INFLUENCE OF COVER CROPS ON POPULATIONS OF SOIL NEMATODES

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ABSTRACT

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A pot experiment was carried out in south Florida to elucidate suppressive or antagonistic effects of several cover crops grown in rotation on soil nematode populations. The crops were two marigolds, *Tagetes patula* L. 'Dwarf Double French Mix' (MDF), and *Tagetes patula* L. 'Lemon Drop' (MLD), Indian mustard (IM) [*Brassica juncea* (L.) Czern.], radish (RD) [*Raphanus sativus* L.], sunn hemp (SH) [*Crotalaria juncea* L., 'Tropic Sun'], velvetbean (VB) [*Mucuna deeringiana* (Bort.) Merr.], a *Meloidogyne*-susceptible cowpea (CP) [*Vigna unguiculata* (L.) Walp, 'Purple Knuckle Hull'], and okra (OK) [*Abelmoschus esculentus* (L.), 'Clemson Spineless 80']. Eight rotation schemes each with 3 rotations were carried out from June, 2002 to December, 2003. Those schemes were MDF-RD-MDF, MLD-CP-MLD, IM-VB-IM, RD-MDF-RD, SH-OK-SH, VB-IM-VB, CP-MLD-CP, and OK-SH-OK. The results showed that marigolds, sunn hemp and velvetbean effectively suppressed root-knot nematodes, but okra, Indian mustard, radish and 'Purple Knuckle Hull' cowpea promoted population growth of the root-knot nematode, *Meloidogyne incognita*. Furthermore, the antagonistic effect of the nematode suppressive crops carried over to reduce the infestation of plant-parasitic nematodes in the following crop. The results indicate that rotating marigold with other ornamental plants or rotating nematode-suppressive cover crops, such as sunn hemp, with field or cash crops may strongly suppress plant-parasitic nematode populations and benefit the following crop.

Key words: Cowpea, Indian mustard, marigold, *Meloidogyne incognita,* okra, radish, root-knot nematodes, sunn hemp, velvetbean.

RESUMEN

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Se llevó a cabo un experimento en macetas en el sur de Florida para elucidar los efectos supresivos o antagonistas de varios cultivos sembrados en rotación sobre las densidades de población de nematodos del suelo. Se consideraron los siguientes cultivos: *Tagetes patula* L. 'Dwarf Double French Mix' (MDF), *Tagetes patula* L. 'Lemon Drop' (MLD), mostaza india (IM) [*Brassica juncea* (L.) Czern.], rábano (RD) [*Raphanus sativus* L.], crotalaria (SH) [*Crotalaria juncea* L., 'Tropic Sun'], *Mucuna deeringiana* (Bort.) Merr. (VB), caupí susceptible a *Meloidogyne* (CP) [*Vigna unguiculata* (L.) Walp, 'Purple Knuckle Hull'], y *Abelmoschus esculentus* (L.) 'Clemson Spineless 80' (OK). Se llevaron a cabo ocho esquemas de rotación, cada uno con tres rotaciones, de junio 2002 a diciembre 2003. Los esquemas fueron MDF-RD-MDF, MLD-CP-MLD, IM-VB-IM, RD-MDF-RD, SH-OK-SH, VB-IM-VB, CP-MLD-CP, y OK-SH-OK, respectivamente. Los resultados indicaron que *T. patula*, *C. juncea* y *M. deeringiana* reducen efectivamente las poblaciones de nematodo del nudo radical, pero que A. *esculentus*, *B. juncea*, *R. sativus* y el caupí 'Purple Knuckle Hull' promovieron el aumento de densidades de población del nematodo del nudo radical *Meloidogyne incognita*. El efecto antagonista de los cultivos supresivos también afectó la infestación de nematodos fitoparásitos el ciclo sigiuiente. Los resultados indican que rotar *T. patula* con otros cultivos ornamentales o rotar cultivos de cobertura supresivos, tales como crotalaria, con otros cultivos puede suprimir efectivamente las poblaciones de nematodos fitoparásitos.

Palabras clave: Abelmoschus esculentus, caupí, crotalaria, mostaza india, *Mucuna deeringiana, Meloidogyne incognita,* nematodo del nudo radical, rábano, *Tagetes patula.*

INTRODUCTION

In tropical or subtropical regions, plant-parasitic nematodes are a major cause of losses in the production of horticultural and field crops. The economic thresholds for different plant-parasitic nematodes species appear to vary greatly, although there is a paucity of research data on economic thresholds for the nematode species in this study. In Florida, extension specialists recommend treatment if any galls were formed by *Meloidogyne incognita* on the previous crop or on 'Clemson Spineless' okra grown as an indicator species, or if juveniles of *Rotylenchulus reniformis* reach 200 per 100 ml of soil at planting (Pernezny *et al.*, 2003). For many years these nematodes have been controlled effectively with nematicides, especially the fumigant, methyl bromide. However, under the Clean Air Act (U.S. Environmental Protection Agency, 1990) and in accordance with the Montreal Protocol (United Nations Environment Program, 1998), use of methyl bromide was phased out in developed countries beginning 1 January 2005 with certain critical use exceptions (CUE) granted temporarily (U.S. Environmental Protection Agency, 2004; Wang *et al.,* 2005). Some chemical and biological alternatives to methyl bromide are being developed, but the use of some chemical alternatives is restricted by local conditions. For example in regions with Karst topography, chemical alternatives, such as 1,3-dichloropropene (1,3-D) are prohibited because they enter the groundwater and contaminate wells (Wang *et al.*, 2005).

Summer cover crops, e.g., sunn hemp, *Crotalaria juncea* L., and velvetbean, *Mucuna deeringiana* (Bort.) Merr., have been shown to suppress the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood (Kloepper *et al.*, 1991; McSorley, 1998, 1999; Vargas-Ayala *et al.*, 2000; Wang *et al.*, 2003, 2005). All cowpea cultivars appear to be susceptible to the reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira (Robinson *et al.* 1997), whereas there are great differences among cowpea cultivars in their susceptibility or resistance to *M. incognita*. *Meloidogyne*-suppressive cowpea cultivars include 'Iron Clay' (McSorley, 1999), 'Tennessee Brown', 'Mississippi Silver' and 'California Blackeye #5', while 'Purple Knuckle' may serve as a host to some extent (Gallaher and McSorley, 1993).

An ornamental plant, French marigold, *Tagetes patula* L., has also shown promise for suppressing soil parasitic nematodes (Steiner, 1941; Tyler, 1938; McSorley and Frederick, 1994; Motsinger *et al.*, 1977). Rotations of marigold have controlled root-lesion nematodes in fields of tobacco (Reynolds *et al.*, 2000), and potato (Ball-Coelho *et al.*, 2003) in Canada. Various *Brassica* species are known to synthesize glucosinolates, which are enzymatically converted to isothiocyanates and other products when tissues are damaged. These materials are toxic to fungi (Charron and Sams, 1999; Chung *et al.*, 2002) and bacteria (Delaquis and Mazza, 1995; Zasada *et al.*, 2003; Zasada and Ferris, 2004). Therefore after soil-incorporation, some *Brassica* species destroy pathogens through biofumigation to protect the following crop (Harvey *et al.*, 2002). Thus, radish and other *Brassica* plants, such as canola (*Brassica napus*) and Indian mustard (*Brassica juncea*), have been used as cover crops in Canada and California

(Omafra, 2002; Thomas, 1999), but effects of these *Brassica* crops on soil nematodes, especially the root-knot nematode, are not clear. Also, crops such as okra, *Abelmoschus esculentus* (L.) Moench, have proven to be very susceptible to parasitic nematodes, especially to root-knot nematodes (Wang *et al.*, 2005, 2006), and their production may be facilitated by use of certain cover crops.

In order for nematode-suppressive crops to benefit the following crop, the suppression must carryover long enough for the following crop to reach the desired level of the development. When this occurs, the cost of soil fumigation may be avoided with economic and environmental benefits (Abdul-Baki *et al.*, 2005; Wang *et al.*, 2003, 2006). Therefore, in sustainable agricultural systems or in organic farming, growing and incorporating nematode suppressive cover crops in rotation with nematode-susceptible cash crops may have the potential to be used as a biological alternative to methyl bromide.

The objectives of this experiment were to elucidate: (1) the influence of various cover crops on soil nematode populations, (2) synergetic or antagonistic effects of different cover crops in rotations on population growth of various taxa of nematodes, especially the root-knot nematode; and (3) the suppressive carry-over effect of certain crops on soil nematode populations during the growth of the following crop.

MATERIALS AND METHODS

Experimental Conditions

The pot experiment was conducted in a screen house from June 2002 to February 2003 at Tropical Research and Education Center, University of Florida, Homestead, Florida (25°31'N, 80° 30'W). The average annual rainfall is 1,499 mm, of which 76% falls between June and October; the annual temperature averages 23.9°C and ranges from 35°C in June to 5°C in January (University of Florida, 2006). Between June 2002 and February 2003, the air temperature ranged from 1.8°C to 35.3°C; the monthly maximum temperature was 27.0°C in August 2002 and monthly minimum temperature was 15.2°C in January 2003; and the relative humidity ranged between 75% in January to 84% in June (University of Florida, 2006).

The Krome soil (loamy-skeletal, carbonatic, hyperthermic Lithic Udorthents) contained 58.8% gravel (>2 mm), and the non-gravel fraction had a distribution of soil particles of 48.4% sand, 30.3% silt and 21.3% clay. Also the soil consists of 60% calcium carbonate ($CaCO_3$), soil organic C 28 g/kg, total N 1.1 g/kg, and ammonium bicarbonate-diethylene triaminepentaacetic acid (AB-DTPA) extractable phosphorus (P) 22.7 mg/kg and potassium (K) 129 mg/kg with pH 7.8.

Experimental Design and Management

A randomized complete block design with 8 rotation schemes was implemented. The 8 crops were marigold (*Tagetes patula* L. 'Dwarf Double French Mix') (MDF), marigold (*Tagetes patula* L. 'Lemon Drop') (MLD), radish (RD) [*Raphanus sativus* L. var. s*ativus*], Indian mustard (IM) [*Brassica juncea* (L.), sunn hemp (SH) [*Crotalaria juncea* L., 'Tropic Sun'], cowpea (CP) [*Vigna unguiculata* (L.) Walp, 'Purple Knuckle Hull'], velvetbean (VB) [*Mucuna deeringiana* (Bort.) Merr.], and okra (OK) [*Abelmoschus esculentus* (L.), 'Clemson Spineless 80']. Each of the 8 crops was planted three times with the following rotations: MDF-RD-MDF; MLD-CP-MLD; IM-VB-IM; RD-MDF-RD; SH-OK-SH; VB-IM-VB; CP-MLD-CP; and OK-SH-OK. Each treatment was replicated 3 times. Before the experiment started and after each rotation, soil samples were collected from each pot to identify and enumerate the nematodes.

Prior to the experiment, tomato roots with root-knot nematode galls were collected from a commercial farm, cut into small pieces (<1 cm) and mixed into potting soil. Okra was then seeded and grown for 4 weeks to increase the root-knot nematode population (Wang *et al.*, 2003). The okra roots with galls were cut into 1-cm pieces, mixed with the soil to obtain a uniform distribution of the nematodes, and 6 kg of soil was placed into each pot. Each pot was 12 cm in diameter and 30 cm tall with a capacity of 3.4 L. The soil was sieved to remove large gravel (>1 cm). Crops were seeded on 6 June 2002 to produce 3 plants per pot for velvetbean; 5 for cowpea, radish, and Indian mustard; and 10 plants per pot of the remaining crops. Drip irrigation was adjusted to deliver 2 L water per h and a timer was used to control irrigation duration and frequency based on plant growth stages. Each succeeding rotation was started immediately after roots had been rated (see below). Then the roots, stems, and leaves were cut into 2-cm long pieces and incorporated into the soil of the same pot used for the previous rotation. Each rotation lasted about 3 months.

Sampling and Analysis

The roots were washed free of soil and examined for galling. Some roots showing lesions were cut into small pieces and left in water for 36 to 48 h to isolate lesion nematode. A soil sample was collected from 3 to 12 cm below the surface in each pot before planting in every rotation. Nematodes from each sample were extracted by means of Cobb's sieving and decanting technique (Cobb, 1918), followed by a modified Baermann funnel method (Hooper, 1986). Plant parasitic nematodes were identified to genus and species following fixation in hot 30 ml/L formaldehyde solution. Some fixed specimens were processed with anhydrous glycerin (Seinhorst, 1959), and examined under a compound microscope for species identification. Nematode identifications were based on the morphology of adult and larval forms confirmed with taxonomic keys (Handoo and Golden, 1992; Mai *et al.*, 1996; Maqbool, 1982; Robinson *et al.*, 1997; Sher, 1966). Nematode density (number in 250 ml of soil) was determined for each species.

Data Analysis

The data were subjected to analysis of variance (ANOVA) and Duncan's multiple range procedures with a general linear model (GLM) for significant differences using SAS version 8.1 (SAS Inst., Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Initial Nematode Population

The total number of nematodes present before the first rotation ranged from 100 to 465 per 250 ml of soil, with an average of 224. The number of second stage juveniles (J2) of the root-knot nematode (*Meloidogyne incognita*) ranged from 37 to 215 with an average of 98 (Table 1).

First Rotation

After the first rotation, the total number of nematodes in every treatment increased (Table 2); however, the population density of the root-knot nematode, *M. incognita,* differed among various crops. Numbers of *M. incognita* were greatest in Indian mustard or okra and somewhat lower with radish, but they were low after one cycle of sunn hemp, cowpea, marigold, or velvetbean.

Table 1. Numbers of nematodes of various taxa in 250 ml of soil prior to the first rotation.

Genera	Range	Mean	% of total
Aphelenchus	0-20	5.7	3.6
Dorylaimids	2.2-13	4.7	3.0
Helicotylenchus	4-55	17.3	10.9
Meloidogyne	37-215	71.6	45.2
Pratylenchus	0-18	4.3	2.7
Quinisulcius	0-20	5.7	3.6
Rhabditids	10.8-55	21.7	13.7
Rotylenchulus	0-60	19.6	12.4
Saprophytes	5.4-45	15.9	10.0
Tylenchus	0-6	2.2	1.4
Parasitic nematodes[z]	41-374	120.7	76.1
Non-parasitic nematodes	59-133	37.9	23.9
Total	100-465	158.6	100.0

[z]Plant-parasitic nematodes include *Helicotylenchus dihystera*, *Heterodera* sp., *Meloidogyne incognita*, *Pratylenchus neglectus*, *Quinisulcius acutus*, *Rotylenchulus reniformis*, and *Tylenchus sandneri*; non-plant parasitic nematodes include *Aphelenchus*, Dorylaimids, *Mononchus* and rhabditids, and saprophytes, respectively, if any identified.

The relative densities of various nematode taxa were strongly affected by the first rotation cycle of the various crops. Prior to the first rotation (Table 1), *M. incognita* juveniles constituted 45% of the total population, *Tylenchus* spp. 1.4%, and rhabditids 13.7%. However after the first rotation (Table 2), the free-living bacterivorous rhabditids became the most numerous in certain rotations, i.e., 78% of the total population with velvetbean, 66% with sunn hemp, and 48% with cowpea. With the two different marigolds in the first rotation, *Helicotylenchus* increased from an average of 10.9% of the initial total population (Table 1) to 44% and 37% of the total (Table 2) with MDF and MLD, respectively. The numbers of *M. incognita* were strongly reduced from 45% of the total before the rotation to 2.7%, 0.6% and 1.0% with velvetbean, sunn hemp, or cowpea in the first rotation, respectively. However in the first rotation with Indian mustard, radish, or okra, the numbers of *M. incognita* remained high, comprising 52.9%, 41.4%, and 50.3% of the total, respectively.

The redistribution in the relative densities of the various nematode taxa after the first rotation resulted from either the susceptibility or the resistance of the various crops to serve as hosts of plant-parasitic nematodes. Initially, the aggregate plant-parasitic nematode taxa constituted 76% of the total population (Table 1), but after the first rotation with the nematode-suppressive crops, the percentage of the aggregate parasitic fauna declined sharply as follows: 4.0% with 'Dwarf Double French Mix' marigold, 15.6% with 'Lemon Drop' marigold, 3.3% with velvetbean, 4.9% with sunn hemp, and 26% with cowpea. This indicates that these five crops possess some resistance to *M. incognita* and in various degrees to the other plant-parasitic taxa. In contrast, Indian mustard, okra, and radish are very susceptible hosts of plant-parasitic nematodes, especially

Table 2. Number of nematodes of various taxa in a 250 ml of soil and % of total nematodes after the first rotation.

	MDF-RD-MDF[x]		MLD-CP-MLD		IM-VB-IM		RD-MDF-RD		SH-OK-SH		VB-IM-VB		CP-MLD-CP		OK-SH-OK	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Aphelenchus	19.3 a[y]	6.6	20.0 a	7.9	10.0 b	1.6	6.7 b	1.8	12.7 ab	3.1	17.3 a	2.5	10.3 b	2.1	9.7 b	1.9
Dorylaimids	18.7 b	6.4	15.7 b	6.2	50.0 a	7.9	16.7 b	4.4	13.3 b	3.2	19.3 b	2.8	9.7 b	2.0	9.7 b	1.9
Helicotylenchus	128.3 a	43.8	93.3 a	36.8	30.7 b	4.8	25.0 b	6.6	16.7 bc	4.1	26.7 b	3.8	26.0 b	5.3	0.0 c	0.0
Heterodera	0.0 b	0.0	0.0 b	0.0	0.0 b	0.0	0.0 b	0.0	0.0 b	0.0	0.0 b	0.0	32.0 a	0.0	0.0 b	0.0
Meloidogyne	10.3 c	3.5	25.7 c	10.1	336.7 a	52.9	156.7 b	41.4	2.3 d	0.6	18.6 c	2.7	5.0 d	1.0	253.3 a	50.3
Pratylenchus	0.0 b	0.0	0.0 b	0.0	5.7 b	0.9	3.3 b	0.9	7.3 b	1.8	1.0 b	0.1	121.7 a	24.9	0.0 b	0.0
Quinisulcius	0.0 a	0.0	0.0 a	0.0	0.0 a	0.0	0.0 a	0.0	2.3 a	0.6	1.0 a	0.1	0.0 a	0.0	0.0 a	0.0
Rhabditids	61.7 bc	21.1	38.3 c	15.1	173.3 b	27.2	93.3 b	24.7	269.3 ab	65.6	545.3 a	78.1	231.7 ab	47.5	176.7 b	35.1
Rotylenchulus	0.0 a	0.0	0.0 a	0.0	0.0 a	0.0	0.0 a	0.0	3.3 a	0.8	0.0 a	0.0	0.0 a	0.0	0.3 a	0.1
Saprophytes	53.0 a	18.1	46.7 a	18.4	30.0 ab	4.7	68.3 a	18.1	78.3 a	19.1	66.7 a	9.6	51.7 a	10.6	51.7 a	10.3
Tylenchus	1.3 b	0.4	14.0 a	5.5	0.0 b	0.0	8.3 a	2.2	5.0 ab	1.2	2.3 b	0.3	0.0 b	0.0	1.7 b	0.3
Parasitic nematodes[z]	11.6 b	4.0	39.7 b	15.6	342.4 a	53.8	168.3 ab	44.5	20.2 b	4.9	22.9 b	3.3	158.7 ab	26.0	255.3 a	50.7
Non-parasitic nematodes	281.0 b	96.0	214.0 b	84.4	294.0 b	46.2	210.0 b	55.5	390.3 ab	95.1	675.3 a	96.7	329.4 b	74.0	247.8 b	49.3
Total	292.6 b	100.0	253.7 b	100.0	636.4 a	100.0	378.3 ab	100.0	410.5 ab	100.0	698.2 a	100.0	488.1 ab	100.0	503.1 a	100.0

[x]Different crops in rotations, MDF = Marigold 'Dwarf Double French Mix', RD = Radish, MLD = Marigold 'Lemon Drop', IM = Indian mustard, SH = sunn hemp, VB = velvetbean, CP = cowpea, and OK = okra.

[y]Nematode numbers (excluding percentages) with same letters within the same row do not differ at $p \leq 0.05$.

[z]Plant parasitic nematodes include *Helicotylenchus dihystera*, *Heterodera* sp., *Meloidogyne incognita*, *Pratylenchus neglectus*, *Quinisulcius acutus*, *Rotylenchulus reniformis*, and *Tylenchus sandneri*; non-plant parasitic nematodes include *Aphelenchus*, Dorylaimids, *Mononchus*, rhabditids, and saprophytes, respectively, if any identified.

M. incognita. After the first rotation of these crops, *M. incognita* constituted 53.8% of the total parasitic fauna with Indian mustard, 50.7% with okra, and 44.5% with radish.

Velvetbean induced greater population growth in the non-parasitic nematode fauna than all other crops except sunn hemp (Table 2). However, it is important to note that some crops, such as sunn hemp, velvetbean, marigold, and cowpea possess an ability to antagonize or resist important plant-parasitic nematodes, especially root-knot nematodes.

Second Rotation

After the second rotation, the total number of nematodes increased in every rotation except cowpea-Marigold II, and okra-sunn hemp. However, the redistribution of various nematode taxa differed with rotation schemes. For instance, after the MDF-RD rotation, the total number of plant-parasitic nematodes (excluding *Helicotylenchus* spp.) increased 18-fold from 12 (Table 2) to 222 (Table 3); which demonstrates the great susceptibility of radish plants to the parasitic nematodes. Likewise after the MLD-CP rotation, the total number of parasitic nematodes (excluding *Helicotylenchus* spp.) increased 3-fold from 40 to 123 with more than 50% of this increase caused by the surge in numbers of *Rotylenchulus reniformis* for which cowpea is a good host (Robinson *et al.*, 1997). In contrast cowpea in this rotation caused the number of *Helicotylenchus* to decrease from 93 (Table 2) to 15 (Table 3), but left the number of *M. incognita* unchanged, i.e., 26 before cowpea (Table 2) and 24 after cowpea (Table 3). After the MDF-RD and MLD-CP rotations the number of the fungivorous *Aphelenchus* spp. increased 3- to 4-fold to become 36% and 25% of the total, respectively.

After the IM-VB rotation the number of *M. incognita* declined 90% from 337 (Table 2) to 33 (Table 3), while *Helicotylenchus* numbers increased 8-fold from 31 (Table 2) to 243 (Table 3), and rhabditid numbers almost doubled from 173 to 320. This shows that velvetbean suppressed the *M. incognita* population, but enhanced population growth of *Helicotylenchus* and of rhabditids. In the RD-MDF rotation, the MDF caused a 2.6-fold decline of *M. incognita* numbers from 157 (Table 2) to 60 (Table 3), and a 6-fold increase in *Helicotylenchus* numbers from 25 (Table 2) to160 (Table 3). After growing okra in the SH-OK rotation, the number of *M. incognita* increased from 2 (Table 2) to 159 (Table 3). Even though sunn hemp suppressed the number of *M. incognita* from 72 (Table 1) to 2 (Table 2), the population of *M. incognita* resurged when okra was planted.

After the VB-IM rotation, the *M. incognita* population did not resurge (Tables 2 and 3), even though in the first rotation Indian mustard served as an excellent host of the root-knot nematode, suggesting that the antagonistic effect of velvetbean persisted to suppress the parasite. However, the *Helicotylenchus* population increased from 27 after the first rotation to 498 after the 2nd rotation with Indian mustard to become dominant (45% of the total) among all nematode taxa (Tables 2 and 3). After the CP-MLD rotation, the number of *M. incognita* remained suppressed. Also MLD suppressed *Heterodera* to a non-detectable level, *Pratylenchus* from 122 to 10, and the non-parasitic rhabditids from 232 (table 2) to below the level of detection (Table 3). Sunn hemp (OK-SH) reduced *M. incognita* over 77% from 253 after the 1st rotation to 58 after the 2nd rotation, but allowed *Pratylenchus* numbers to increase to 65% (Tables 2 and 3).

Third Rotation

In 6 of the 8 rotation schemes, the total number of nematodes decreased after the third rotation compared to the second one (Table 4). In some schemes, the following

Table 3. Number of nematodes of various taxa in a 250 ml of soil after the second rotation.

	MDF-RD-MDF[x]		MLD-CP-MLD		IM-VB-IM		RD-MDF-RD		SH-OK-SH		VB-IM-VB		CP-MLD-CP		OK-SH-OK	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Aphelenchus	226.7 a[y]	35.8	115.0 ab	25.0	81.7 ab	10.5	46.7 b	11.0	75.3 ab	10.6	118.3 ab	10.6	2.3 b	1.2	0.0 b	0.0
Dorylaimids	51.7 a	8.2	70.0 a	15.2	33.3 ab	4.3	13.3 b	3.2	88.3 a	12.4	70.0 a	6.3	41.7 ab	21.4	1 8.3 b	4.6
Helicotylenchus	101.7 bc	16.1	15.0 c	3.3	243.3 b	31.2	160.0 b	37.8	6.7 c	0.9	498.3 a	44.8	8.3 c	4.3	0.0 c	0.0
Meloidogyne	58.3 ab	9.2	23.5 b	5.1	33.3 b	4.3	60.0 ab	14.2	158.7 a	22.3	22.0 b	2.0	3.5 c	1.8	58.0 ab	14.5
Mononchus sp.	61.7 ab	9.7	5.0 c	1.1	0.0 c	0.0	2.3 c	0.6	81.7 a	11.5	6.7 c	0.6	5.0 c	2.6	0.0 c	0.0
Pratylenchus	0.0 c	0.0	5.0 bc	1.1	0.0 c	0.0	0.0 c	0.0	0.0 c	0.0	0.0 c	0.0	10.0 b	5.1	258.3 a	64.6
Quinisulcius	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhabditids	26.7 b	4.2	76.7 b	16.7	320.0 a	41.0	108.3 ab	25.6	196.7 ab	27.6	301.7 a	27.1	0.0 bc	0.0	2 5.0 b	6.3
Rotylenchulus	0.0 b	0.0	71.0 a	15.5	0.0 b	0.0	0.0 b	0.0	0.0 b	0.0	0.0 b	0.0	0.0 b	0.0	0.0 b	0.0
Saprophytes	106.7 a	16.8	75.0 ab	16.3	68.3 ab	8.8	30.0 ab	7.1	105.0 a	14.7	95.0 a	8.5	121.7 a	62.6	40.0 ab	10.0
Tylenchus	0.0 a	0.0	3.3 a	0.7	0.0 a	0.0	2.3 a	0.6	0.0 a	0.0	0.0 a	0.0	2.0 a	1.0	0.0 a	0.0
Parasitic nematodes[z]	221.7 ab	35.0	122.8 b	26.7	276.7 ab	35.5	224.7 ab	53.1	247.0 ab	34.7	527.0 a	47.4	28.8 c	14.8	316.3 ab	79.1
Non-parasitic nematodes	411.7 b	65.0	336.7 bc	73.3	503.4 a	64.5	198.3 bc	46.9	465.3 b	65.3	585.0 a	52.6	165.6 bc	85.2	83.3 c	20.9
Total	633.4 ab	100.0	459.5 ab	100.0	780.0 a	100.0	423.0 b	100.0	712.3 a	100.0	1112.0 a	100.0	194.5 b	100.0	399.6 b	100.0

[x]Different crops in rotations, MDF = Marigold 'Dwarf Double French Mix', RD = Radish, MLD = Marigold 'Lemon Drop', IM = Indian mustard, SH = sunn hemp, VB = velvetbean, CP = cowpea, and OK = okra.

[y]Nematode numbers (excluding percentages) with same letters within the same row do not differ at $p \leq 0.05$.

[z]Plant parasitic nematodes include *Helicotylenchus dihystera*, *Heterodera* sp., *Meloidogyne incognita*, *Pratylenchus neglectus*, *Quinisulcius acutus*, *Rotylenchulus reniformis*, and *Tylenchus sandneri*; non-plant parasitic nematodes include *Aphelenchus*, Dorylaimids, *Mononchus*, rhabditids, and saprophytes, respectively, if any identified.

Table 4. Number of nematodes of various taxa in a 250 ml of soil after the third rotation.

	MDF-RD-MDF[x]		MLD-CP-MLD		IM-VB-IM		RD-MDF-RD		SH-OK-SH		VB-IM-VB		CP-MLD-CP		OK-SH-OK	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Aphelenchus	87.0 a[y]	27.8	15.0 b	3.1	10.7 b	3.2	1.7 c	0.5	125.0 a	29.0	0.0 c	0.0	1.0 c	0.2	1.3 c	0.4
Dorylaimids	41.7 c	13.3	78.3 bc	15.9	33.3 c	9.9	101.7 b	30.2	300.0 a	69.7	40.0 c	7.7	76.7 bc	13.6	77.0 bc	24.0
Helicotylenchus	0.0 c	0.0	0.0 c	0.0	35.0 b	10.4	0.0 c	0.0	0.0 c	0.0	163.3 a	31.4	0.0 c	0.0	0.0 c	0.0
Meloidogyne	12.6 bc	4.0	16.8 bc	3.4	68.0 bc	20.2	98.0 ab	29.1	21.0 bc	4.9	38.0 bc	7.3	15.0 bc	2.7	125.0 a	38.9
Mononchus	17.3 b	5.5	71.7 a	14.6	46.7 ab	13.8	51.7 a	15.4	28.3 ab	6.6	70.0 a	13.5	65.0 a	11.5	1.0 b	0.3
Pratylenchus	3.5 b	1.1	0.0 b	0.0	0.0 b	0.0	0.0 b	0.0	1.0 b	0.2	0.3 b	0.1	27.5 a	4.9 b	0.0 b	0.0
Quinisulcius		0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0
Rhabditids		0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0
Rotylenchulus		0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0
Saprophytes	173.3 ab	55.4	200.0 ab	40.7	135.0 b	40.0	115.0 b	34.2	68.3 b	15.9	221.7 ab	42.6	340.0 a	60.4	210.0 ab	65.4
Tylenchus	46.7 a	14.9	40.0 a	8.1	50.0 a	14.8	23.3 ab	6.9	24.0 ab	5.6	25.0 ab	4.8	21.7 ab	3.9	13.3 b	4.1
Parasitic nematodes[z]	80.1 bc	24.5	128.5 b	26.1	199.7 ab	59.2	173.0 ab	51.4	74.3 bc	17.3	296.6 a	57.0	129.2 b	22.9	139.3 b	43.4
Non-parasitic nematodes	232.9 ab	75.5	363.2 a	73.9	137.6 b	40.8	163.7 b	48.6	356.4 a	82.7	223.7 ab	43.0	434.1 a	77.1	181.7 ab	56.6
Total	313.0 b	100.0	491.7 ab	100.0	337.3 b	100.0	336.7 b	100.0	430.7 ab	100.0	520.3 a	100.0	563.3 a	100.0	321.0 b	100.0

[x]Different crops in rotations, MDF = Marigold ‘Dwarf Double French Mix’, RD = Radish, MLD = Marigold ‘Lemon Drop’, IM = Indian mustard, SH = sunn hemp, VB = velvetbean, CP = cowpea, and OK = okra.

[y]Nematode numbers (excluding percentages) with same letters within the same row do not differ at $p \leq 0.05$.

[z]Plant parasitic nematodes include *Helicotylenchus dihystera*, *Heterodera* sp., *Meloidogyne incognita*, *Pratylenchus neglectus*, *Quinisulcius acutus*, *Rotylenchulus reniformis*, and *Tylenchus sandneri*; non-plant parasitic nematodes include *Aphelenchus*, Dorylaimids, *Mononchus*, rhabditids, and saprophytes, respectively, if any identified.

taxa were scarcely encountered: *Helicotylenchus*, *Pratylenchus*, *Quinisulcius*, *Rotylenchulus reniformis*, and rhabditids. This decline in the total number of nematodes was caused mainly by the decrease in the air temperature, which obviously influences plant growth and the reproduction of nematodes (Wang *et al.*, 2005). However, in the MLD-CP-MLD scheme the taxa with population increases were: *Mononchus* (14-fold), *Tylenchus* (21.7-fold) and saprophytes (2.7-fold), while in the CP-MLD-CP scheme the taxa with increases in population numbers were: *Meloidogyne* (4.3-fold), *Mononchus* (13-fold), *Pratylenchus* (2.8-fold), *Tylenchus* (10.9-fold) dorylaimids (1.8-fold) and saprophytes (2.8-fold). Clearly 'Purple Knuckle Hull' cowpea does not suppress plant-parasitic nematodes as reliably or as effectively as marigold, sunn hemp and velvetbean.

After the third rotation in the MDF-RD-MDF scheme, the root-knot nematode population, *M. incognita*, reduced from 58 (Table 3) to 13 (Table 4); and saprophytes increased from only 17% of the total population after the second rotation to become dominant taxa comprising 55% of the total (Tables 3 and 4). The composition of the nematode population after the third rotation in the MLD-CP-MLD scheme was very similar to that in MDF-RD-MDF with low numbers of *M. incognita* and dominance of the saprophytes (Table 4).

In the IM-VB-IM scheme after the third rotation compared to the second one, plant-parasitic nematodes increased from 36% to 59% of the total nematode population, and *M. incognita* increased from 4.3% to 20.2% of the total. Also populations of *Tylenchus* and *Mononchus* increased substantially, but *Helicotylenchus* population declined (Tables 3 and 4).

After the third rotation compared to the second one in the RD-MDF-RD scheme the number of *Aphelenchus* and *Helicotylenchus* nematodes declined while nematodes in the following taxa increased: *Meloidogyne* (1.6-fold), *Mononchus* (22.5-fold), *Tylenchus* (10.1-fold), rhabiditids (7.6-fold) and saprophytes (3.8-fold). This result indicates that radish is a highly susceptible host of plant-parasitic nematodes, and provides a favorable environment for non-parasitic nematodes.

After the SH-OK-SH third rotation, the number of *Helicotylenchus*, *Pratylenchus*, *Quinisulcius*, *Rotylenchulus reniformis* and rhabditids were very low or non-detectable, and the nematode counts decreased as follows: *Meloidogyne* from 159 to 21, *Mononchus* from 82 to 28, and saprophytes from 105 to 68 (Tables 3 and 4). By contrast the number of *Aphelenchus* increased 1.7-fold, and dorylaimids increased 3.4-fold from densities after the third rotation (Tables 3 and 4).

After the third rotation in the VB-IM-VB scheme the numbers of *Aphelenchus*, *Pratylenchus*, *Quinisulcius*, *Rotylenchulus reniformis* and rhabditids were very low or non-detectable and other nematode counts decreased as follows: *Helicotylenchus* by 67% (498 vs. 163), dorylaimids by 1.8-fold. In contrast, after the second and third rotations the nematode counts increased as follows: *Mononchus*, 10.4-fold; saprophytes, 2.3-fold, but *M. incognita* increased from 22 to 38 (Tables 3 and 4). The result indicates that velvetbean is somewhat inconsistent in suppressing root-knot nematode under the experimental condition, since it provided strong suppression in the IM-VB-IM rotation scheme (Tables 2 and 3).

In the CP-MLD-CP scheme the number of *Aphelenchus*, *Helicotylenchus*, *Quinisulcius*, *Rotylenchulus reniformis* and rhabditids were very low or non-detectable after the third rotation (Table 4), while the numbers of other taxa increased as follows: *Meloidogyne* (4.3-fold), *Mononchus* (13-fold), *Pratylenchus* (2.8-fold), *Tylenchus* (10.9-fold) dorylaimids (1.8-fold) and saprophytes (2.8-fold) (Tables 3 and 4). This shows that 'Purple Knuckle Hull' cowpea is quite susceptible to *Meloidogyne.*

After the third rotation in the OK-SH-OK scheme the number of *Aphelenchus, Helicotylenchus, Mononchus, Pratylenchus, Quinisulcius, Rotylenchulus reniformis, Tylenchus* and rhabditids were very low or non-detectable (Table 4). However, in relation to nematode densities after the second rotation, after the third rotation the populations of the following taxa increased: *Meloidogyne* (2.2-fold), dorylaimids (4.2-fold), and saprophytes (5.3-fold) (Tables 3 and 4).

The parasitic nematodes, especially root-knot nematodes, showed a relatively consistent redistribution after either velvetbean or 'Purple Knuckle Hull' cowpea had been grown in the rotation schemes of VB-IM-VB and CP-MLD-CP compared to the previous rotation. Thus the total numbers of parasitic nematodes comprised 47% vs. 57% of the total nematode population after the second and the third rotations of VB-IM-VB, and 15% vs. 23% after the second and the third rotations of CP-MLD-CP. The corresponding numbers of root-knot nematodes were 22 vs. 38 per 250 ml of soil in the VB-IM-VB scheme, and 4 vs. 15 in the CP-MLD-CP scheme (Tables 3 and 4). However, the numbers of other nematodes, e.g., *Mononchus* and *Tylenchus* after velvetbean, and *Mononchus, Pratylenchus* and *Tylenchus* after 'Purple Knuckle Hull' cowpea increased under these two rotations. This indicates these two cover crops suppress parasitic nematode taxa to the same extent.

The distributions of plant-parasitic and non-parasitic nematodes after the third rotation in various schemes indicate the susceptibilities of these 8 plant species to the plant-parasitic nematodes, as well as the favorable conditions they provide to non-parasitic nematode taxa, differ greatly among plant species. Sunn hemp and both marigold cultivars suppress nematode population strongly, velvetbean is at least moderately nematode suppressive, and 'Purple Knuckle Hull' cowpea is not consistently nematode suppressive, while okra, Indian mustard, and radish serve as favorable hosts of the root-knot nematode, *M. incognita.* The distribution and redistribution of soil nematodes after various rotations clearly showed that some biological approaches, such as growing cover crops or rotating cash crops with nematode-suppressive crops, especially sunn hemp and marigold, can sufficiently suppress plant-parasitic nematodes, especially the root-knot nematode, *Meloidogyne incognita,* to reduce damage to a susceptible cash crop. This conclusion is consistent with other findings including those of Wang *et al.* (2003, 2005, 2006) who reported sunn hemp as a promising summer cover crop in tropical or subtropical regions for root-knot nematode (*Meloidogyne incognita*) suppression in tomato and okra production. Hackney and Dickerson (1975), McKenry (1988), Reynolds *et al.* (2000), and Ball-Coelho *et al.* (2003) reported that lesion nematodes (*Pratylenchus* spp.) were suppressed by marigold. The current research indicates that marigold, especially the 'Dwarf Double French Mix', can effectively suppress root-knot nematode, *M. incognita.*

Relative Changes of Root-knot and Other Plant- Parasitic Nematodes After Each Rotation

The relative changes in density of various nematode populations after each rotation (Table 5) showed that 'Dwarf Double French Mix' marigold and sunn hemp consistently suppressed total parasitic nematode populations in all 3 rotations. In contrast, 'Lemon Drop' marigold and velvetbean suppressed the total parasitic nem-

Table 5. Percent of parasitic and root-knot nematode populations in different crop rotations

	MDF-RD-MDF[y]	MLD-CP-MLD	IM-VB-IM	RD-MDF-RD	SH-OK-SH	VB-IM-VB	CP-MLD-CP	OK-SH-OK
1st rotation								
Change in % of parasitic nematodes	-90.4	-67.1	183.7	39.4	-83.3	-81.0	5.0	111.5
Change in % of *Meloidogyne* J2	-85.6	-64.1	370.3	118.9	-96.8	-74.0	-93.0	253.8
2nd rotation								
Change in % of parasitic nematodes	1810.9	209.4	-19.2	33.5	1122.8	2201.2	-81.8	23.9
Change in % of *Meloidogyne* J2	466.3	-8.6	-90.1	-61.7	6798.7	18.3	-30.0	75.7
3rd rotation								
Change in % of parasitic nematodes	-63.9	4.6	-27.8	-23.0	-69.9	-43.7	348.1	-56.0
Change in % of *Meloidogyne* J2	-78.4	-28.5	104.0	63.3	-86.8	72.7	328.6	115.5

[y]Different crops in rotations, MDF = Marigold 'Dwarf Double French Mix', RD = Radish, MLD = Marigold 'Lemon Drop', IM = Indian mustard, SH = sunn hemp, VB = velvetbean, CP = cowpea, and OK = okra.
[z]Plant parasitic nematodes include *Helicotylenchus dihystera*, *Heterodera* sp., *Meloidogyne incognita*, *Pratylenchus neglectus*, *Quinisulcius acutus*, *Rotylenchulus reniformis* and *Tylenchus sandneri*; non-plant parasitic nematodes include *Aphelenchus,* Dorylaimids, *Mononchus,* rhabditids, and saprophytes, respectively, if any identified.

atode population in the first rotation, but only provided weak suppression in the third rotation during the cool winter months. In the first rotation 'Purple Knuckle Hull' cowpea strongly suppressed the root-knot nematode population, as well as the other plant-parasitic taxa with the exception of *Helicotylenchus*, *Heterodera*, and *Pratylenchus*. However 'Purple Knuckle Hull' cowpea was generally ineffective in suppressing populations of any parasitic taxon in the third rotation during the cool winter months. 'Iron Clay' cowpea was consistently antagonistic to parasitic nematodes in other studies (McSorley and Parrado, 1983; McSorley *et al.*, 1994; Barker and Koenning, 1998; McSorley, 1999). However, the 'Purple Knuckle Hull' cowpea did not show a consistent suppression to plant-parasitic nematodes in this study, which indicates the importance in choosing not only the cover crop species but also the proper cultivar.

These results further indicate that sunn hemp and marigold ('Dwarf Double French Mix') have potential to suppress plant-parasitic nematodes, and to protect susceptible cash crops. Therefore, in tropical or subtropical vegetable production systems, growing sunn hemp as a cover crop during the summer period can improve soil fertility and control plant-parasitic nematodes (Wang *et al.*, 2003, 2005). In home gardens or nurseries, rotating ornamental plants with marigold or sunn hemp may provide a practical approach to reduce or control soil nematodes.

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