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Effect of Soils from Six Management Systems on Root-knot Nematodes and Plant Growth in Greenhouse Assays

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Abstract: The effects of soil management systems on root-knot nematode (*Meloidogyne incognita*) eggs and gall incidence on tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus*) following tomato were evaluated. Soil was collected from a replicated field experiment in which six management systems were being assessed for vegetable production. Soil management systems were conventional production, organic production, bahiagrass (*Paspalum notatum*) pasture, bahiagrass: *Stylosanthes* (*Stylosanthes guianensis*) pasture, bare ground fallow, and weed fallow. Soil was collected from field plots and used in greenhouse experiments. Identification of egg-parasitic fungi and the incidence of root-knot nematode galling were assessed both on tomato and cucumber planted in the same pots following the removal of tomato plants. Organic, bare ground fallow and conventional production treatments reduced galling both on tomato and on cucumber following tomato. Although no treatment consistently enhanced egg-parasitic fungi, management system did affect egg viability and the types of fungi isolated from parasitized eggs.

Key words: biological control, cropping systems, cucumber, *Cucumis sativus,* fungal egg parasites, *Lycopersicon esculentum, Meloidogyne incognita,* root-knot nematode, tomato.

In the production of many vegetables in Florida, damage from root-knot nematodes (*Meloidogyne* spp.) can substantially limit yield (Duncan and Noling, 1998). Florida accounts for 27% of the fresh market tomatoes (*Lycopersicon esculentum*) grown in the United States, producing an annual crop valued at more than $500 million from 17,400 ha (FASS, 2004). Growers rely primarily on fumigation with methyl bromide to control soilborne pests. However, methyl bromide has been implicated as a major ozone-depleting substance and its use in the United States is being phased out (Federal Register, 2000; WMO, 1998). Currently there is no single alternative fumigant or chemical pesticide as effective as methyl bromide. Therefore, management of soilborne pests in the post methyl bromide era may require the use of multiple tactics. These may include a combination of fungicides, herbicides, insecticides, other fumigants, and non-chemical alternatives such as rotation cropping systems, resistant crops, organic amendments, and biological control (Chellemi, 2001; Gilreath et al., 1999; Locascio et al., 1997).

To keep their business profitable, many tomato growers in Florida have adopted a production strategy that spreads the cost of soil fumigation and fertilization over two crops. This is done by mowing the first crop at the end of production, leaving the raised polyethylene mulched beds in place, and planting a second crop. This practice enables growers to use residual fertilizer from the first crop and spread the cost of soil fumigation, plastic mulch, and, in some cases, drip irrigation tubing over two crops. In the past, methyl bromide has provided adequate control of nematodes and weeds, enabling production of the second crop. The practice of growing a second crop using infrastructure from the previous fumigated crop is called double-cropping. In Florida, the first crop is often tomato, and the second crop is cucumber (*Cucumis sativus*) or watermelon (*Citrullus lanatus*). Cucurbits are preferred because they are inexpensive to grow and are only distantly related to tomato taxonomically, thereby avoiding some potential pest problems. Both crops, however, serve as hosts for many species of *Meloidogyne,* and large populations sometimes develop in the double-crop scenario.

Alternatives to chemical nematicides include the use of crop rotations, fallow periods, and organic amendments. Crop rotation is widely used and very effective at reducing nematode multiplication and crop damage compared to continuous cultivation of susceptible crops (Chellemi, 2002; McSorley et al., 1994; Widmer et al., 2002). Nematodes with narrow host ranges can be controlled by infrequently growing host crops in rotation with non-host crops such as bahiagrass (*Paspalum* spp.) or pangola grass (*Digitaria eriantha*), or by using strip-tillage in existing bahiagrass pasture (Chellemi, 2002). Control using crop rotation is more difficult for nematodes with wide host ranges, such as *Meloidogyne incognita;* where the choice of non-host crops may be limited and not economically feasible; or where mixed populations of nematodes occur (McSorley and Dickson, 1995).

Leaving land fallow can greatly reduce root-knot nematode populations (Johnson and Campbell, 1977; Johnson et al., 1997; McSorley, 1998; Weaver et al., 1995). Neither bare fallow nor growing non-host crops will eliminate root-knot nematodes from infested soil, although numbers can often be decreased substantially. There are several disadvantages to leaving land fallow including the loss of crop production, increased soil erosion, and increased oxidation of soil organic matter (Whitehead, 1998). Supplementing soil organic matter with amendments can reduce disease caused by nematodes directly by affecting soil properties and indirectly

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by improving plant health, changing root physiology, enhancing populations of antagonistic microorganisms, and inducing disease resistance (Van Loon et al., 1998). Amending soil with animal manures and agricultural by-products has reduced *Meloidogyne* spp. numbers on a variety of crops (Rodríguez-Kábana et al., 1987). Certain amendments increase phenolic compounds in soil, which can be nematotoxic and affect soil microbial populations by increasing saprophytic fungi and decreasing plant pathogenic fungi (Kokalis-Burelle and Rodríguez-Kábana, 1994a, 1994b; Kokalis-Burelle et al., 1994). Organic amendments also increase plant vigor, enabling plants to withstand nematode attack (Singh et al., 1986).

A variety of fungi can be isolated from nematode eggs, including commonly found opportunistic genera such as *Fusarium, Penicillium,* and *Trichoderma,* as well as others that are rarely isolated from soil (Domsch et al., 1980; Rodríguez-Kábana and Morgan-Jones, 1988). Soil management strategies can affect the types of fungi isolated from eggs (Culbreath et al., 1984). Morgan-Jones et al. (1983, 1984) found that the *Meloidogyne arenaria* juveniles within healthy nonparasitized eggs were not affected by 24-hour exposure to 2% gluteraldehyde and 3-hour exposure to 1% osmium tetroxide, which are standard conditions for fixation for electron microscopy. Larvae in parasitized eggs were successfully fixed, indicating that unaffected nematode egg cuticles were impermeable and demonstrating the potential of fungal egg parasites for reducing nematode populations directly, and for contributing to the susceptibility of nematode eggs to environmental factors.

The objectives of this research were to determine the effects of soils collected from replicated field plots testing different soil management strategies on root-knot nematode egg viability and galling on tomato and cucumber planted in succession in a greenhouse trial. The susceptibility of eggs to fungal parasites was evaluated, and egg parasitizing fungi were identified to genus.

Methods and Materials

Field experimental design: Soil samples were collected from a field experiment evaluating six soil management systems used in commercial tomato production. The experiment was located at the USDA, ARS Header Canal Farm, Fort Pierce, Florida. The farm had been in conventional tomato production for 10 year before the start of the experiment. The soil type was Riviera fine sand (loamy, siliceous, hyperthermic, Arenic Glossaqualfs). Soil organic matter was 1.7%; soil pH 7.4; and the soil texture was 96% sand, 2% silt, and 2% clay. Each management system was replicated six times and arranged in a randomized complete block design. Each replicate plot was 0.16 hectare. The soil management systems were (i) conventional tomato production, (ii) organic production, (iii) bahiagrass pasture (*Paspalum notatum* var Agentine), (iv) pasture comprised of a mixture of bahiagrass and *Stylosanthes guianensis* (var savanna stylo), (v) bare ground fallow, and (vi) weed fallow. The conventional tomato production treatment consisted of a broadcast application of 1,3-dichloropropene plus chloropicrin (Telone C-35) at 205 liters/ha and the herbicides napropamide (Devrinol) and trifluralin (Treflan) applied at 2.2 and 0.6 kg/ha, respectively. Pesticides were applied in August 2000. In September, tomato (*Lycopersicon esculentum* 'Florida 91') was transplanted in raised, fertilized beds covered by polyethylene plastic mulch. The crop was managed according to commercial production guidelines (Maynard and Hochmuth, 1999). The organic production treatment was started in July 2000 and included 22.4 tonnes/ha of chicken litter and 67.4 tonnes/ha of partially composted urban plant debris. Sunn hemp (*Crotalaria juncea* 'Tropic Sunn') was planted as a cover crop in August, and Japanese millet (*Echinochloa crusgalli* 'Frumentaea') was planted as a cover crop in March. The bahiagrass and bahiagrass/*Stylosanthes* pasture treatments were established by seeding in August 2000. In the bare-ground fallow treatment, soil was kept free of plant material by disking at 30-day intervals. Disking was started in July 2000. In the weed fallow treatment, soil was undisturbed, allowing a natural succession of weed communities to become established.

Soil sampling and greenhouse experimental design: Soil samples for greenhouse experiments were collected in June 2001. Each plot was sampled using a zigzag pattern typical for collecting soil within fields. A standard 2.5-cm-diam. soil probe was inserted to an average depth of 12 to 20 cm, with 15 to 20 cores/plot, and combined for 1 bulk sample/plot and a total of 36 samples. Soil samples from each plot were placed in 7.8-liter pots on greenhouse benches in a randomized complete block design with six replications. Adequate space was maintained between pots to eliminate cross contamination during watering. At the same time, two 3- to 4-week-old root-knot nematode susceptible 'Improved Bonny Best' tomato seedlings were planted per pot. Plants were watered daily and fertilized (20-10-20, NPK, plus micro nutrients) once per week through the irrigation system. Imidacloprid at 0.53 ml/liter (Bayer CropScience, Research Triangle Park, NC) was mixed with bifenthrin at 0.4 ml/liter (FMC, Philadelphia, PA) and applied once during the experiment to control whiteflies (*Bemisia tabaci*). M-Pede insecticidal soap at 10.6 ml/liter (Dow AgroSciences, Indianapolis, IN) was also sprayed twice to control shore flies (*Scatella* spp.). A second identical experiment was begun one week after the first experiment using freshly collected soil from the same replicated field plots.

Preparation of alginate films: Alginate films containing root-knot nematode eggs were used to bait nematode egg parasitic fungi from the soil (Rodríguez-Kábana et

al., 1994a). Nematode eggs were extracted from tomato culture plants in the greenhouse using a modification of Hussey and Barker's NaClO method (1973). Galled roots were cut into 1- to 3-cm pieces, and approximately 20 g of roots were placed in a 1-liter bottle containing 400 ml of water and 200 ml of 1.8% NaOCl solution. The bottle was stoppered and shaken for 3 minutes, and its contents were passed successively through 250-, 75-, and 20-µm-pore sieves. Eggs were retained on the 20-µm-pore sieve and rinsed thoroughly with water. Two alginate films were placed in each pot in the greenhouse, 1 week after planting for the first sampling and 6 weeks after planting for the second sampling. Films remained in the pots for 48 hours. Control films were placed in sterile water for 48 hours.

Evaluation of nematode eggs and juveniles: After being removed from the pots and rinsed free of soil, each film was placed in a sterile petri dish containing sterile water and stored in an incubator at 24 °C in the dark. Films were maintained in the incubator for 5 days and then evaluated for egg mortality and parasitism. Root-knot nematode eggs were counted (50 eggs/film) using an inverted microscope and determined to be nonviable, viable, or parasitized by fungi. Eggs were considered viable if they appeared to be experiencing normal cell division and juvenile development, or if developing or active juveniles were observed. Eggs were considered as non-viable if cellular contents appeared abnormally vacuolated or disrupted and were considered parasitized if fungal hyphae were observed growing on or in the egg.

Fungal isolation: Fungi from parasitized eggs were isolated using potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) containing antibiotics (chloramphecinol at 25 mg/liter + streptomycin sulfate at 25 mg/liter; Sigma Chemical Co., St Louis, MO). Ten parasitized eggs were extracted from each film using a microscope and placed (1/dish) on PDA. Petri dishes were stored in an incubator at 25 °C in the dark for 5 to 15 days, depending on fungal growth rate. When necessary to remove contamination, clean hyphal tips of the fungus were re-isolated on new PDA. Once the cultures were determined to be pure, they were stored in a 30% glycerol solution at −80 °C.

Fungal identification: Each fungal isolate was identified to genus using taxonomic keys based upon morphological criteria (Barnett and Hunter, 1998; Watanabe, 2002) and using Biolog Filamentous Fungi micro plates (BioLog, Inc., Hayward, CA). Fungi were grown, incubated, and inoculated into microplates according to BioLog protocol.

Plant growth and disease evaluations: Tomato plants were removed from soil 42 days after transplanting. Plant measurements were recorded, and roots were washed free of soil, weighed, and rated for galling and root condition. Root condition, considered as a general indicator of root disease, was assessed using a subjective scale of 1 to 5 with 1 = 0% to 20% discolored roots, 2 = 21% to 40%, 3 = 41% to 60%, 4 = 61% to 80%, and 5 = 81% to 100%. Root galling was assessed using a root-gall index based on a scale of 1 to 10, with 1 = no galls and 10 = severe (100%) galling (Zeck, 1971).

As described above, in Florida cucumber is commonly grown following a primary crop such as tomato. To evaluate the effects of management strategies on root-knot nematode infestation in the second cucumber crop, 4-week-old 'Spacemaster' cucumber transplants were placed in pots following the removal of tomatoes. Cucumbers were grown for 36 days, removed from soil, and rated for root condition and galling as described for tomato. Cucumbers were watered daily, fertilized weekly, and scouted for insects as described for tomatoes.

Statistical analysis: Tomato and cucumber growth and disease data, as well as nematode egg viability and fungal colonization data, were statistically analyzed according to standard procedures for analysis of variance (general linear model) and mean separation (least significant difference) (SAS Institute, Cary, NC). All differences referred to in the text were significant at the 5% level of probability.

Results

Nematode development: When alginate films containing *M. incognita* eggs were placed into soil 7 days after transplanting tomato seedlings, egg mortality ranged from 20.7% to 32.3% in test 1 and 23.7% to 34.3% in test 2 (Table 1). Soil management strategy affected mortality in test 1 ($P = 0.004$). Egg mortality was highest in the bare-ground fallow treatment and lowest in the organic treatment in test 1. In test 2, mortality was lowest in the bare-ground fallow treatment and highest in the bahiagrass: *Stylosanthes* and weed fallow treatments. The level of fungal parasitism was low and unaffected by treatment in both tests (Table 1).

Alginate films containing eggs placed into soil 42 days after transplanting had higher overall mortality and fungal parasitism rates than films placed in soil 7

Table 1. Mortality and fungal parasitism of *Meloidogyne incognita* eggs placed into soil 7 days after transplanting tomato seedlings.

	Mortality (No. of eggs) ($n = 50$)		Fungal Parasitism (No. of eggs) ($n = 50$)	
Treatments	Test 1	Test 2	Test 1	Test 2
Bare fallow	16.2 a[a]	11.8 b	1.0 a	1.5 a
Weed fallow	14.0 ab	17.0 a	0.7 a	2.3 a
Bahiagrass	14.2 ab	13.0 ab	0.8 a	0.8 a
Bahia/*Stylosanthes*	12.3 ab	17.2 a	0.7 a	2.0 a
Organic	10.3 b	15.2 ab	0.5 a	2.0 a
Conventional	12.2 ab	12.3 ab	0.0 a	1.0 a
LSD (0.05)	4.5	4.9	1.2	1.9

[a] Means with the same letter are not significantly different.

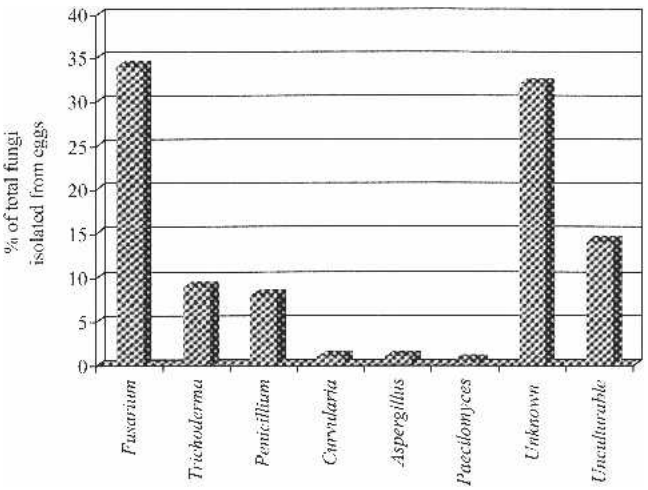

FIG. 1. Predominant fungal genera isolated from *Meloidogyne incognita* eggs.

days after transplanting (data not shown). However, no differences in mortality or fungal parasitism among treatments were observed.

Association of fungal genera among six different production systems: Overall, more culturable fungi were isolated from nematode eggs incubated in soil collected from the bahiagrass: *Stylosanthes* and organic treatments than from eggs incubated in soil collected from the bare-ground fallow treatment (data not shown). The most common genera of fungi isolated from root-knot nematode eggs were *Fusarium* followed by *Trichoderma, Penicillium, Curvularia, Aspergillus,* and *Paecilomyces* (Fig. 1). Only *Penicillium* and *Aspergillus* differed in the frequency of their isolation among treatments, with *Penicillium* isolated more frequently in the weed fallow and bahiagrass: *Stylosanthes* treatments, and *Aspergillus* isolated more frequently in the organic treatment (data not shown). A large percentage of the egg-colonizing fungi were not associated with the genera listed and remain to be identified, or were not able to be grown in culture (Fig. 1).

Nematode infestation and tomato growth: For test 1, root condition was improved with the bare-ground treatment compared to bahiagrass: *Stylosanthes* (Table 2). Gall ratings were lower for the organic and conventional treatments compared with the weed fallow and both treatments containing bahiagrass (Table 2). There were no differences in top weight and root weight among the different treatments in test 1 (Table 2).

In test 2, root condition was unaffected by treatment. Root galling was less in the organic, conventional, and bare-ground fallow treatments than in the bahiagrass: *Stylosanthes,* bahiagrass, and weed fallow treatments (Table 2). The organic and bahiagrass: *Stylosanthes* treatments had 22.5% more plant top weight than the bare-ground fallow and conventional treatments (Table 2). Root weights for the bare-ground fallow treatment were 25.5% lower than those for the weed fallow treatment (Table 2). There were no differences among production systems for tomato plant length and stem diameter in either test (data not shown).

Nematode infection of cucumber following tomato: In test 1, there were no differences in root condition ratings on cucumber with bare-ground fallow, bahiagrass, organic and conventional treatments (Table 3). However, the bahiagrass: *Stylosanthes* treatment had healthier root condition ratings than the weed fallow treatment (Table 3). Galling on cucumber following tomato in test 1 was less in the organic and bare-ground fallow treatments than the bahiagrass treatment (Table 3). In test 2, there were no differences in root-condition ratings among treatments (Table 3). However, the organic, bare-ground fallow, and conventional treatments had reduced galling caused by root-knot nematodes in cucumber planted after tomato compared to the bahiagrass and weed fallow treatments (Table 3).

DISCUSSION

In this study, soil from field plots under different soil management strategies was evaluated in the greenhouse to compare the effects of management strategy on *Meloidogyne* eggs and galling of tomato and cucumber planted after tomato. When the soil was planted with tomato and then with cucumber, galling on cucumber caused by root-knot nematodes was less in bare-

TABLE 2. Plant growth and disease data 42 days after planting.

Treatments	Root condition[a]		Gall rating[b]		Top weight (g)		Root weight (g)	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Bare fallow	1.7 c[c]	1.4 a	1.2 bc	0.4 c	227.4 a	200.7 b	33.9 a	29.0 b
Weed fallow	2.8 ab	1.6 a	3.1 a	3.5 a	208.5 a	225.1 ab	34.4 a	36.5 a
Bahia	2.7 ab	1.6 a	3.6 a	3.0 ab	230.6 a	238.2 ab	36.5 a	33.4 ab
Bahia/*Stylosanthes*	3.0 a	1.7 a	2.4 ab	1.8 b	200.4 a	247.4 a	27.7 a	33.1 ab
Organic	2.1 bc	1.6 a	0.92 c	0.0 c	261.3 a	244.1 a	30.2 a	35.2 ab
Conventional	2.3 abc	1.4 a	0.6 c	0.2 c	232.1 a	199.8 b	33.6 a	31.3 ab
LSD (0.05)	0.7	0.5	1.3	1.3	79.4	39.9	11.6	6.6

[a] 0 = excellent condition (healthy root system); 5 = poor condition (rotten roots).
[b] Rating values were 0 to 10, with 0 = no galls and 10 = severe (100%) galling.
[c] Means with the same letter are not significantly different.

TABLE 3. Cucumber root disease 36 days after planting following tomato.

Treatments	Root condition[a]		Gall rating[b]	
	Test 1	Test 2	Test 1	Test 2
Bare fallow	1.6 ab[c]	1.4 a	1.7 b	1.8 c
Weed fallow	1.7 a	1.7 a	2.5 ab	7.0 a
Bahia	1.7 ab	1.4 a	2.9 a	4.5 b
Bahia/*Stylosanthes*	1.4 b	1.4 a	1.8 ab	3.7 bc
Organic	1.5 ab	1.4 a	1.7 b	2.0 c
Conventional	1.7 ab	1.6 a	2.2 ab	1.8 c
LSD (0.05)	0.3	0.3	1.2	2.2

[a] 0 = roots in excellent condition whereas 5 = poor root condition.
[b] Rating values were 0 to 10, with 0 = no galls and 10 = severe (100%) galling.
[c] Means with the same letter are not significantly different.

ground fallow and organic treatments compared to weed fallow and bahiagrass treatments. This effect, however, was not due to an increase in egg-parasitizing fungi. The bare-ground fallow treatment effect may have been due to the reduction of weeds that serve as hosts for root-knot nematodes. The reduction in root galling in the organic treatment probably resulted from the production of ammoniacal nitrogen following the addition of poultry waste (Rodríguez-Kábana, 1986; Rodríguez-Kábana, et al., 1987).

The increase in root galling in the bahiagrass treatments was unexpected as bahiagrass has been shown to be an effective rotation crop for controlling root-knot nematode populations (Dickson and Hewlett, 1989; Rodríguez-Kábana et al., 1994b). The effectiveness of bahiagrass rotations for reducing root-knot nematode populations, however, is enhanced when used for two or more consecutive seasons (Rodríguez-Kábana et al., 1994b).

Many physical, chemical, and microbial factors affect the establishment of nematode populations in soil. Further investigation is under way to confirm egg parasitism of the fungal isolates and determine the specific mechanisms involved in the parasitism of root-knot nematode eggs by these fungi. Whether differences occur among fungal genera with respect to the mechanisms involved in egg parasitism is of particular interest. A greater understanding of the interactions among soil microorganisms and nematodes will lead to the implementation of more effective strategies for nematode control.

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