniformis* were extracted using the sodium hypochlorite method (7). Females used as inoculum for the infectivity studies were extracted from soil by a modified centrifugal-sugar flotation technique (8). The fungus *R. solani* was isolated from cotton seedlings exhibiting seedling blight symptoms from a field in Richland Parish, Louisiana. Procedures for isolation, maintenance, as well as production of fungal inocula on oat grains have been reported (20). The experimental design used in all experiments was a randomized complete block, and data were subjected to analysis using the SAS General Linear Model procedure (21). Experiments were repeated at least once, and data were pooled and analyzed.

Culture filtrates of R. solani: To determine the effect of *R. solani* on nematode egg hatching, the fungus was grown in compartmentalized dishes (100 × 15 mm)

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commonly referred to as "I plates." One half (side 1) of each dish contained 24 ml of potato dextrose broth (PDB) and the other half (side 2) contained 24 ml of sterile deionized distilled water (SDDW). A single 1-cm-d PDA disc cut from the growing edge of a 3-day-old culture of *R. solani* was introduced into side 1 and was allowed to grow over the SDDW of side 2. After 7 days, the mycelium was removed from side 2 and discarded and the aqueous contents were removed and sterilized by vacuum filtration through a 0.45-μm-pore filter. Side 1 of control plates received PDA discs minus fungus, and contents were collected from side 2 after 7 days and filter sterilized. Eight-tenth ml of the crude filtrate was pipetted into polystyrene cell wells containing 0.2 ml of the nematode egg suspension (40–50 eggs/well). Treatments were replicated five times, and eggs were incubated at room temperature (22–25 C). Egg hatching was monitored at 2-day intervals, until hatching ceased. Data are expressed as percentage of cumulative egg hatch, i.e., (total numbers of juveniles observed at each interval/total numbers of eggs at day zero) × 100.

Exudates from cotton seedlings: Seedlings of Deltapine 90 (DP 90) cotton were produced in greenhouse germination trays containing a 3:2:1 mixture of methyl bromide treated loamy soil (80.8% sand, 4.7% silt, 14.5% clay, pH adjusted to 6.5), autoclaved sand, and soilless potting media. After 15 days, seedlings were removed from the trays, the root system was washed twice in SDDW, and seedlings of uniform weight (1.25 ± 0.10 g) were selected.

A sterile glass slide (7.5 cm × 2.5 cm) was placed inside a sterile Petri dish (15 × 1.5 cm), and one end of the slide was elevated by placing a sterile glass rod (0.75 cm-d × 7.5 cm long) beneath it. A single seedling was placed onto the slide with the cotyledonary portion elevated. The entire dish was inclined 10 degrees by placing another glass rod under the dish. Five ml of SDDW (pH 5.5) was pipetted into the bottom of the dish, where it pooled and covered the seedling root system. A total of 20 Petri dishes were used for the experiment. Seedlings in half of the dishes were inoculated by placing an oat grain colonized by *R. solani* onto the glass slide next to the hypocotyl region of the seedling. Seedlings in control plates received noncolonized oat grains. A single piece of sterile Whatman No. 1 filter paper (15 cm-d) was folded in half, moistened with 3 ml of SDDW, and placed in the lower end of each lid to provide moisture for fungus growth. Dishes were incubated at ambient temperature (22–25 C) under supplemental light from plant Grow-Lux fluorescent bulbs (ca. 16 $\mu E/M^2/sec$ at bench surface), which provided a 14:10 hours light:dark photoperiod each day. After 24 hours, Petri dishes were opened and the filter paper was remoistened with additional 2 ml of SDDW. After 48 hours, symptoms of *Rhizoctonia* infection were apparent on inoculated hypocotyl tissue of seedlings, and filter papers were removed from all dishes. After 4 days, lengths of lesions on inoculated plants were measured and the aqueous contents of all dishes were removed and pooled into two samples representing *Rhizoctonia*-infected or noninfected plants. The volume of each sample was adjusted to 30 ml by the addition of SDDW and the pH was determined. Samples were filter sterilized by passing through a 0.2-μm-pore filter.

To collect nematode eggs, tomato roots infected with *R. reniformis* were washed and cut into 7–10 cm lengths. From the root segments, 50–60 individual egg masses were removed with the aid of a stereomicroscope and placed into an autoclaved test tube. Five ml of 0.5% NaOCl were then added to the tube and it was shaken vigorously for 5 minutes. Under a laminar flow hood, the egg suspension was poured over a sterile 45-μm-pore sieve, nested in a 25-μm-pore sieve fitted to the bottom of a storage dish (80 × 100 mm). Eggs were removed from the 25-μm-pore sieve by rinsing with SDDW. One-tenth ml of the egg suspension (50–60 eggs) was pipetted into polystyrene cell wells containing 0.9 ml of one of the following: filtrate

from infected seedlings, filtrate from noninfected seedlings, a SDDW control (pH 5.5), a SDDW control adjusted to the pH of filtrates from infected and noninfected seedlings (pH 6.7) using 5-mM phosphate buffer (pH 7.0). Each treatment was replicated six times. Eggs were incubated at 25 C and hatched juveniles were counted at 2-day intervals until hatching ceased. The experiment was repeated once.

Nematode infectivity: Root systems of 15-day-old seedlings of cotton (DP 90) produced under greenhouse conditions as described were washed in SDDW, and seedlings of uniform weight (1.39 ± 0.24 g) were selected.

Twenty-five grams of methyl bromide-treated soil mixture were weighed and 15 g were layered at the bottom of a sterile Petri dish (15 × 1.5 cm). One cotton seedling was placed in each dish so that the root system was spread across the layer of soil and the remaining 10 g of soil was used to cover the root system. Thirty-two such dishes were prepared and divided into two groups. Seedlings in one group were inoculated with *R. solani* by placing an infested oat grain next to the hypocotyl at the soil line. Each seedling in the second group received a noninfested oat grain. Then each seedling was inoculated with 100 infective female reniform nematodes by pipetting aqueous suspension containing the nematodes onto the soul surface. The soil was wetted with 2.5 ml of SDDW, and dishes were covered and incubated as described.

Soil was wetted with an additional 2.5 ml of SDDW after 48 hours. Four seedlings from each group were harvested at intervals of 24, 48, 72, and 96 hours after inoculation. Infectivity was monitored by counting female reniform nematodes present on the entire root system with the aid of a stereomicroscope. To monitor the presence of the fungus, hypocotyl lesion length was measured at each harvest interval. In a repeat of this experiment, an additional treatment was included that consisted of wounding the hypocotyl of another group of 16 seedlings by scraping a 5-cm region of the hypocotyl with a sterile razor blade.

Results

Culture filtrates of R. solani: Fungal filtrate produced no effect on the percentage of cumulative egg hatch at any of the observation intervals (Table 1). Cumulative egg hatch on day 14 in filtrates obtained from the SDDW side of compartmentalized "I plates" with the fungus was 48.5% compared with 50.2% in SDDW filtrates collected from controls.

Exudates from cotton seedlings: At 4 days, the lesion length on the hypocotyls of seedlings inoculated with *R. solani* was 38.9 ± 3.4 mm. There were no differences in the numbers of juveniles observed in the root exudates of *R. solani*-infected and noninfected seedlings and water controls 2 days after the experiment was initiated (Table 2). On day 4, there were no differences in the numbers of juveniles present in wells containing root exudates of *Rhizoctonia*-infected and noninfected seedlings. Also,

Table 1. Hatching of eggs of *Rotylenchulus reniformis* in crude culture filtrates of *Rhizoctonia solani* produced over water in compartmentalized "I plates."

	Days after initiation						
Treatment	2	4	6	8	10	12	14
Fungus present	0.2	7.9	21.8	32.1	40.0	46.3	50.2
Fungus absent	0.3	9.6	20.6	31.8	39.2	45.5	48.5
Source							
Fungus	NS	NS	NS	NS	NS	NS	NS

Data are percentages of cumulative egg hatch at each interval and are composite means of two trials of the same experiment. NS = nonsignificant based on *F* test.

TABLE 2. Hatching of eggs of *Rotylenchulus reniformis* in exudates of roots of Deltapine 90 cotton seedlings not inoculated and inoculated with *Rhizoctonia solani*.

Treatment†	Days after initiation						
	2	4	6	8	10	12	14
No fungus (pH 6.7)	0.7 a	14.5 ab	32.9 a	48.7 a	57.6 a	60.9 a	61.2 a
Fungus (pH 6.7)	0.6 a	16.1 a	33.9 a	50.2 a	60.6 a	63.7 a	64.0 a
SDDW (pH 6.7)	0.5 a	13.2 b	23.9 b	40.3 b	47.9 b	50.6 b	50.9 b
SDDW (pH 5.5)	0.9 a	12.1 b	23.6 b	40.6 b	49.8 b	52.5 b	52.8 b

Data are percentages of cumulative egg hatch at each interval and are composite means of two trials of the same experiment.
† SDDW = sterile deionized distilled water.
Means in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple-range test.

cumulative egg hatch in water controls did not differ from that which occurred with exudates from seedlings that received a noncolonized oat grain. From day 6 to day 14, increases ($P \leq 0.05$) were observed in egg hatching in root exudates compared with water controls. The numbers of juveniles that hatched from eggs in exudates from seedlings with and without *R. solani* did not differ. Numbers of juveniles observed in the two water controls of pH 5.7 and 6.7 did not differ.

Nematode infectivity: Lesion lengths on hypocotyls of seedlings infected with *R. solani* were 0, 11.9 ± 1.9 mm, 17.8 ± 2.1 mm, and 23.6 ± 2.3 mm, respectively, at the 24-, 48-, 72-, and 96-hour intervals. At 24 and 48 hours, there were no differences in the numbers of infective females that entered the root systems of *R. solani*-infected and noninfected seedlings (Table 3). At the 72- and 96-hour intervals, however, the numbers of infective females that entered root systems of *Rhizoctonia*-infected seedlings were greater ($P \leq 0.05$) than the numbers that penetrated healthy root systems. At 24 and 48 hours, numbers of females observed on the root systems of wounded, Rhizoctonia-infected and noninfected seedlings did not differ (Table 4). At 72 and 96 hours, numbers of females recovered from the root systems of seedlings infected with *R. solani* were greater ($P \leq 0.05$) than the numbers from both wounded and healthy seedlings. Numbers of females on root systems of wounded and healthy seedlings did not differ.

TABLE 3. Number of sessile *Rotylenchulus reniformis* females per root system of Deltapine 90 cotton not infected or infected with *Rhizoctonia solani*.

Treatment	Hours after initiation			
	24	48	72	96
No fungus	8.1 a	13.8 a	20.0 b	28.6 b
Fungus	7.5 a	14.3 a	32.0 a	47.0 a

Values are composite means of two trials of the same experiment.
Means in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple-range test.

DISCUSSION

In preliminary studies, crude *R. solani* filtrate collected from PDB inhibited the hatching of eggs of *R. reniformis*. However, the suggestion that medium could also in-

TABLE 4. Number of sessile *Rotylenchulus reniformis* females per root system of Deltapine 90 cotton as influenced by the presence or absence of *Rhizoctonia solani* or hypocotyl wounding.

Treatment	Hours after initiation			
	24	48	72	96
No fungus	7.0 a	11.8 a	18.3 b	26.5 b
Fungus	6.3 a	13.3 a	30.5 a	44.8 a
Wounded†	5.6 a	12.3 a	20.0 b	28.0 b

Values are composite means of two trials of the same experiment.
Means in columns followed by the same letter are not significantly different ($P < 0.05$) according to Duncan's multiple-range test.
† Wounding accomplished by scraping a 5-cm region of the hypocotyl with a sterile razor blade.

fluence egg hatching was confirmed in a subsequent experiment, and PDB was found to be more inhibitory to egg hatching than the fungal filtrate. A similar effect was observed by Metha et al. (14) with *R. solani* and the root-knot nematode, *Meloidogyne javanica.* They found that PDB was more inhibitory to the hatching of eggs of *M. javanica* than the filtrates of *R. solani.* Rambir Singh et al. (18) also observed that filtrates of *R. solani* grown on PDB were inhibitory to the hatching of eggs of *M. javanica.*

Attempts to produce cultures of *R. solani* over water by using "I plates" for the purpose of eliminating substrate effects on egg hatching were successful. Although there was abundant mycelial growth, the experiment did not show any effect of fungus on egg hatching. If the fungus does produce compound(s) that directly affect egg hatching, they are not produced over water culture, or concentrations produced are below levels necessary to affect egg hatching.

Root exudates from cotton seedlings had a pronounced effect on nematode egg hatching. Although other investigators (9) have shown that the malvaceous hose, okra (*Abelmoschus esculentus*) produces exudates that enhance hatching of reniform nematode eggs, our data constitute the first report of this type for cotton. The fact that there were no differences in egg hatching between pH 5.5 or 6.7 demonstrates that a pH effect in our system was minimal. Reports on the effects of pH on nematode egg hatching are variable. It has been previously reported that pH influences egg hatching of *M. incognita* (11). Conversely, similar work with cyst nematode (*Heterodera glycines*) indicated that pH had no effect on egg hatching (10).

In this study, increased numbers of infective females were observed after 72 hours in the root systems of *Rhizoctonia*-infected seedlings. Wounding had no detectable influence on infectivity of females. This observation constitutes the first report of *R. solani*-related enhancement of reniform nematode infectivity on cotton and is one of the few reports of this phenomenon for any crop. Infectivity studies by Edmund (3) with *P. penetrans* and *F. oxysporum* on alfalfa showed enhanced penetration by second-stage juveniles within 5 days of fungal infection. This time frame corresponds closely to our findings for *R. reniformis* and *R. solani.* In subsequent work with the *P. penetrans–F. oxysporum* system, increased attractiveness of alfalfa roots to *P. penetrans* was attributed to the release of more CO_2, resulting from fungal infection (4). Pectinolytic and cellulolytic enzymes play a major role in the pathogenicity of *R. solani* on cotton hypocotyls (2,23). Increased respiratory rate, which would result in increased CO_2 evolution, in bean (*Phaseolus vulgaris*) hypocotyl tissues infected with *R. solani* has been reported by Bateman and Daly (1). It is likely, then, that changes in the physiology of *Rhizoctonia*-infected cotton hypocotyls affects root metabolism, CO_2 production, and subsequent attractiveness of the roots to nematodes. Also, Riddle and Bird (19) demonstrated that juveniles of *R. reniformis* oriented their movement toward inorganic salts such as $MgCl_2$ and NaCl. It is possible that, as the result of enhanced root parasitism, quantitative changes in electrolyte concentrations in the root exudates render root systems more attractive to nematodes. Van Gundy et al. (22) documented this with *M. incognita,* which, like *R. reniformis,* is a sedentary endoparasite. They showed that the nematode induced leakage of exudates from tomato roots that contained high concentration of Mg and Na.

The increased egg production and enhanced soil population densities consistently observed in our greenhouse studies in the presence of *R. solani* probably resulted from enhanced infectivity of females. Results of our research provide increased impetus for seedling disease management in cotton. Additionally, management of this early season seedling disease can reduce the potential for *R. solani-*

related enhancement of reniform nematode reproduction.

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