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Effect of *Crotalaria juncea* Amendment on Squash Infected with *Meloidogyne incognita*[1]

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Abstract: Two greenhouse experiments were conducted to examine the effect of *Crotalaria juncea* amendment on *Meloidogyne incognita* population levels and growth of yellow squash (*Cucurbita pepo*). In the first experiment, four soils with a long history of receiving yard waste compost (YWC+), no-yard-waste compost (YWC−), conventional tillage, or no-tillage treatments were used; in the second experiment, only one recently cultivated soil was used. Half of the amount of each soil received air-dried residues of *C. juncea* as amendment before planting squash, whereas the other half did not. *Crotalaria juncea* amendment increased squash shoot and root weights in all soils tested, except in YWC+ soil where the organic matter content was high without the amendment. The amendment suppressed the numbers of *M. incognita* if the inoculum level was low, and when the soil contained relatively abundant nematode-antagonistic fungi. Microwaved soil resulted in greater numbers of *M. incognita* and free-living nematodes than frozen or untreated soil, indicating nematode-antagonistic microorganisms played a role in nematode suppression. The effects of *C. juncea* amendment on nutrient cycling were complex. Amendment with *C. juncea* increased the abundance of free-living nematodes and *Harposporium anguillulae,* a fungus antagonistic to them in the second experiment but not in the first experiment. Soil histories, especially long-term yard waste compost treatments that increased soil organic matter, can affect the performance of *C. juncea* amendment.

Key words: free-living nematode, nematode-trapping fungi, organic amendments, root-knot nematode, soil ecosystem, soil nutrient, sunn hemp, tillage.

As a cover crop, sunn hemp (*Crotalaria juncea*) has many beneficial qualities. It grows rapidly and is frequently used as a green manure to enhance soil organic matter and nitrogen content (Marshall, 2002; Rotar and Joy, 1983). In addition, it is particularly versatile for nematode management. *Crotalaria juncea* is a poor host to many important plant-parasitic nematodes, including *Meloidogyne incognita* (McSorley, 1999; Santos and Ruano, 1987), *M. javanica* (Araya and Caswell-Chen, 1994; McSorley, 1999; Silva et al., 1990), *M. arenaria* (McSorley, 1999), *Rotylenchulus reniformis* (Caswell et al., 1991; Silva et al., 1989; Wang et al., 2002), and *Pratylenchus brachyurus* (Charchar and Huang, 1981). Root and leaf exudates from *C. juncea* contain allelopathic compounds such as monocrotaline and pyrrolizidine alkaloids that are toxic to nematodes (Rich and Rahi, 1995). When incorporated into the soil, sunn hemp residue is able to enhance the activity of some nematode-antagonistic microorganisms (Quiroga-Madrigal et al., 1999; Rodríguez-Kábana and Kloepper, 1998; Wang et al., 2001). On the other hand, *C. juncea* amendment enhances population densities of free-living nematodes (Venette et al., 1997; Wang et al., 2002; Wang et al., 2003) that are important for soil nutrient cycling (Ingham et al., 1985).

We were particularly interested in using *C. juncea* hay as an organic fertilizer, where the hay was harvested and stored until used, rather than as a green manure cover crop. Not only does stored hay offer great flexibility in timing of application, but one can also control the quality of the hay (age of the plant at harvest) and quantity of the hay (based on nutrient element contents) to achieve specific fertilization needs for a cash crop (Marshall et al., 2002). Using cover crop hay as an organic fertilizer is especially appropriate for tropical cover crops, which have great potential for nematode management (McSorley and Gallaher, 1992; McSorley et al., 1994; Wang et al., 2002). Farmers usually have limited time to fit in a summer cover crop schedule if summer is their peak season for cash crop production. Marshall (2002) reported that incorporating *C. juncea* hay into squash (*Cucurbita pepo*) field at planting achieved squash yield equivalent to plots fertilized with ammonium nitrate. Amendment with *C. juncea* can also impact plant-parasitic nematodes. *Rotylenchulus reniformis* suppression by *C. juncea* was influenced by duration of fallowing, time after planting, nematicide application, initial numbers of plant-parasitic nematodes, and nematode-antagonistic fungi (Wang et al., 2003). The current research evaluated the effect of *C. juncea* against *M. incognita* under various edaphic histories achieved through long-term agronomic practices. The specific objectives of this research were to determine the effect of *C. juncea* amendment on the growth of squash infected by *M. incognita* and on the reproduction of *M. incognita* in soils with different management histories. We manipulated soil microorganisms to further understand the effect of *C. juncea* amendment on *M. incognita.*

MATERIALS AND METHODS

Experiment I (soil history experiment): Soil history. Four loamy sands with different histories of long-term agricultural practices were collected in March 2001, from two field sites previously used for long-term yard-waste

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compost (5 years) and tillage (25 years) studies at the former University of Florida Green Acres Agronomy Field Research Laboratory in Alachua County, Florida. All soils were Arredondo loamy sand (loamy, siliceous, hyperthermic, Grossarenic Paleudult, with 94% sand, 2% silt, and 4% clay). These soils are characterized as having previous histories of yard-waste compost (YWC+), no yard-waste compost (YWC–), no-till (NT), and conventional till (CT). The YWC+ soil was collected from field plots amended with 269 Mg/ha/yr of composted yard-waste plant materials including sticks, clippings, and wood fragments each year from 1993 to 1998. The site was planted with two cycles of corn (*Zea mays*) and cowpea (*Vigna unguiculata*) as intercycle cover crops during 1998 and 1999, fallowed with weeds, and remained undisturbed until soil collection in March 2001. Organic matter content of this site was 8.44%. The YWC-soil was collected from the same experimental sites as the YWC+ (McSorley and Gallaher, 1996) but from field plots never amended with yard-waste compost. Organic matter content in this area was 2.42%. The NT soil was collected from a cotton (*Gossypium hirsutum*) field that was continuously under no-till practices for the previous 25 years as part of a double-cropping rotation involving cotton or soybean (*Glycine max*) in the summer and oat (*Avena sativa*) in the winter (McSorley and Gallaher, 1993). Organic matter in this area was 2.87%. The CT soil was from the same site as NT soil, but from areas rototilled twice before planting summer crops for the previous 25 years. This soil contained 2.10% organic matter. Collected soil (25 to 30 kg from each site) was sieved through a 2-mm-pore sieve and homogenized for use in the greenhouse experiment.

Soil manipulation treatment. Each field soil was divided into three portions to receive different thermal treatments to manipulate the soil microorganisms. Treatments included microwave heating (Chen et al., 1995) (500 g soil at a time in 2 microwaveable plastic containers with 2-cm depth of soil, at 700 W for 3 minutes), frozen (–17 °C for 1 week), and untreated. Microwaving reduces indigenous nematodes and other soil microorganisms (including nematode antagonistic fungi) (Chen et al., 1995). Freezing reduces indigenous nematodes and other soil fauna that could be feeding on nematode antagonistic fungi but preserves the fungi (Ko and Schmitt, 1996). After designated thermal treatments, all soils were stored at 22 °C for less than a week prior to potting up in the greenhouse.

Soil amendment. Crotalaria juncea hay was harvested from a crop grown in another site during fall 2000. The hay was air-dried, ground into fine dust, and stored over the winter (>3 months). Each soil history and treatment combination (4 soil history × 3 soil treatments) was either amended or not amended with *C. juncea* hay at 1 g dry hay/100 g dry soil. The soil (454 g dry weight equivalent) was placed into a 12.7-cm-diam. plastic pot. Thus, the experiment was a 4 × 3 × 2 factorial (soil history × soil treatment × *C. juncea* amendment) design. Pots were arranged in randomized complete block with six replications in a greenhouse. Temperature in the greenhouse ranged from 15 °C to 32 °C during March to May 2001.

Ten days after adding *C. juncea,* a 3-day-old squash (*C. pepo* 'Yellow-Crookneck') seedling germinated on a wet paper towel was planted in each pot. Nematodes were obtained from a greenhouse culture on pepper (*Capsicum annuum* 'California Wonder'). The eggs were extracted from root systems in 0.35% NaOCl (Hussey and Barker, 1973) and incubated on Baermann trays for 7 days to obtain second-stage juveniles (J2) (Rodríguez-Kábana and Pope, 1981). One week after planting, 200 *M. incognita* J2 suspended in water were delivered to each pot by injecting 3 ml of the nematode suspension into three 1-cm-deep holes surrounding the squash seedling.

Plants were watered daily and fertilized weekly with 15-30-15 (N: P_2O_5: K_2O) of Miracle-Gro (Scotts Miracle-Gro Product Inc., Marysville, OH) using an equal amount of water or fertilizer for each plant. Squash flowers were removed as soon as they appeared to prevent uneven fruiting and development among the plants. Safer brand insecticidal soap (Safer Inc., Bloomington, MN) was sprayed on the foliage according to label instructions to manage silverleaf whiteflies (*Bemisia* spp.). Nevertheless, all plants showed silver leaf symptoms by the end of the experiment.

Nematode assay: The experiment was terminated 8 weeks after *M. incognita* inoculation. Shoot and root fresh weights and root gall indices were recorded. Root gall index for *M. incognita* was rated on a modified scale of 0 to 7 where 0 = no gall, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls, 5 = 25% to 50% root system galled, 6 = 50% to 75% root system galled, and 7 = > 75% root system galled (Netcher and Sikora, 1990; Taylor and Sasser, 1978). Soil from each pot was placed in a plastic bag and mixed well; 100 cm^3 was used to extract nematodes by sieving and centrifugal flotation (Jenkins, 1964). Numbers of each genus of plant-parasitic nematode and total number of free-living nematodes (not plant-parasitic) were counted using an inverted microscope.

Assay for nematode-antagonistic fungi. A subsample of each of the four soils was assayed for nematode-antagonistic fungi before *C. juncea* amendment. At the end of the experiment, nematode-antagonistic fungi were quantified only from the frozen soil treatment in three of the replications for the YWC+ and YWC– soil amended or not with *C. juncea.* Microwaved soil was not examined for nematode antagonistic fungi because few fungi survive in the microwaved soil (Chen et al., 1995). Ten grams of soil from each sample was suspended in 20 ml sterile distilled water. A 10-fold-dilution series with three dilution levels (0.500, 0.050, and 0.005 g of soil/ml) was prepared. A 100-µl aliquot of each dilution was plated on ¼-strength cornmeal agar (CMA/4) (Jaffee and Muldoon, 1995) containing 100 ppm streptomycin. One hundred *Heterorhabditis* spp. were added to each plate as bait for nematode-trapping fungi. The dishes were stored at 22 °C for 3 weeks. The entire surface of agar in each dish was then examined with an inverted microscope. The presence and absence of species of nematode-trapping fungi were recorded, and the fungal propagules numbers were estimated by the Most Probable Number program (Woomer et al., 1990). Nematode-trapping fungi were identified to species level according to a key to the nematode-destroying fungi (Cooke and Godfrey, 1964).

Statistical analysis. Most data were subjected to a 4 × 3 × 2 (soil history × soil treatment × amendment) factorial analysis of variance (ANOVA) in a randomized complete block design using SAS (SAS Institute, Cary, NC). Nematode-trapping fungal numbers were subjected to a 2 × 2 (soil history × amendment) factorial ANOVA. The Waller-Duncan k-ratio ($k = 100$) t-test was used to separate treatment means where appropriate.

Experiment II (vegetable soil experiment): The experiment was repeated in fall 2001 with the same procedures as in the soil history experiment, but only one soil from a different site was used and was inoculated with a higher nematode inoculum level. A different soil was used because the site from which soils for the soil history experiment were collected was closed by the University of Florida in summer 2001. Therefore, soil of similar texture was collected for Experiment II from the University of Florida, Experimental Designs Field Teaching Laboratory, Gainesville, Florida. The soil was Millhopper sand (loamy, siliceous, hyperthermic, Grossarenic Paleudult, 92% sand, 3% silt, and 5% clay, 1.95% organic matter). This field was rotated with various vegetable crops. Rye (*Secale cereale* L.) was intercropped with lupine (*Lupinus angustifolius* L.) in the season before soil collection. This soil received the manipulation and amendment treatments as described in the soil history experiment. Therefore, the experimental design was a randomized complete block, arranged in a 3 × 2 (soil treatment × amendment) factorial design with six replications. Three-day-old germinated squash seedlings were transplanted on 24 September 2001, and soil was infested with *M. incognita* 1 week after germination. Due to the low recovery of *M. incognita* at the termination of the soil history experiment, plants in the vegetable soil experiment were inoculated with 800 J2/pot. Plant maintenance and data collection were as described in the soil history experiment, and the experiment was terminated 8 weeks after nematode inoculation. Data analysis was similar to the soil history experiment, except that most of the data were subjected to a 3 × 2 (soil treatment × amendment) factorial ANOVA, whereas the nematode-trapping fungal propagules numbers and soil nutrient analysis data were subjected to a one-way ANOVA.

RESULTS

Cj effects on plant growth: Crotalaria juncea amendment increased squash root weight in all the soils tested in both experiments ($P \leq 0.01$) (Tables 1,2). However, the effect of *C. juncea* amendment (Cj) on squash shoot weight varied according to soil history, as indicated by an interaction ($P \leq 0.01$) between Cj and soil history effects. *Crotalaria juncea* amendment improved squash shoot weight in all the soils tested (Tables 1,2,3) except in YWC+ soil (Table 3). Manipulation of soil microorganisms by microwaving or frozen treatments did not affect the impact of *C. juncea* on shoot weights of squash, as indicated by the absence of any interaction between Cj and soil treatment effects.

Cj effects on nematodes: Numbers of *M. incognita* and root-gall rating were suppressed by *C. juncea* amend-

TABLE 1. Shoot and root weight of squash, root-gall index, and numbers of nematodes in soils with history of yard-waste compost (YWC+), no yard-waste compost (YWC−), no-tillage (NT), and conventional tillage (CT) receiving microwave (M), frozen (F), or untreated control (C) treatments, and amended or not with *Crotalaria juncea* (Cj+ or Cj−) in the soil history experiment.

				Nematodes per 100 cm^3 soil			
	Root weight (g)	Shoot weight (g)	Root-gall index	Root-knot	Lesion	Ring	Free-living
Cj							
+	3.7 a	24.2 a	4.1 b	7 b	7 a	17 a	3,589 a
−	2.8 b	19.3 b	4.7 a	15 a	7 a	17 a	3,109 a
Soil history							
CT	3.0 ab	19.4 c	4.6 b	15 a	15 a	17 ab	4,273 a
NT	3.8 a	19.3 c	5.5 a	14 a	6 b	7 b	3,248 ab
YWC−	2.6 b	21.6 b	3.5 c	9 ab	1 c	27 a	2,814 b
YWC+	3.6 a	26.8 a	4.0 c	5 b	7 b	18 ab	3,061 ab
Treatment							
M	3.7 a	24.2 a	4.9 a	14 a	1 c	4 b	5,594 a
F	3.2 a	21.7 b	4.0 b	6 b	5 b	3 b	2,026 b
C	2.9 a	19.4 c	4.4 b	12 ab	15 a	45 a	2,428 b

Means are averages of 72, 36, and 48 replications for the main factors: Cj, soil history, and treatment, respectively. For each main factor, means in a column followed by the same letters are not different according to Waller-Duncan k-ratio t-test ($P \leq 0.05$) or analysis of variance where appropriate.

TABLE 2. Shoot and root weight of squash, root-gall index, and population densities of nematodes in soil receiving microwave (M), frozen (F), or untreated control (C) treatments, amended or not amended with *Crotalaria juncea* (Cj+ or Cj−) in the vegetable soil experiment.

	Root weight (g)	Shoot weight (g)	Root-gall index	Nematodes per 100 cm^3 soil	
				Root-knot	Free-living
Ci					
+	4.2 a[z]	17.7 a	5.8 a	348 a	2,708 a
–	2.4 b	9.0 b	6.1 a	184 b	873 b
Treatment					
M	4.6 a	11.0 b	7.1 a	505 a	4,221 a
C	3.1 b	14.9 a	5.4 b	100 c	424 c
F	2.4 c	14.2 a	5.3 b	194 b	726 b

[z] Means are averages of 18 and 12 replications for the main factors: Cj and treatment, respectively. For each main factor, means in a column followed by the same letters are not different according to analysis of variance or Waller-Duncan *k*-ratio *t*-test ($P \leq 0.05$) where appropriate.

ment in the soil history experiment ($P \leq 0.01$) (Table 1). However, when a higher level of *M. incognita* was used in the vegetable soil experiment, Cj amendment resulted in higher numbers of *M. incognita* than in unamended soil ($P \leq 0.001$) (Table 2), but root-gall index was not affected by Cj ($P > 0.05$) (Table 2). Other plant-parasitic nematodes present at moderate levels were lesion (*Pratylenchus* spp.) and ring (*Mesocriconema* spp.) nematodes. *Crotalaria juncea* amendment did not affect the numbers of these nematodes (Tables 1,2).

Numbers of free-living nematodes were generally higher in the soil history experiment than in the vegetable soil experiment (Table 1,2). The number of free-living nematodes was not affected by Cj in the soil history experiment (Table 1) but was increased by Cj in the vegetable soil experiment ($P \leq 0.01$) (Table 2). Soil history and manipulation treatment did not interact ($P > 0.05$) to alter the effects of Cj on numbers of free-living nematodes in either experiment.

Soil history effect: Generally, YWC+ and YWC- soils supported heavier squash shoot weights, lower root-knot nematode populations, and a lower root-gall index than CT and NT soils (Table 1). YWC+ had higher squash shoot and root weight than YWC- ($P \leq 0.05$) (Table 1). No-till soil had a higher root gall index and lower lesion nematode numbers as compared to CT ($P \leq 0.05$), but otherwise there were no differences in the parameters measured between CT and NT soils. The numbers of lesion and free-living nematodes were highest in CT soil among the four soils tested ($P \leq 0.05$).

TABLE 3. Shoot weight of squash in yard-waste compost (YWC+), no-yard-waste compost (YWC−), no-tillage (NT), or conventional tillage (CT) treated soils. Each soil received three treatments: microwave (M), frozen (F), or untreated control (C), and was either amended or not amended with *Crotalaria juncea* (Cj+ or Cj−) in the soil history experiment.

	YWC+	YWC−	NT	CT
Cj				
+	27.4 a[z]	24.6 a	22.2 a	22.6 a
–	26.2 a	18.6 b	16.3 b	16.2 b
Treatment				
M	32.5 a	22.3 ab	19.9 a	21.9 a
F	23.3 b	23.7 a	19.8 a	20.0 ab
C	24.6 b	18.7 b	18.2 a	16.2 b

[z] Means are averages of 18 and 12 replications for the main factors: Cj and treatment, respectively. For each main factor, means in a column followed by the same letters are not different according to analysis of variance or Waller-Duncan *k*-ratio *t*-test ($P \leq 0.05$) where appropriate.

Soil manipulation treatment effect: Manipulation of soil organisms by different thermal treatments affected most parameters measured in both experiments, except on root weight in the soil history experiment (Tables 1,2). The soil manipulation treatment effect on squash shoot weight differed among soils ($P \leq 0.001$) (Tables 1,2). Microwave treatment resulted in a higher shoot weight than the other soil treatments in the soil history experiment (Table 1) except in NT soil (Table 3), but it resulted in the lowest shoot weight in the vegetable soil experiment (Table 2). Microwave treatment produced higher root-gall index and free-living nematodes than the frozen and control treatments in both experiments ($P \leq 0.001$) (Tables 1,2) and resulted in larger numbers of root-knot nematodes in the vegetable soil experiment ($P \leq 0.05$) (Table 2). In contrast, microwave and frozen treatments had lower numbers of lesion and ring nematodes than the control in the soil history experiment (Table 1).

Cj effects on nematode-antagonistic fungi: No nematode-antagonistic fungus was detected prior to Cj amendment in both experiments. At the end of the experiment, the nematode-antagonistic fungi present in the soil history experiment were mainly nematode-trapping fungi including *Arthrobotrys brochopaga* (forms constricting rings), *Monacrosporium parvicollis* (forms adhesive knobs), *A. oligospora* and *A. superba* (both form adhesive 3-dimensional nets), and a few unidentified nematode-endoparasitic fungi. The nematode-antagonistic fungi present in the vegetable soil experiment were mainly the endoparasitic fungus, *Harposporium anguillulae;* low numbers of *A. oligospora,* a nematode-trapping fungus; and two unidentified fungi, one with adhesive knobs and one with adhesive branches, without conidial spores. The nematode-trapping fungi (NTF) were placed in two groups based on their predaceous activity: saprophytic and parasitic (Cooke, 1963). The saprophytic NTF form 3-dimensional nets in response to the presence of nematodes. Under low nematode numbers, they remain saprophytic. The parasitic NTF spontaneously form constricting rings, non-constricting rings, adhesive branches, or adhesive knobs without the presence of nematodes. An interaction ($P \leq 0.01$) between Cj and soil history was observed for NTF numbers. The amendment increased total propagule numbers of NTF in the YWC+ but not in the YWC- soil in the soil history experiment (Table 4). Numbers of saprophytic NTF were higher ($P \leq 0.10$) in YWC+ than in

TABLE 4. Numbers of nematode-antagonistic fungi (propagules/g soil) in soils with history of yard-waste compost (YWC+) and no-yard-waste compost (YWC−), followed by frozen treatment with or without *Crotalaria juncea* amendments (Cj+ or Cj−) in the soil history experiment.

	YWC+		YWC−		Means		Means	
Fungi	Cj+	Cj−	Cj+	Cj−	Cj+	Cj−	YWC+	YWC−
SNTF[z]	18.42[y]	7.52	2.68	0	10.55[x]	3.76	12.97[x]	1.34 @
PNTF	2.98	1.32	6.50	8.49	4.74	4.91	2.15	7.49
Total NTF	38.07 a	11.82 b	10.51 b	12.49 b	24.29	12.16**	24.95	11.50**
Harposporium anguillulae	15.35	2.98	0	0	7.68	1.49	9.16	0.0
Other endoparasites	1.32	0	1.32	4.00	1.32	2.00	0.66	2.66
Total endoparasites	16.67	2.98	1.32	4.01	9.00	3.49	9.80	2.67

[z] NTF = Nematode-trapping fungi, SNTF = saprophytic NTF, PNTF = parasitic NTF.

[y] Means are averages of 3 replications for each Cj amendment in each soil history. Only means of Total NTF showed significant differences among the Cj × Soil history treatments. In this case, means followed by the same letters are not different among the Cj × Soil history treatments according to Waller-Duncan *k*-ratio *t*-test ($P \le 0.05$).

[x] Means are averages of 6 replications for each Cj amendment and soil history, with @ and ** indicating significant difference between the corresponding value of Cj pair or YWC pair at $P \le 0.1$ and $P \le 0.01$, respectively.

YWC- soil (Table 4). Numbers of NTF in the vegetable soil experiment (range from 1.78 to 5.35 propagules/g soil) were relatively low compared to those in the soil history experiment (Table 4) and were not increased by Cj treatment ($P > 0.05$). However, Cj increased *H. anguillulae* (a nematode endoparasitic fungus) from 6.05 propagules/g soil in the unamended control to 79.24 propagules/g soil in this experiment ($P \le 0.05$).

DISCUSSION

Increased plant growth following *C. juncea* amendment is commonly reported in many crops (Hiremath and Patel, 1998; Marshall, 2002). In the current experiment, *C. juncea* amendment increased shoot and root weight of squash in most of the soils tested except in YWC+ soil that already had an unusually high organic matter content (8.44%). Likely, this was because soil nutrient and organic matter reserves in unamended YWC+ soil were already sufficient for optimum squash growth. This can be observed from the heavier shoot weight of squash in the unamended YWC+ than in the nonamended soil in YWC- ($P \le 0.05$). Effects of Cj on *M. incognita* also behaved differently between these experiments. These results could be attributed to differences in various soil edaphic factors and soil biology listed below.

Initial M. incognita numbers: Numbers of *M. incognita* were lower in *C. juncea*-amended soil in the soil history experiment than in the vegetable soil experiment. A higher inoculation level of *M. incognita* in the vegetable soil experiment might have resulted in too high of an *M. incognita* population for Cj amendment to overcome. A similar result has been reported for suppression of *R. reniformis* by Cj amendment in soil with different *R. reniformis* numbers (Wang et al., 2002). Differences in shoot and root weight between *C. juncea*-amended and nonamended soils were greater in the vegetable soil experiment than the soil history experiment, indicating that Cj increased plant growth more in the vegetable soil experiment. However, the better root growth in *C. juncea*-amended soil in the vegetable soil experiment also might have increased the carrying capacity for *M. incognita,* thus resulting in higher *M. incognita* numbers.

Nematode-antagonistic fungi: Most of the nematode-antagonistic fungi in the soil history experiment, including *Monacrosporium parvicollis, Arthrobotrys brochopaga, A. oligospora,* and *A. superba* can prey on *M. incognita.* Stirling et al. (1998a, 1998b) demonstrated that NTF such as *A. dactyloides* and *A. oligospora* suppressed *M. incognita* under field conditions. However, in the vegetable soil experiment, the most abundant nematode-antagonistic fungus was the endoparasitic fungus, *H. anguillulae,* which infects only bacterivorous nematodes (Barron, 1977). Although Cj could enhance both types of nematode-antagonistic fungi, enhancement of NTF by Cj in the soil history experiment may have resulted in *M. incognita* suppression by the amendment, whereas enhancement of *H. anguillulae* in vegetable soil experiment did not affect the *M. incognita* population. However, in comparing the two soils examined in the soil history experiment, Cj amendment enhanced the total number of NTF in YWC+ soil but not in YWC− soil. This could be due to a higher initial saprophytic NTF present in the YWC+ soil compared to YWC− soil, although initial nematode-antagonistic fungal assay of both soils was unable to detect any NTF propagules. Based on the dilutions used in the current experiment, the lowest number of propagules detectable by the assay method described was 3.63 propagules/g soil. According to Cooke (1963), the parasitic NTF are more effective trappers than the saprophytic NTF, but the higher abundance of saprophytic NTF in the *C. juncea*-amended soil still might have resulted in greater suppression of *M. incognita* than in the nonamended soil. Efficiency of various NTF in trapping *M. incognita* should be evaluated further.

Microwaving of soil may eliminate most nematode-antagonistic fungi and other beneficial microorganisms (Chen et al., 1995). Final numbers of *M. incognita* were greater in microwaved soil in both experiments and, in the vegetable soil experiment, microwaved soil had lower squash shoot weight, more severe root-galling, and heavier root weight. However, in spite of having the highest root-gall index among the soil manipulation treatments, microwaved soil in the soil history experiment had the highest squash shoot weight. It is possible that besides the lower *M. incognita* inoculum in the soil history experiment, the efficiency of nutrient cycling due to the high population of free-living nematodes in this high OM soil might improve plant vigor.

Nutrient cycling: Free-living nematodes play an important role in nutrient cycling (Ingham et al., 1985). Population densities of free-living nematodes were stimulated by *C. juncea* amendment in the vegetable soil experiment but not in the soil history experiment. This is partially consistent with previous reports that Cj enhances bacterivorous nematode numbers (Venette et al., 1997; Wang et al., 2002). Substantial numbers of free-living nematodes were already present in the soil history experiment, even in unamended soil, and probably were a result of the higher organic matter content of the soils in this experiment (5.4% in YWC+ and 1.9% in YWC−). Adding *C. juncea* amendment in the soil history experiment did not further increase the already high population densities of the free-living nematodes. Because soil organic matter content in the vegetable soil experiment was low (1.43%), adding *C. juncea* hay to this soil increased ($P \leq 0.05$) soil organic matter (1.73%) and thus increased population densities of free-living nematodes.

Nematode-antagonistic fungi also regulate the rate of soil nutrient cycling, perhaps in an indirect way. Bouwman et al. (1994) found that *A. oligospora* may contribute directly to C mineralization and indirectly to N mineralization by reducing the numbers of bacterivorous nematodes. Greater numbers of *H. anguillulae* in the vegetable soil experiment may have reduced the numbers of free-living nematodes in the soil, thus slowing the N mineralization by the nematodes. This phenomenon actually might be beneficial as it can prevent overgrazing by nematodes on bacteria, thus prolonging the nutrient cycling period.

In conclusion, the ability of *C. juncea* amendment to increase squash growth, suppress *M. incognita* infection, and improve soil nutrients varied according to soil edaphic and biological factors. These factors included soil history and fertility (especially soil organic matter), initial numbers of *M. incognita,* and abundance and types of nematode-antagonistic fungi. Many of these factors will influence the population densities of free-living nematodes involved in soil nutrient cycling. In this research, field history, such as previous addition of composts to build up soil organic matter, had more influence on the performance of a *C. juncea* amendment than conventional or no-tillage treatments. Whereas this study has demonstrated the complexity of a few soil ecosystem components that are affected by agricultural practices, there are many more soil fauna, microorganisms, and associated processes involved in the soil ecosystem that remain to be examined.

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