Effects of *Trichoderma* spp. on Maize Growth and *Meloidogyne arenaria* Reproduction

G. L. WINDHAM, Research Plant Pathologist, USDA-ARS, Crop Science Research Laboratory, Mississippi State, MS 39762; M. T. WINDHAM, Assistant Professor, Department of Entomology and Plant Pathology, University of Tennessee, Knoxville 37916; and W. P. WILLIAMS, Research Geneticist, USDA-ARS, Crop Science Research Laboratory, Mississippi State, MS 39762

ABSTRACT

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The effects of *Trichoderma harzianum* and *T. koningii* on two commercial maize hybrids grown with and without *Meloidogyne arenaria* were investigated in the greenhouse. Root necrosis was not observed on plants grown in soil infested with either of the *Trichoderma* spp., *M. arenaria*, or a combination of either fungal isolate with the nematode. Shoot and root growth of both hybrids in *Trichoderma*-infested soil was significantly enhanced ($P = 0.05$), regardless of the presence of *M. arenaria*, compared to plant growth in treatments without *Trichoderma*. When plants were grown in soil infested with *T. harzianum* or *T. koningii*, the reproduction of *M. arenaria* on the root-knot-susceptible maize hybrid was suppressed, whereas reproduction on the root-knot-resistant hybrid was not affected.

Trichoderma harzianum Rifai and *T. koningii* Oud. have been used as antagonists to control a number of plant pathogens (4,6–8,10,13,16). Crop losses due to diseases caused by *Fusarium* spp. (16), *Pythium* spp. (8), *Sclerotium rolfsii* Sacc. (6), and *Rhizoctonia solani* Kühn (6) have been reduced in soil infested with *T. harzianum*. *T. koningii* has been used to limit damping-off (10) and seed rot caused by *Pythium* spp. (8). In addition, *T. harzianum* and *T. koningii* have been used to enhance plant growth (1–3,14,19).

Trichoderma spp. have also been associated with a seedling disease of maize (*Zea mays*) in southwestern Ontario (12,17). Lesions resulting from *T. koningii* infections in mesocotyl, primary root, and first-whorl root tissues have been observed (17). McFadden and Sutton (12) stated that *Trichoderma* at inoculum densities of 10^4–10^5 propagules per gram of soil resulted in root lesions in 70–100% of maize seedlings.

Effects of *T. harzianum* and *Meloidogyne incognita* (Kofoid & White) Chitwood on root development of tobacco and cotton have previously been investigated (15,20). *T. harzianum* may be an important component of a cotton wilt complex, along with *M. incognita* and *F. oxysporum* f. sp. *vasinfectum* Snyd. & Hans. (20). Adding an "antagonist" such as *Trichoderma* to a cropping system can increase plant disease under greenhouse conditions. *T. harzianum* has been shown to induce extensive root necrosis in tobacco plants colonized by *M. incognita* (15). The damage attributed to *T. harzianum* infections under these conditions was as extensive as that incited by *P. ultimum* Trow.

T. harzianum isolate T-12 (8) and *T. koningii* isolate T-8 (8) have been used to control plant pathogens and enhance the growth of other crops. The objectives of this research were 1) to determine if these isolates would detrimentally affect maize plants infected with *M. arenaria* (Neal) Chitwood and 2) to determine if they would induce disease symptoms in maize similar to those observed in maize infected with *T. koningii* (17).

MATERIALS AND METHODS

The maize hybrids used in this study were Pioneer Brand 3110 (susceptible to *M. arenaria*) and Northrup King Brand 508 (resistant to *M. arenaria*) (18). *T. koningii* isolate T-8 and *T. harzianum* isolate T-12 were provided by G. E. Harman (New York State Agricultural Experiment Station, Geneva). Thalli of T-8 and T-12 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 (14). *Trichoderma* inoculum was quantified on a selective medium (5). The population densities of the T-8 and T-12 preparations were 3.2×10^8 and 2.8×10^9 cfu/g, respectively. The inoculum was thoroughly mixed with the soil before planting. Seed was planted in clay pots 10-cm in diameter containing a 1:1 mixture of heat-sterilized sandy loam soil and river sand.

M. arenaria was increased on tomato (*Lycopersicon esculentum* Mill. cv. Floradel) in the greenhouse. After 8–10 wk, nematode egg inoculum was collected from tomato roots with NaOCl (9). Seedlings were inoculated when 10 days old; a water suspension containing 3,000 eggs was transferred by pipette into each pot.

The soil treatments included T-8, T-12, T-8 plus *M. arenaria*, T-12 plus *M. arenaria*, and *M. arenaria*. Soil without *Trichoderma* amendments or *M. arenaria* inoculum served as controls. Baker et al (1) demonstrated that uninfested peat-bran amendments or autoclaved *Trichoderma* peat-bran amendments added to growth media were treatments and not controls, and these treatments were not included in this study. Treatments were arranged in a randomized complete block design with six replications for each treatment. The plants were grown in a greenhouse at an average temperature of 29 ± 6 C. The experiment was repeated in the greenhouse at an average temperature of 29 ± 4 C.

At 10-day intervals beginning 10 days after inoculation with *M. arenaria* and continuing until 50 days after inoculation, a soil sample 8 mm in diameter was taken at a depth of 5 cm in each pot and air-dried. *Trichoderma* population densities in each soil sample were estimated on a selective medium and expressed as colony-forming units per gram of air-dried soil.

Plant height was measured 25 days after inoculation with *M. arenaria*. Plant shoots were harvested, dried, and weighed 50 days after inoculation with *M. arenaria*. Roots were carefully washed free of soil, weighed, and rated for necrosis by the classification system of Powell et al (15). Roots were cut into 1-cm segments, and *M. arenaria* eggs were extracted from each root system

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with NaOCl (9) and counted. Data from both repetitions were combined for analyses of variance. Treatments were compared by least significant differences ($P = 0.05$).

RESULTS AND DISCUSSION

The major effect of the *Trichoderma* treatments on maize seedlings was enhanced plant growth. All maize plants in the *Trichoderma*-infested soils had enhanced growth. The *Trichoderma* treatments resulted in increased plant growth (shoot height) of both hybrids ($P = 0.05$) by 25 days after inoculation with *M. arenaria* (Table 1). The nematode treatment had no effect on plant height. The enhanced growth was still evident when the experiment was terminated 50 days after inoculation with *M. arenaria*. Plants in all *Trichoderma* treatments, regardless of the presence of *M. arenaria*, had greater shoot dry weights than the controls. Increases in shoot growth of Northrup King Brand 508 ranged from 39% with T-8 to 63% with T-12, and increases in Pioneer Brand 3110 ranged from 67% with T-12 to 76% with T-8 plus *M. arenaria*. Treatment with *M. arenaria* had no effect on the shoot growth of either hybrid. Enhanced plant growth induced by these *Trichoderma* spp. has previously been reported (14,19). Windham et al (19) concluded that these fungi produce a growth-regulatory factor.

Maize plants treated with T-8 or T-12 had larger root systems than the controls. Increases in root weight of Northrup King Brand 508 ranged from 41% with T-8 plus *M. arenaria* to 50% with T-12, and increases in Pioneer Brand 3110 ranged from 24% with T-12 plus *M. arenaria* to 47% with T-8. The nematode treatment had no effect on root weight. The treatments combining *Trichoderma* and *M. arenaria* had no detrimental effect on maize roots. Root necrosis in all *Trichoderma* treatments was negligible, and these data are not presented.

Trichoderma populations in soil treated with T-8 and T-12 were highest in samples collected 10 days after inoculation with *M. arenaria* (Fig. 1). Population densities were 10 times higher at the first sampling (10 days after inoculation) than at the beginning of the experiment. At 20 days after inoculation, however, they were 100 times lower than at 10 days, and they remained stable for the next 30 days.

The soil in the control pots had an initial *Trichoderma* population near 200 cfu/g. Population levels increased 10-fold in the control pots after 10 days and remained relatively stable throughout the remainder of the experiments. The two maize hybrids tested and the presence or absence of root-knot nematodes had no influence on *Trichoderma* populations.

Nematode egg production on the nematode-susceptible Pioneer Brand 3110 was affected by the *Trichoderma* treatments (Table 2). Egg production on Pioneer Brand 3110 was reduced by 47% in the T-12 treatment and by 53% in the T-8 treatment. Reproduction on the resistant Northrup King Brand 508 was much lower than on Pioneer Brand 3110

Table 1. Effects of *Trichoderma koningii* (T-8) and *T. harzianum* (T-12) on plant height and shoot and root growth of maize grown in pots with and without *Meloidogyne arenaria*

	Northrup King Brand 508			Pioneer Brand 3110		
Treatment	Plant height[a] (cm)	Shoot dry weight[b] (g)	Root fresh weight (g)	Plant height (cm)	Shoot dry weight (g)	Root fresh weight (g)
Control	61.0	6.8	19.5	60.3	5.9	20.2
T-8	71.0	9.5	28.9	74.2	10.0	29.7
T-12	71.7	11.1	29.3	71.3	9.9	27.5
T-8 + *M. arenaria*	75.6	10.9	27.6	70.5	10.4	26.6
T-12 + *M. arenaria*	72.6	10.1	29.0	71.3	10.3	25.2
M. arenaria	58.9	6.5	18.3	60.9	7.4	17.7
LSD ($P = 0.05$)	5.1	2.1	7.0	4.5	1.8	4.7

[a]Plant height was measured 35 days after planting.
[b]Shoot and root growth were measured 60 days after planting.

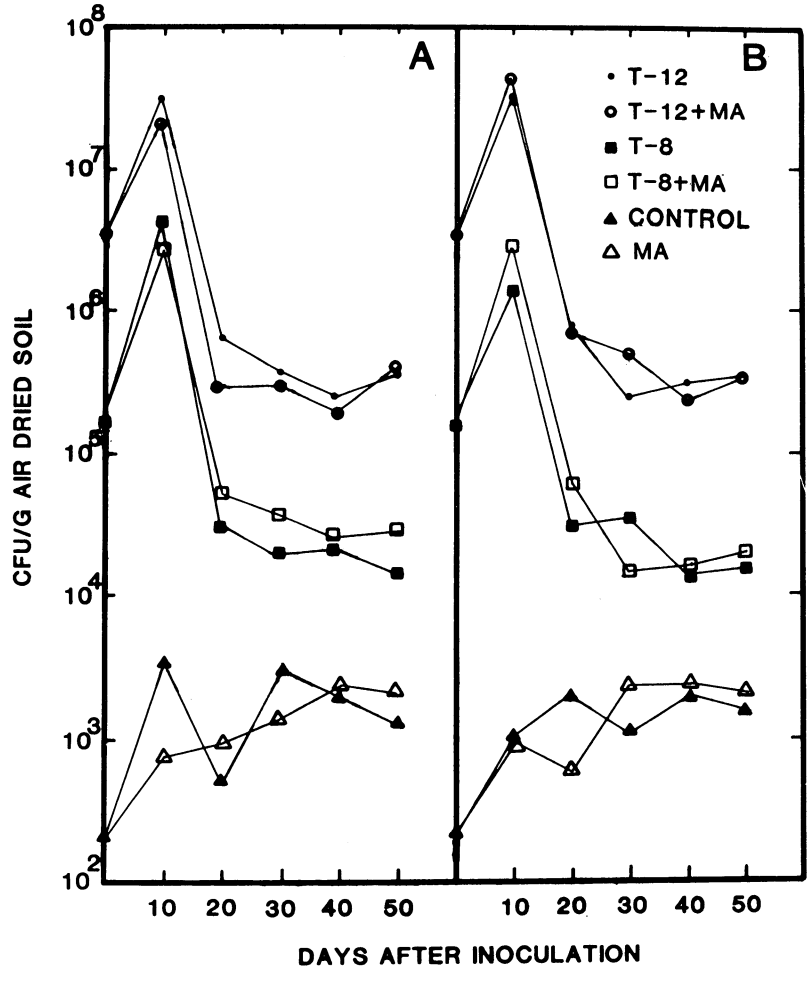

Fig. 1. Colony-forming units (CFU) of *Trichoderma* spp. in soil inoculated with *T. harzianum* (T-12) and *T. koningii* (T-8) and infested and uninfested with *Meloidogyne arenaria* (MA). Maize hybrids in these experiments were Pioneer 3110 (**A**) and Northrup King 508 (**B**).

Table 2. Reproduction of *Meloidogyne arenaria* on maize hybrids grown in soil with and without *Trichoderma koningii* (T-8) and *T. harzianum* (T-12)

	Number of eggs per gram of fresh root	
Treatment	Northrup King Brand 508	Pioneer Brand 3110
Control	55	4,779
T-8	58	2,244
T-12	21	2,538
LSD ($P = 0.05$)	35	1,215

and was not affected by the *Trichoderma* treatments.

In previous studies (12,17), isolates of *T. koningii* and *T. harzianum* were reported to be pathogenic on maize hybrids and to cause necrotic lesions on roots and mesocotyls. In this study, *T. koningii* isolate T-8 and *T. harzianum* isolate T-12 were not pathogenic, and root necrosis and lesions were absent. The population densities of T-8 and T-12 at the beginning of the experiments were equal to or higher than the population densities of the *Trichoderma* isolates that caused root disease of maize seedlings (12). Therefore, isolates of *T. koningii* may be used in research involving biocontrol of root pathogens of maize seedlings, providing that high population densities of the *Trichoderma* isolate do not result in detrimental plant responses similar to those previously reported (12,17). Differences in *Trichoderma* pathogenicity observed in this study and in previous studies (12,17) may be due to different environmental conditions, maize genotypes, or genetic variability of *Trichoderma* isolates within the same species complex.

Trichoderma spp. in combination with root-knot nematodes have produced synergistic interactions resulting in root necrosis of tobacco and cotton (15,20). *T. harzianum* caused extensive decay of *Meloidogyne*-infected roots of tobacco and increased wilt symptoms in cotton. However, in our study, no additive or synergistic interactions were observed. The root-knot nematode treatments did not predispose corn plants to the *Trichoderma* spp. and did not increase root necrosis.

Nematode reproduction on susceptible maize was lower in soils with *Trichoderma* amendments. Since an increase in root growth was also noted, the suppression of nematode reproduction per gram of root tissue may have been due to a dilution of nematode numbers in the greater root masses. However, the greater root masses of susceptible maize should have resulted in a larger number of potential nematode infection sites, and infection at a larger number of sites would result in greater reproduction.

The decline of reproduction by *M. arenaria* on *Trichoderma*-infested plants is not indicative of a parasitic relationship. In previous studies, where *Trichoderma* was demonstrated to be parasitic on plant root pathogens, *Trichoderma* populations increased and pathogen populations decreased (11). However, it is possible that *Trichoderma* isolates could antagonize *M. arenaria* by producing antinematodal compounds that directly affect nematodes or make the roots less attractive and thus limit nematode penetration.

Further studies will be conducted to determine if T-8 and T-12 actually limit nematode reproduction, or if the apparent lower reproduction is simply due to the dilution of nematode numbers as a result of enhanced plant growth. In any case, the growth-enhancing effect of these fungi on maize should be studied in microplots and field studies. Even if growth enhancement is the only effect on the plant, it would probably make maize plants more tolerant to root-knot nematodes and other nematode pests.

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LITERATURE CITED

1. Baker, R., Elad, Y., and Chet, I. 1984. The controlled experiment in the scientific method with special emphasis on biological control. Phytopathology 74:1019-1021.
2. Baker, R., Paulitz, T., Windham, M., and Elad, Y. 1986. Enhancement of growth of ornamentals by a biological control agent. Page 8 in: Res. Bull. Colo. Greenhouse Grow. Assoc.
3. Chang, Y.-C., Chang, Y.-C., Baker, R., Kleifeld, O., and Chet, I. 1986. Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Dis. 70:145-148.
4. Chet, I., and Baker, R. 1980. Induction of suppressiveness to *Rhizoctonia solani* in soil. Phytopathology 70:994-998.
5. Elad, Y., Chet, I., and Henis, Y. 1981. A selective medium for improving quantitative isolation of *Trichoderma* spp. from soil. Phytoparasitica 9:59-67.
6. Elad, Y., Chet, I., and Katan, J. 1980. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70:119-121.
7. Hadar, Y., Chet, I., and Henis, Y. 1979. Biological control of Rhizoctonia solani damping-off with wheat bran culture of *Trichoderma harzianum*. Phytopathology 69:64-68.
8. Hadar, Y., Harman, G. E., and Taylor, A. G. 1984. Evaluation of *Trichoderma koningii* and *T. harzianum* from New York soils for biological control of seed rot caused by *Pythium* spp. Phytopathology 74:106-110.
9. Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Rep. 57:1025-1028.
10. Lifshitz, R., Windham, M. T., and Baker, R. 1986. Mechanism of biological control of preemergence damping-off of pea by seed treatment with *Trichoderma* spp. Phytopathology 76:720-725.
11. Liu, S., and Baker, R. 1980. Mechanism of biological control in soil suppressive to *Rhizoctonia solani*. Phytopathology 70:404-412.
12. McFadden, A. G., and Sutton, J. C. 1975. Relationships of populations of *Trichoderma* spp. in soil to disease in maize. Can. J. Plant Sci. 55:579-586.
13. Papavizas, G. C., Lewis, J. A., and Abd-El Moity, T. H. 1982. Evaluation of new biotypes of *Trichoderma harzianum* for tolerance to benomyl and enhanced biocontrol capabilities. Phytopathology 72:126-132.
14. Paulitz, T., Windham, M., and Baker, R. 1986. Effect of peat:vermiculite mixes containing *Trichoderma harzianum* on increased growth response of radish. J. Am. Soc. Hortic. Sci. 111:810-816.
15. Powell, N. T., Meléndez, P. L., and Batten, C. K. 1971. Disease complexes in tobacco involving *Meloidogyne incognita* and certain soil-borne fungi. Phytopathology 61:1332-1337.
16. Sivan, A., Ucko, O., and Chet, I. 1987. Biological control of Fusarium crown rot of tomato by *Trichoderma harzianum* under field conditions. Plant Dis. 71:587-592.
17. Sutton, J. C. 1972. *Trichoderma koningii* as a parasite of maize seedlings. Can. J. Plant Sci. 52:1037-1042.
18. Windham, G. L., and Williams, W. P. 1987. Host susceptibility of commercial corn hybrids to *Meloidogyne arenaria* and *M. incognita*. Ann. Appl. Nematol. 1:13-16.
19. Windham, M. T., Elad, Y., and Baker, R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76:518-521.
20. Yang, H., Powell, N. T., and Barker, K. R. 1976. The influence of *Trichoderma harzianum* on the root-knot Fusarium wilt complex in cotton. J. Nematol. 8:81-86.