INTERACTION OF *TRICHODERMA HARZIANUM*, *MELOIDOGYNE INCOGNITA*, AND *MELOIDOGYNE ARENARIA* ON *TRIFOLIUM REPENS*†

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RESUMEN

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Plántulas de 6 semanas de edad del cultivar susceptible (Regal) y del cultivar tolerante (SC-1) de trébol blanco, se inocularon con *M. incognita*, *M. arenaria* o *Trichoderma harzianum* y con cada nematodo en combinación con el hongo. Las plántulas se mantuvieron 12 semanas bajo condiciones de invernadero. Al término del ensayo se evaluó el crecimeinto de las plantas y raíces y la producción de huevos de nematodos. No se observaron diferencias en el crecimiento de las plantas. *Trichoderma harzianum* no causó lesiones en las raíces, tanto en la presencia o la ausencia de los dos nematodos. La reproducción de *M. incognita* en ambos cultivares de trébol blanco se vió significativamente disminuída en macetas inoculadas con *T. harzianum* (un 38% en el cv. Regal y 58% en el cv. SC-1). *Trichoderma harzianum* tuvo poco efecto sobre la reproducción de *M. arenaria*, logrando una disminución del 8% y 29% en Regal y SC-1, respectivamente.

Palabras clave: control biológico, *Meloidogyne incognita*, *Meloidogyne arenaria*, trébol blanco, *Trichoderma harzianum*, *Trifolium repens*.

Root-knot nematodes, *Meloidogyne* spp., are associated with a number of disease complexes (12,13,17) and have long been known for their ability to predispose plants to infection by other organisms, especially soil-borne pathogens such as *Fusarium* spp. (16,23). However, root-knot nematodes also predispose plants to infection by fungi that are normally innocuous or even beneficial soil saprophytes. The fungus *Trichoderma harzianum* Rifai is not pathogenic to tobacco or cotton in the absence of *Meloidogyne* spp. However, in the presence of *M. incognita* (Kofoid & White) Chitwood, *T. harzianum* causes severe necrosis on roots of tobacco (13,17) and cotton (23). In addition, *T. harzianum*, in the presence of *M. incognita*, accentuates *Fusarium* wilt on cotton (23).

The interaction of *Meloidogyne* spp. with *T. harzianum* does not always have a detrimental effect on the host plant. *Trichoderma harzianum* isolate T-12 in combination with *M. arenaria* stimulated growth of two maize cultivars (*Zea mays*) and no root necrosis was observed (21). Reproduction of *M. arenaria* on a root-knot nematode susceptible maize hybrid was also reduced in the presence of *T. harzianum*. Additional studies are needed to clarify the interaction of *Meloidogyne* spp. with *T. harzianum* isolate T-12, which is commonly used as a biocontrol agent for soil-borne fungi (5,7,8,9,11,14,18). Since

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maize is fairly tolerant to damage by *Meloidogyne* spp., the interaction of *T. harzianum* isolate T-12 with *Meloidogyne* spp. on a plant that is highly susceptible to root-knot nematodes needs to be defined. The objectives of this study were to determine the combined effects of *T. harzianum* and two *Meloidogyne* spp. on the growth of white clover (*Trifolium repens* L.) and to evaluate the effect of the fungus on nematode reproduction.

A race 4 population of *M. incognita* and a race 2 population of *M. arenaria* (Neal) Chitwood were increased on tomato (*Lycopersicon esculentum* Mill. cv. Floradel) in greenhouse pot cultures. After 8 to 10 weeks, eggs were collected from tomato roots with NaOCl and resuspended in water (10).

Trichoderma harzianum isolate T-12 was provided by G. E. Harman (New York State Agricultural Experiment Station, Geneva). Thalli of *T. harzianum* were added to 3.8-L jars containing sterilized media of equal parts by volume of wheat bran, sphagnum peat moss, and water (1) and were incubated as described by Paulitz *et al.* (15). *Trichoderma* inoculum was quantified on a selective medium (4), and the population density was determined to be 1.0×10^8 colony forming units per gram of substrate.

The white clovers used were Regal (a root-knot susceptible cultivar) (19,20) and SC-1 (a root-knot tolerant germplasm) (6). Seeds were germinated on water agar at 22 °C and transplanted into Cell-paks (Ball Seed Company, West Chicago, Illinois, U.S.A.) containing a methyl bromide-sterilized mixture of sandy loam soil and river sand (80% sand, 6% clay, 14% silt). A commercial preparation of *Rhizobium leguminosarum* biovar *trifolii* Jordan was broadcast over the seedlings and watered into the soil.

When clover seedlings in Cell-paks were 6 weeks old, they were transplanted to 10-cm-diam clay pots containing 620 g of soil mixture described previously. Pots receiving *T. harzianum* were inoculated by thoroughly mixing 2 g of the *T. harzianum* wheat bran inoculum with the soil in each pot prior to transplanting. One week after transplanting, each pot was inoculated with 5 000 eggs of *M. arenaria* or *M. incognita*, which were applied to the soil surface in a water suspension with a pipette.

The soil treatments included *T. harzianum* alone, *M. incognita* alone, *M. arenaria* alone, *T. harzianum* plus *M. incognita*, and *T. harzianum* plus *M. arenaria*. Soil with neither *Trichoderma* nor *Meloidogyne* inoculum served as a control. Treatments were arranged in a randomized complete block design with 10 replications for each treatment. The plants were grown in a greenhouse at an average temperature of 28 °C.

Shoot growth was determined 4, 8, and 12 weeks after inoculation with nematodes. At 12 weeks the experiment was terminated. Clover roots were carefully washed free of soil, weighed, and rated for necrosis by the classification system of Powell *et al.* (17). Roots were cut into 1-cm segments, and root-knot nematode eggs were extracted from each root system with NaOCl (10) and counted. Data from two repetitions of the experiment were combined for analysis of variance.

Trichoderma harzianum did not have a detrimental effect on growth of either Regal or SC-1 white clover regardless of the presence of *M. incognita* or *M. arenaria*. Root necrosis was not observed on any white clover roots from *T. harzianum* infested pots. This was the same reaction found previously with maize (21), whereas in other studies, root systems of cotton (23) and tobacco (13,17) became severely

deteriorated when grown in soil infested with *T. harzianum* and *M. incognita*. Differences that have been observed in interactive effects of *T. harzianum* and root-knot nematodes on crop plants could be crop specific, but also could be the result of differences between the strains of *T. harzianum* used.

No difference was observed in shoot growth in *T. harzianum* infested pots regardless of the presence or absence of either *Meloidogyne* sp. *Trichoderma harzianum* commonly stimulates shoot growth (1,21,22); however, that did not occur in this study. The only difference in root growth was between Regal plants grown in *T. harzianum* infested pots and plants grown in pots infested with *T. harzianum* and *M. incognita*. White clover root mass was 19% greater in soil infested with both *T. harzianum* and *M. incognita* than in soil infested only with *T. harzianum*.

Reproduction of *M. incognita*, as measured by eggs per gram of root, was reduced 38% on Regal (root-knot susceptible) and 58% on SC-1 (root-knot tolerant) clover as a result of adding *T. harzianum* inoculum ($P \leq 0.05$) (Table 1). When measured as total eggs per pot, reproduction of *M. incognita* was reduced by 24% on Regal and 61% on SC-1 ($P \leq 0.01$). *Trichoderma harzianum* had less of an effect on *M. arenaria* reproduction. Suppression of reproduction was not statistically significant on Regal or SC-1; however, eggs per gram of root and total eggs per pot were numerically less on both white clovers. In an earlier study, *M. arenaria* reproduction was suppressed by *T. harzianum* on a root-knot susceptible maize hybrid (21).

This is the first report of *M. incognita* reproduction being suppressed by a *Trichoderma* sp. The typical, synergistically detrimental effect of *T. harzianum* and *M. incognita* on susceptible hosts was not observed on white clover. White clover is an exceptionally good host for *Meloidogyne* spp. and heavy infections can lead to plant death. Another *T. harzianum* isolate may have induced a synergistic reaction, but the T-12 isolate did not.

Our results with white clover confirm that wheat bran inoculum of *T. harzianum* isolate T-12 can suppress reproduction by root-knot nematodes. Reduced nema-

Table 1. Effect of *Trichoderma harzianum* wheat bran inoculum on reproduction of *Meloidogyne incognita* and *M. arenaria* on two white clover genotypes after 12 weeks in greenhouse pots.

Clover genotype	Nematode	Control	*T. harzianum*	Difference (%)	F test
		Eggs/g of fresh root			
Regal	*M. incognita*	1 408	879	–38	*
	M. arenaria	2 657	2 453	–8	NS
SC-1	*M. incognita*	975	410	–58	**
	M. arenaria	3 049	2 176	–29	NS
		Eggs/pot			
Regal	*M. incognita*	44 906	34 517	–24	NS
	M. arenaria	87 370	85 512	–3	NS
SC-1	*M. incognita*	31 687	12 396	–61	**
	M. arenaria	83 797	61 412	–27	NS

Data are means of 20 replications.
*Significant at $P \leq 0.05$; **Significant at $P \leq 0.01$; NS = not significant.

tode reproduction on white clover inoculated with *T. harzianum* was not associated with root growth suppression or root necrosis, indicating that the fungus did not reduce nematode reproduction by limiting the number of feeding sites available. Various other mechanisms could be involved. *Trichoderma* spp. produce chitinases that could facilitate parasitism of *Meloidogyne* eggs (2). *Trichoderma longibrachiatum* Rifai can produce nematotoxic concentrations of acetic acid (3). Other metabolic byproducts of microfloral activity on the inoculum substrate also could be involved. The use of *Trichoderma* species as nematode biocontrol agents and the mechanism of nematode suppression deserve further study.

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