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Detection of Suppressiveness against *Rotylenchulus reniformis* in Soil from Cotton (*Gossypium hirsutum*) Fields in Texas and Louisiana

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Abstract: Rotylenchulus reniformis is a major problem confronting cotton production in the central part of the cotton belt of the United States of America. In this study, the hypothesis that natural antagonists in some cases are responsible for unusually low densities of the nematode in certain fields was tested by assaying soils from 22 selected fields for the presence of transferable agents in pots containing cotton plants. In one field, soil from four different depth ranges was tested. In the first of two types of assays, 1 part nematode infested soil was added to 9 parts test soil that was left untreated or autoclaved before mixing; this mixture was used to fill pots. In the second type of assay, 1 part test soil was added to 9 or 19 parts pasteurized fine sand, and nematodes were introduced in aqueous suspension. In three experiments representing both types of assay, transferable or autoclavable agent(s) from four fields in South Texas suppressed nematode populations by 48, 78, 90 and 95%. In one experiment, transferable agents in five fields in Louisiana suppressed populations from 37 to 66%. Identification and evaluation of these agents for biological control of *R. reniformis* merits further study.

Key words: biological control, cotton, *Gossypium hirsutum*, *Rotylenchulus reniformis*, reniform nematode, soil suppressiveness.

The reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, is considered the most important nematode of cotton within the central cotton-producing states of Louisiana, Mississippi and Alabama and causes total annual losses to USA cotton production estimated to exceed US$100M (Blasingame, 2006). Nematicides and crop rotation are the primary approaches currently available for management. In many cases, growers recover only a fraction of the profit lost to this nematode (Starr and Page, 1990; Robinson, 2007, 2008).

Little is known about natural antagonists of *R. reniformis*. However, *R. reniformis* has been detected in 3,213 fields in Louisiana, and, in about 15 of these fields, the population density of the nematode was observed to remain inexplicably low despite conducive cropping history and soil characteristics, suggesting the presence of potent biological control agents (C. Overstreet, pers. comm.). In other fields, most commonly in the Lower Rio Grande Valley of Texas, population densities in the upper 30 cm of the soil profile are markedly less than in deeper soil, in striking contrast to the more common situation where most nematodes occur within the top 20 cm of soil where root density is greatest.

We hypothesize that in at least some cases, inexplicably low population densities of *R. reniformis* that have been consistently observed in some cotton fields are the result of suppression by transferable agents in the soil (Stirling, 1991; Westphal and Becker, 2000, 2001; Westphal, 2005). Our specific objective in this study was to test this hypothesis by assaying soil from 22 selected fields in Louisiana, the Mississippi Delta, the Texas High Plains, and the Lower Rio Grande Valley of Texas for the presence of transferable agents suppressing population buildup by *R. reniformis* in pots.

Materials and Methods

Soil collection: Soil was collected from 22 cotton fields in Louisiana, Mississippi and Texas. In 18 fields, the reniform nematode (*Rotylenchulus reniformis*) was present in lower population densities than expected based on soil texture, cropping history and infestation levels in other local fields. In some cases, soil samples from different depths in the same field were tested. Unless otherwise stated, soil was collected 15 or more cm deep, or as deep as necessary to be moist and friable. For shipment and storage, the soil was kept at 15 or 20°C within plastic bags to prevent drying.

Experiment 1: In the first of two types of assays, conducted in a greenhouse at Weslaco, TX, in 1999, 1 part nematode-infested soil was added to 9 parts test soil. The latter was either autoclaved or left untreated before mixing. This soil mixture was used to fill 1.2-liter pots. There were four replicate pots/soil origin with autoclaved and four with untreated soil. Autoclaved soil was autoclaved for 30 min on each of two consecutive d. Pots were planted with cotton cv. Suregrow 125 and maintained in a greenhouse for 14 wk, at which time soil was removed, nematodes were extracted by Baermann funnel, and roots were gently washed and weighed. All soil was from fields in the Lower Rio Grande Valley of Texas and included: cotton fields (#1, #2 and #4), an area near a cotton field but uncultivated for 10 yr (#3) and a cotton field 0- to 30-cm deep (#5-A) and 45- to 105-cm deep (#5-B). Soil textures for these fields are presented in Table 1. Nematode densities are

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TABLE 1. Effects of autoclaving soil from cotton fields in Hidalgo County in the Lower Rio Grande Valley of Texas, on suppressiveness against *Rotylenchullus reniformis*. [Experiment 1].

		Final nematode population density and root fresh weight[a]				
		Vermiform in soil per gram soil		Root fresh weight in grams		Calculated suppresion (%)[b]
Field[c]	Soil texture	Untreated	Autoclaved	Untreated	Autoclaved	
#1	Silt loam	62 (12)	297 (62)	2.6 (0.2)	2.3 (0.1)	79*
#2	Loam	15 (2)	219 (33)	1.8 (0.1)	2.0 (0.1)	93*
#3	Sandy loam	136 (19)	110 (18)	4.0 (0.4)	3.2 (0.3)	–24
#4	Loam	34 (10)	256 (27)	2.2 (0.2)	2.1 (0.4)	87*
#5-A	Sandy clay loam	25 (3)	87 (6)	1.9 (0.2)	3.1 (0.2)	71*
#5-B	Sandy clay loam	109 (32)	222 (80)	1.9 (0.2)	2.3 (0.3)	51*

[a] Nematode population densities measured 14 wk after growing cotton in pots containing a soil mixture of 1 part soil from #5-B and 9 parts soil from #1, 2, 3, 4, 5-A or 5-B that had been left untreated or autoclaved before mixing.

[b] The calculated suppression was the percent of reduction of nematode numbers in untreated soil compared to autoclaved portions of the same soil.

* Asterisk indicates significant difference of the log-transformed [$\log_{10}(x+1)$] population density means for a particular soil at $P = 0.05$; values in parentheses are the standard errors of the means.

[c] Field #3, denoted "Brush", had not been cultivated for 10 yr. #5-A is soil 0- to 30-cm and #5-B is soil 46- to 107-cm deep from Field #5, the North farm at Weslaco, TX.

expressed per gram soil, and means for autoclaved vs. untreated soil were separated by LSD to test for nematode suppressiveness.

Experiments 2 and 3: In a second type of assay, conducted in an environmentally controlled chamber at College Station, TX, in 2006, 1 part test soil was added to 19 (Experiment 2) or 9 (Experiment 3) parts steam-pasteurized fine sand supplemented with vermiculite and balanced nutrients (Robinson et al., 2004, 2007). This mixture was used to fill 0.5-liter pots that were planted with susceptible cotton cv. Fibermax 832.

Treatments in Experiment 2 included soil from two fields (#6 and #7) on the Texas High Plains, one field (#9) in the Mississippi Delta, and two fields (#5 and #8) from the Rio Grande Valley. Field #5, which was the same Field #5 tested in 1999, was represented by newly collected soil from the 0 to 15 cm (#5-C) and 23 to 38 cm (#5-D) depths. Field #5 is also referred to as North farm. Field #8 was on a different farm about 10 km away. Experiment 3 also included new collections of soil from Field #8 and the two depths of Field #5 tested in Experiment 2 (referred to as #5-E and #5-F in Experiment 3), plus soil from 13 fields in six parishes of Louisiana.

In both experiments there were two controls: Fibermax 832 and the resistant accession *G. barbadense* GB713 planted in sand with no test soil added. In Experiment 3, there were 12 instead of six replicates of the Fibermax 832 control.

Two weeks after planting, each pot was inoculated with 4,000 vermiform *R. reniformis* previously propagated in the greenhouse on cotton and tomato, and 7 wk after inoculation, three 15-cm^3 cores were removed from each pot to evaluate nematode populations in pots (Robinson et al., 2007). Nematode population densities were measured by counting vermiform stages collected by Baermann funnel extraction and compared by Dunnett's test with the Fibermax 832 sand-only control. Roots were not weighed, but plants were confirmed to be comparable in size, and plant heights were measured at the end of Experiment 3.

TABLE 2. Testing various soils for suppressiveness against *Rotylenchulus reniformis*, by testing for suppressiveness transferability [Experiment 2].

				Final nematode population density[a]		
Field[b]	Soil texture of added soil	State	County or parish	Vermiform/g soil	Relative to control (%)	Calculated suppression (%)
#5-C	Sandy clay loam	TX	Hidalgo	19	20	80*
#5-D	Sandy clay loam	TX	Hidalgo	61	64	36
#6	Loam	TX	Lubbock	181	191	–90
#7	Fine sandy loam	TX	Dawson	79	83	18
#8	Sandy clay loam	TX	Hidalgo	56	59	42
#9	Clay Loam	MS	Washington	36	38	62
Control	None (Fibermax 832)	—	—	95	100	0
Control	None (resistant GB713)	—	—	22	23	77*

[a] Nematode population levels were measured in pots containing cotton plants 7 wk after inoculating with 4,000 vermiform *Rotylenchulus reniformis*. Pots contained a mixture of 1 part test soil and 19 parts fine sand supplemented with vermiculite and fertilizer.

[b] #5-C and #5-D, respectively, are soil collected 0- to 15-cm and 23- to 38-cm deep in Field #5.

* Asterisk indicates significant difference from 100% at $P < 0.05$ by Dunnett's test.

TABLE 3. Testing various soils for suppressiveness against *Rotylenchulus reniformis*, by testing for suppressiveness transferability [Experiment 3].

Field[b]	Soil Texture of added soil	State	County or parish	Sample identifier	Plant height (% of control)	Final nematode population density[a]		Calculated suppression (%)
						Vermiform/g soil	Relative to control (%)	
#5-E	Sandy clay loam	TX	Hidalgo	North farm	101	14	5	95**
#5-F	Sandy clay loam	TX	Hidalgo	North farm	104	212	71	29
#8	Sandy clay loam	TX	Hidalgo	South farm	100	157	52	48**
#9	Clay loam	MS	Washington	USDA Stoneville	99	307	102	–2
#10	Silt loam	LA	Tensas	Padgett 11D-306	100	312	104	–4
#11	Silt loam	LA	Tensas	Padgett NE La	93	387	129	–29
#12	Silt loam	LA	Tensas	St. Joe	88	272	91	9
#13	Silt loam	LA	Rapides	Poole 06-47	97	178	59	41**
#14	Silt loam	LA	Morehouse	Holley 05-2695	88	189	63	37*
#15	Silt loam	LA	Rapides	Poole 06-33	96	101	34	66**
#16	Silt loam	LA	Morehouse	Turner 06-1154	87	162	54	46**
#17	Silt loam	LA	Rapides	Lee 06-1148	89	287	96	4
#18	Silt loam	LA	East Baton Rouge	Burden plantation	95	226	75	25
#19	Silt loam	LA	Rapides	Poole 06-53	102	209	70	30
#20	Silt loam	LA	Rapides	Poole 06-55	96	147	49	51**
#21	Silt loam	LA	Concordia	Vanielden 06-197	92	424	141	–41
#22	Silt loam	LA	Franklin	Thornhill- 06-1182	97	330	110	–10
Control (susceptible)	None (Fibermax 832)	—	—		—	300	100	0
Control (resistant)	None (GB713)	—	—		—	27	9	91**

[a] Nematode population levels were measured in pots containing cotton plants 7 wk after inoculating with 4,000 vermiform *Rotylenchulus reniformis*. Pots contained a mixture of 1 part test soil and 9 parts fine sand supplemented with vermiculite and fertilizer.
[b] #5-E and #5-F, respectively, are soil collected 0- to 15-cm and 23- and 38-cm deep in Field #5.
*,** Asterisks indicate significant differences from 100% at $P < 0.05$ and 0.01, respectively, by Dunnett's test.

RESULTS AND DISCUSSION

Experiment 1: Autoclaving increased final nematode populations by four- to 18-fold for all soils except #3, which had not been cultivated for 10 years. For other soils, the calculated suppressiveness was 51 to 93% ($P \leq 0.05$) (Table 1). Consequently, even though there were appreciable differences in physical and, most likely, chemical characteristics of the soils in which plants were grown, these differences did not influence the effect of autoclaving on nematode population increase.

Experiment 2: The resistant GB713 control in sand with no added test soil suppressed the nematode population 77% (Table 2). Soil #5-C (North farm, 0- to 15-cm deep), which in this experiment was present as 5% of the soil of the pot rather than as 90% in Experiment 1, suppressed the population 80% compared to the Fibermax 832 control (Table 2).

Experiment 3: Uniform plant heights indicated that adding 10% test soil to sand had a negligible effect on plant growth (Table 3). Soil #5-E, which in this experiment was present at twice the concentration as the comparable soil (#5-C) in Experiment 2 (Table 2), suppressed the nematode population by 95% compared to the Fibermax control ($P = 0.01$), suggesting a dosage effect. Soil from five fields in Louisiana suppressed populations 37 to 66% ($P \leq 0.05$) (Table 3).

Altogether, suppressiveness was detected in soil from five fields in the Lower Rio Grande Valley of Texas and five fields in Louisiana. The strongest suppression detected (95%) was for soil from field #5 at the North farm in the Lower Rio Grande Valley (LRGV), collected from the upper 15 cm. In Experiments 2 and 3, suppression by soil from the upper 15 cm of Field #5 was comparable to or better than that achieved with the highest level of resistance to *R. reniformis* known in *G. barbadense* (Robinson et al., 2004). Also in Experiments 1 and 2, the suppression (51 and 36%) measured for deeper soil in Field #5 was significantly less than that for the 0- to 15-cm layer.

Vertical distributions of *R. reniformis* in more than 200 graduated vertical samples taken to a depth of 122 cm in 17 fields in Texas, Louisiana, Arkansas, Louisiana, Mississippi, Alabama and Georgia indicate that the mean depth of *R. reniformis* in cotton tends to be several centimeters deeper than the mean root depth. In most cases, nematode density, like root density, was greatest within the top 30 cm of soil and diminished with depth (Robinson et al., 2005a, 2005b, 2006). However, in at least four fields of the Texas Lower Rio Grande Valley, vertical distribution patterns for *R. reniformis* were atypical compared to other areas (Robinson and Cook, 2001; Westphal et al., 2004; Robinson et al., 2006), suggesting that the nematodes in the upper profiles were suppressed in the four fields.

Further research is needed to determine the organ-

ism(s) involved in population suppression of *R. reniformis* in some of the more suppressive fields identified in this study. We predict that the antagonist(s) responsible for suppression in the North farm and other Lower Rio Grande Valley fields will be at their greatest densities near the surface.

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