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Potential of Leguminous Cover Crops in Management of a Mixed Population of Root-knot Nematodes (*Meloidogyne* spp.)

Kingsley Osei,[1] Simon R. Gowen,[1] Barbara Pembroke,[1] Rick L. Brandenburg,[2] David L. Jordan[3]

Abstract: Root-knot nematode is an important pest in agricultural production worldwide. Crop rotation is the only management strategy in some production systems, especially for resource poor farmers in developing countries. A series of experiments was conducted in the laboratory with several leguminous cover crops to investigate their potential for managing a mixture of root-knot nematodes (*Meloidogyne arenaria, M. incognita*, *M. javanica*). The root-knot nematode mixture failed to multiply on *Mucuna pruriens* and *Crotalaria spectabilis* but on *Dolichos lablab* the population increased more than 2- fold when inoculated with 500 and 1,000 nematodes per plant. There was no root-galling on *M. pruriens* and *C. spectabilis* but the gall rating was noted on *D. lablab*. Greater mortality of juvenile root-knot nematodes occurred when exposed to eluants of roots and leaves of leguminous crops than those of tomato; 48.7% of juveniles died after 72 h exposure to root eluant of *C. spectabilis*. The leaf eluant of *D. lablab* was toxic to nematodes but the root eluant was not. Thus, different parts of a botanical contain different active ingredients or different concentrations of the same active ingredient. The numbers of root-knot nematode eggs that hatched in root exudates of *M. pruriens* and *C. spectabilis* were significantly lower (20% and 26%) than in distilled water, tomato and *P. vulgaris* root exudates (83%, 72% and 89%) respectively. Tomato lacks nematotoxic compounds found in *M. pruriens* and *C. spectabilis*. Three months after inoculating plants with 1,000 root-knot nematode juveniles the populations in pots with *M. pruriens, C. spectabilis* and *C. retusa* had been reduced by approximately 79%, 85% and 86% respectively; compared with an increase of 262% nematodes in pots with *Phaseolus vulgaris*. There was significant reduction of 90% nematodes in fallow pots with no growing plant. The results from this study demonstrate that some leguminous species contain compounds that either kill root-knot nematodes or interfere with hatching and affect their capacity to invade and develop within their roots. *M. pruriens, C. spectabilis* and *C. retusa* could be used with effect to decrease a mixed field populations of root-knot nematodes.

Key words: Crotalaria spectabilis, *Crotalaria retusa*, *Dolichos lablab*, *Mucuna pruriens*, *Phaseolus vulgaris*, nematicidal compounds, phytoalexins.

Phytoparasitic nematodes are among the most difficult crop pests to control (Chitwood, 2002). They damage economic crops throughout the world but are particularly important in the sub tropics and tropics where environmental conditions and cropping practices favour their development (Luc et al., 2005). The root-knot nematodes, *Meloidogyne* (Goeldi) spp., are the most damaging largely because of their wide host ranges; in fact there are few valuable crop species that are not attacked by these species (Williams-Woodward and Davis, 2001). In Ghana, *Meloidogyne* spp. are a threat to the vegetable growers and particularly the tomato industry where up to 33% crop losses can occur (Addoh, 1971). Of the many species three are widely found in the tropics *Meloidogyne arenaria* (Neal), Chitwood, *M. incognita* (Kofoid and White), Chitwood and *M. javanica* (Treub), Chitwood, all being recorded as key pests and accounting for 90% of the damage caused by root-knot nematodes (Castagnone-Sereno, 2002). A further problem related to their host preferences is the number of common agricultural weeds that will support their reproduction (Luc et al. 2005).

The symptoms of *Meloidogyne* spp. damage are root galling, fewer feeder roots, stunted growth, loss of vigour, wilting during the hottest time of the day, chlorosis and low and poor quality yields (Sikora and Fernandez, 2005). Use of chemicals and crop rotation are widely used strategies for their control but these have their limitations. Use of chemical option, once favoured by commercial growers (Hague and Gowen, 1987), is in decline because of the withdrawal of the more toxic compounds and the efforts of regulatory authorities to reduce their use; policies driven by the risks of exposure to users and residues in food and the environment. The synthetic chemicals still available are less effective or lack broad spectrum activity (Chitwood, 2002). Crop rotation will always be a difficult strategy to implement because of the wide host ranges of *Meloidogyne* spp. (Sikora and Fernandez, 2005).

The use of non-host cover crops can provide a reliable means of suppressing root-knot nematodes. The feasibility of using crops in a management strategy has been explored but most of the experimental work has been with single nematode species (Reddy et al., 1986; Rodríguez-Kábana et al., 1992a 1992b; Quénéhervé et al., 1998). Under field conditions where mixed populations of *Meloidogyne* spp. are often encountered the potential of using cover crop remains uncertain because

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[1]Former graduate student, Professor, and Professor, Department of Agriculture, University of Reading, School of Agriculture, Policy, and Development, Box 237, Earley Gate, RG6 6AR, Reading, UK.

[2]William Neal Reynolds Distinguished Professor, Department of Entomology, North Carolina State University, Box 7613, Raleigh, NC 27695.

[3]Professor, Department of Crop Science, North Carolina State University, Box 7620, Raleigh, NC 27695.

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of the risk of favouring the development of one species. Ghanaian farmers commonly use cover crops in their farming systems. The cover crops in the current study were chosen based upon utility in a sustainable tropical agricultural system; for checking soil erosion and restoring the fertility of soils (Osei-Bonsu et al., 1996). Secondly, their seeds are readily available for propagation. Again, *Mucuna* is used for food in some African countries (Rachie and Roberts, 1974). In addition to these, *M. pruriens* provides excellent hay for livestock, and its seeds are used as feed supplement after processing (Maasdorp et al., 2001). The goal of this research was to evaluate the ability of *M. pruriens, C. spectabilis* and *C. retusa* to reduce mixed populations of *Meloidogyne* spp. The specific goals of this research were to: (i) evaluate reproduction of *Meloidogyne* spp. on the cover crops, (ii) screen eluants for toxicity against *Meloidogyne* spp., and (iii) determine if cover crop root leachates inhibit *Meloidogyne* spp. egg hatch.

Materials and Methods

Root-knot nematode culture: Meloidogyne arenaria, M. incognita and *M. javanica* collected from a field in Kumasi, Ghana were identified through perennial patterns (Jepson, 1987) and cultured on tomato (*Solanum lycopersicum* L.). Infected roots of tomato cv. Tiny Tim were kept separate and sent to the UK. Three egg masses, one each of the species were combined and cultured on the dwarf determinate tomato cultivar Tiny Tim in the glasshouse at 20-30^{0}C. Six weeks after inoculation, when two generations had occurred under these optimum conditions (McLeod et al., 2001), eggs were extracted from the tomato roots by shaking for 3 min in 0.05 % NaOCl solution and rinsing for 2 min under running tap water (Stanton and O' Donnel, 1994). Extracted eggs were incubated at 22^{0}C on modified Baermann trays (Rodríguez-Kábana and Pope, 1981) for collection of infective second- stage juveniles (J2). These nematodes were the basis of a stock culture maintained on tomato and the *Meloidogyne* species were not further characterized before use in the various experiments. The growing medium used was multi-purpose peat-based compost and loam-based compost (John Innes No.2) (supplied by Roffey Brothers, Bournemouth, Dorset, UK) mixed in a ratio of 1:1.

Effect of Meloidogyne *spp. inoculum level on reproduction on three leguminous cover crops.* Three legumes, *Mucuna pruriens* L., *Dolichos lablab* L. and *Crotalaria spectabilis* Roth were direct seeded into 5,200 cm^3 of compost in plastic pots with one seed per pot. *M. pruriens* seeds were scarified before sowing. One day after sowing, pots were inoculated with freshly collected J2 at four densities (Pi = 0, 500, 1,000, 1,500) through five small holes made in a circle 5 cm from the seeds. Pots were arranged in a randomised complete block design and replicated four times.

Seedling height was measured at 6 weeks and plants were destructively sampled after twelve weeks; fresh shoot biomass and root galling index based on Zeck's 0-10 scale (Sikora and Fernandez, 2005) were recorded. The soil from the pots was thoroughly mixed and J2 were extracted from 200 cm^3 sub-samples in trays using a method described by Whitehead and Hemming (1965). Reproduction factor (RF), defined as the density of final nematode population (juveniles in soil) relative to the initial inoculum level; RF = Pf/Pi was determined. The root systems were washed, chopped into 1 cm sections and a 1- g sub-sample was taken, cleared in diluted sodium hypochlorite bleach (5.25 % NaOCl) for 4 min. After rinsing the roots were covered with acidified lactoglycerol plus 0.05 % methyl blue stain and boiled for 30 sec. in a microwave oven. The roots were placed on microscope slides and juveniles teased from the tissue were counted under the microscope (Bridge et al., 1982). The experiment was repeated once, however results were pooled as the interaction value of experiment x treatment was not significant. Means were subjected to analysis of variance (ANOVA) using Genstat 8.1. (Lawes Agricultural Trust, VSN International). Means separation was done using Tukey's multiple comparisons at ($P < 0.01$).

Effect of root and leaf eluants of leguminous cover crops on Meloidogyne *spp. juveniles.* Root and leaf samples of 3- month old plants of *M. pruriens, D. lablab, Crotalaria spectabilis* and tomato cv. Tiny Tim (control) were freeze dried using the "Lyo Swift Modular System®". Samples were frozen before freeze drying. Powdered 5- g samples were placed between two cellulose disks in the stainless steel sample holder (25- mm diam, 65- mm length) of the Soxflo (SoF) instrument® (Scientific and Technical Supplies Ltd, Newmarket, UK) and firmly compressed by hand (Brown and Muller-Harvey, 1999). The sample holder was then inserted into the SoF apparatus which consists of (i) a solvent reservoir at the top that is connected to a small pump, (ii) an apparatus for securing the sample holder and (iii) a connector for a round-bottom flask to collect the eluant. Each of the 5- g powdered samples was extracted for 1 h with 70 ml of sterilized distilled water.

One hundred freshly hatched J2 in 2- ml suspension were placed in 2 ml of each eluant. Treatments were arranged in a randomized complete block design. Immobile nematodes were counted under stereo microscope x50 after 24, 48 and 72 h. The immobile nematodes were removed from the suspension and were then transferred to sterilized distilled water for 24 h to determine if effects were reversible. The nematodes did not recover and were considered dead.

The experiment included three replications and was repeated once. Results were pooled and means were log transformed ln (x+1) before analysis using Genstat 8.1 statistical package. Means separation was by Tukey's multiple comparisons at ($P < 0.01$).

Effect of leguminous cover crops root exudates on hatching of Meloidogyne *spp. eggs.* Two seeds each of *M. pruriens,*

Crotalaria retusa L., *C. spectabilis, Phaseolus vulgaris* L. and tomato (control) were sown directly in plastic pots (10 x 7 x 4.5 cm) and grown for 8 weeks. Root exudates were collected daily during the eighth week into 100- ml beakers by flushing the pots with 20- ml tap water. The water that passed through the pots was strained over a 350- μm sieve to remove extraneous solid material.

One hundred eggs in 2- ml water suspension was immersed into 2 ml of exudates in a 9- cm Petri dish. Tomato exudates and sterilized distilled water were the control treatments. The number of eggs that hatched was counted over 3, 5 and 7 days. The treatments were replicated four times and the experiment was repeated once. Results were pooled and means used in analysis, while means were separated using Tukey's multiple comparisons at ($P < 0.01$).

Effect of some leguminous cover crops on a soil population of Meloidogyne *spp.* Twenty plastic pots were filled with 5,200 cm^3 of compost. Each was inoculated with 1,000 J2 in the middle of the pot, placed in a hole and then covered and 1 day later, seed of *M. pruriens, C. retusa, C. spectabilis* and *P. vulgaris* were sown to simulate what happens under field conditions. Four of the inoculated pots were left unplanted (fallow-control). The treatments were arranged in a randomised complete block design and replicated four times. All pots were managed in a similar manner. After 3 months the soil in each pot was mixed thoroughly and nematodes were extracted from 200 cm^3 samples and population levels calculated for the entire pot (5,200 cm^3). The experiment was repeated once, results were pooled and means used in analysis. Means separation was done using Tukey's multiple comparisons at ($P < 0.01$).

Results

Effect of Meloidogyne *spp. inoculum level on reproduction on three leguminous cover crops. Dolichos lablab* was relatively susceptible to the *Meloidogyne* spp. and after 12 weeks, the density of J2 per pot increased by 248% and 208% in treatments inoculated with 500 and 1,000 J2 respectively. The populations recovered from the *M. pruriens* and *C. spectabilis* pots were less than those applied (Table 1). Significantly higher numbers of juveniles were extracted from 1 g of *D. lablab* root; there was no significant difference between *M. pruriens* and *C. spectabilis* ($P < 0.01$) (Table 2). No root galling occurred on either *M. pruriens* or *C. spectabilis* but *D. lablab* recorded an approximate mean galling index of 6 on a 0-10 scale at the three inoculum levels (data not presented).

Height and shoot biomass of *M. pruriens* and *C. spectabilis* were not affected by the different levels of nematodes, however in *D. lablab,* there was a highly significant difference between treated and untreated ($P < 0.01$) (Figure 1).

Effect of root and leaf eluants of leguminous cover crops on Meloidogyne *spp. juveniles.* Compared to tomato, the leaf eluants of the three cover crops caused highly significant mortality from 24 to 72 h of exposure ($P < 0.01$). *Crotalaria spectabilis* root eluant resulted in the highest mortality of approximately 49%. *Mucuna pruriens* leaf was the next most active (44%) and *D. lablab* leaf eluant recorded 41%. Whilst the root eluants of *M. pruriens* and *C. spectabilis* were toxic that of *D. lablab* was less active not being significantly different to that of the tomato (Table 3).

Effect of leguminous cover crops root exudates on hatching of Meloidogyne *spp. eggs.* Root exudates of *M. pruriens, C. spectabilis* and *C. retusa* had a significant effect on hatching of eggs of *Meloidogyne* spp., however the exudates of another legume *P. vulgaris* had no effect; the resulting hatch being not significantly different to that occurring with eggs immersed in tomato exudates or distilled water (Figure 2). The numbers of eggs hatched increased in all treatments from the 3rd to the 7th day.

Effect of some leguminous cover crops on a soil population of Meloidogyne *spp.* When sown into soil infested with *Meloidogyne* spp. juveniles, only *P. vulgaris* supported reproduction in the subsequent 3 months (Table 4). There was significant decline ($P < 0.01$) in nematode densities in the *M. pruriens, C. retusa, C. spectabilis* and fallow treatment pots (79%, 85%, 86% and 90%), respectively.

Table 1. Juveniles per pot and reproduction factor of *Meloidogyne* spp. on leguminous cover crops 12 weeks after applying different levels of inoculum.

	Inoculum (J2/pot) Pi					
	500		1,000		1,500	
Crop	Pf	Pf/Pi	Pf	Pf/Pi	Pf	Pf/Pi
M. pruriens	170 (2.2)*	0.3 a	194 (2.2)	0.2 a	229 (2.3)	0.2 a
D. lablab	1241 (3.1)	2.5 b	2080 (3.3)	2.1 b	1452 (3.1)	1.0 b
C. spectabilis	88 (1.9)	0.2 a	114 (2.0)	0.1 a	158 (2.2)	0.1 a
LSD (ln(x+1)	(0.32)		(0.27)		(0.28)	
P-value	(0.001)		(0.001)		(0.001)	

Data are means of four replications. Mean separation was done using Tukey's multiple comparisons ($P < 0.01$).
* $\sqrt{(x + 1)}$ transformed data used in ANOVA in parenthesis
Pi = initial inoculum levels (500, 1,000, 1,500). Pf = final nematode density. Pf/Pi = reproduction factor.

Table 2. Juveniles (J2) g^{-1} root of leguminous cover crops 12 weeks after application of different inoculum levels of *Meloidogyne* spp.

	Inoculum (J2/pot) Pi		
Crop	500	1,000	1,500
M. pruriens	46 (1.7) * a	126 (2.1) b	97 (2.0) a
D. lablab	338 (2.5) b	673 (2.8) c	859 (2.9) b
C. spectabilis	24 (1.4) a	49 (1.7) a	88 (1.9) a
LSD (ln(x+1)	(0.12)	(0.05)	(0.09)
P-value	(0.01)	(0.01)	(0.01)

Data are means of four replications. Mean separation was done using Tukey's multiple comparisons ($P < 0.01$).
* $\sqrt{(x + 1)}$ transformed data used in ANOVA in parenthesis
Pi = initial inoculum levels (500, 1,000, 1,500).

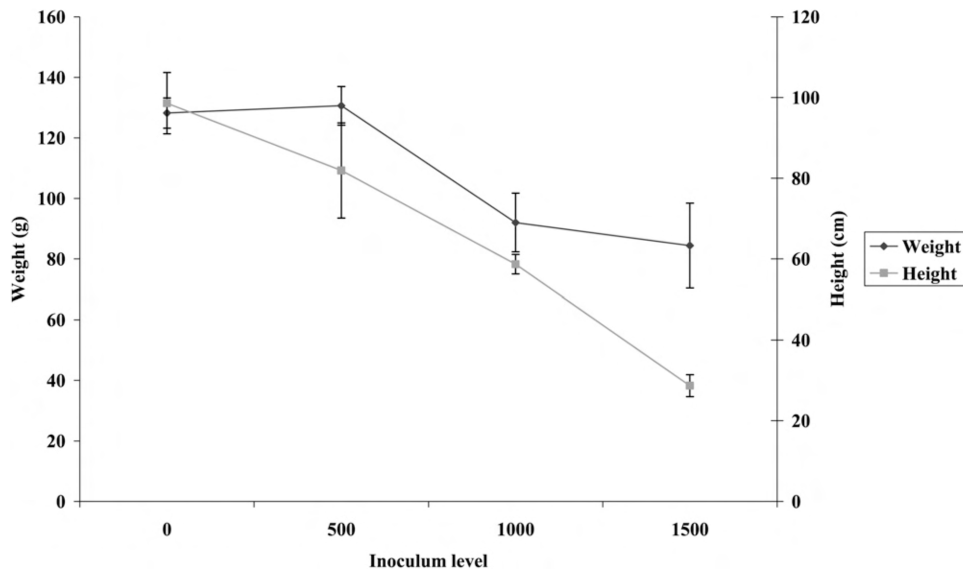

Fig. 1. Effect of different inoculum levels of *Meloidogyne* spp. on *Dolichos lablab* shoot height at 6 weeks and fresh weight after 12 weeks.

Discussion

Mucuna pruriens and *Crotalaria spectabilis* were shown to be effective in reducing a Ghanaian mixed population of *Meloidogyne arenaria, M. incognita* and *M. javanica* in the current study. Hitherto, previous workers had shown the efficacy of these cover crops against single species of the three *Meloidogyne* species in laboratory experiments. In the field, however, *Meloidogyne* species exist in mixed populations. It was against this background that McSorley and Dickson (1995) pointed out that a cover crop has to be effective against mixed populations of *Meloidogyne* spp. if this was to be a recommended management practice. Some *Meloidogyne* juveniles entered the roots but there was no galling response and egg masses did not develop. McSorley and Dickson (1995) and Quénéhervé et al. (1998) showed that *Mucuna deeringiana* was effective in reducing populations of *M. incognita* in Florida and Martinique, respectively, but *M. arenaria* and *M. javanica* did produce limited numbers of galls and egg masses in pot-grown *Mucuna* plants in Florida (McSorley et al., 1994). In Alabama, *C. spectabilis* reduced populations of *M. arenaria* (Rodríguez-Kábana et al., 1992a). McSorley et al. (1994) found no galls or egg masses on roots of pot-grown *C. spectabilis* in soil inoculated with Florida populations of *M. arenaria, M. incognita* and *M. javanica.* Potentially nematotoxic compounds such as monocrotaline, identified in *C. spectabilis* inhibited movement of *Meloidogyne* spp. juveniles (Fassuliotis and Skucas 1969). Nogueira et al. (1996) reported that triacontanol and tricontanyl tetracosanoate from leaves and roots of *Mucuna aterrima* prevented *M. incognita* hatching. From the results of these experiments, it would seem that use of cover crops is a promising management strategy for the mixture of *Meloidogyne* spp. found in Ghana. However, care must be taken in making such recommendations since not all cover crops will be effective. Although soil scientists and agronomists might favour the growth of leguminous cover crops for soil conservation, nitrogen fixation and their use as fodder, the results from this study indicate that it would not be wise to use *Dolichos lablab* or the bean *Phaseolus vulgaris* if *Meloidogyne* spp. are a threat in the cropping system. It must be pointed out that, the three cultures (*M. arenaria, M. incognita* and *M. javanica*) used might not have been a uniform mix throughout the studies and that individual species might have increased or decreased in the cultures over time.

Leguminous plants contain numerous chemicals some of which are potentially nematotoxic or can influence nematode behaviour. Our results have shown that the compounds extracted from leaves and roots and those released as exudates from roots influence hatching and

Table 3. Mortality (%) of a mixed *Meloidogyne* spp. juveniles placed in root and leaf eluants of leguminous crops and tomato over time. Numbers are mortality based on 100 juveniles.

	Incubation interval (hours)		
Crop (Root)	24	48	72
M. pruriens	21.7 (1.3) *	36.5 (1.6)	42.0 (1.6) bc
D. lablab	4.3 (0.6)	7.5 (0.9)	11.5 (1.1) a
C. spectabils	33.7 (1.5)	47.0 (1.7)	48.7 (1.7) d
Tomato	2.0 (0.3)	5.0 (0.7)	7.0 (0.9) cd
Crop (Leaf)			
M. pruriens	30.5 (1.5)	36.5 (1.6)	43.7 (1.6) cd
D. lablab	30.0 (1.5)	38.5 (1.6)	41.0 (1.6) bc
C. spectabilis	22.5 (1.4)	30.5 (1.5)	33.3 (1.5) b
Tomato	2.7 (0.4)	4.7 (0.7)	12.0 (1.1) a
LSD (ln(x+1)	(0.22)	(0.14)	(0.13)
P-value	(0.01)	(0.01)	(0.01)

Data are means of three replications. Mean separation was done using Tukey's multiple comparisons ($P < 0.01$).

* $\sqrt{(x + 1)}$ transformed data used in ANOVA in parenthesis

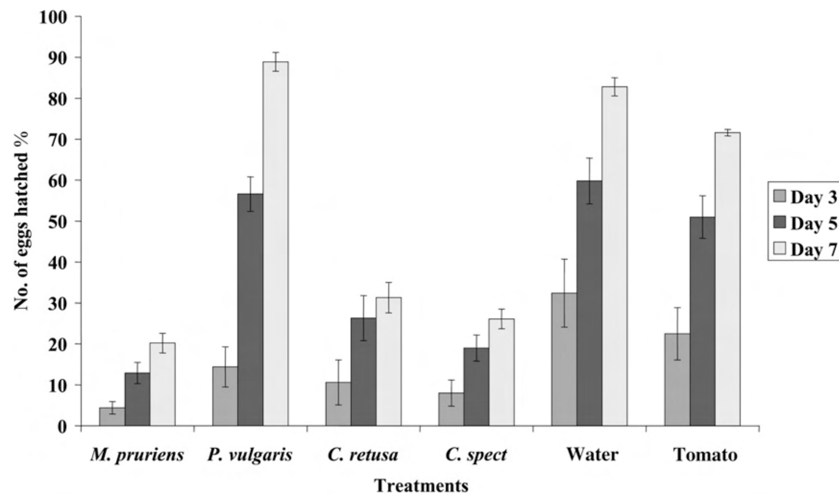

FIG. 2. Hatching of *Meloidogyne* spp. eggs in root exudates of leguminous cover crops over 3, 5 and 7 days. Data are means of four replications. Mean separation was done using Tukey's multiple comparisons at ($P < 0.01$).

mobility of the hatched juveniles. *Mucuna pruriens* and *C. spectabilis* suppressed hatching of *M. incognita* eggs. While only 20% and 26% of eggs hatched in *M. pruriens* and *C. spectabilis* exudates, significantly higher (83%, 72% and 89%) hatched in distilled water (control), tomato and *P. vulgaris* exudates, respectively. These results were in concert with the findings of Bharadwaj and Sharma (2007) in which no hatching occurred within 48 h when *M. incognita* eggs were placed into water soluble extract of *Ocimum sanctum* with the control treatment recording 35% hatching during the same period. Although these studies were done in confined Petri dish arenas in the laboratory, they show that these chemicals have some nematicidal activity which might explain the effects observed under field conditions. The identity of most of these chemicals and their precise modes of action are unknown (Ferraz and Grassi de Freitas, 2004).

It is recorded that an indirect effect of growing some leguminous cover crops is the development of a rhizosphere microflora which is antagonistic to plant parasitic nematodes (Kloepper et al., 1991; Vargas-Ayala et al., 2000). However, the decline in the *Meloidogyne* spp. in pots containing *Mucuna pruriens* and the *Crotalaria* species was less than that in the fallow (no plant control) suggesting that no such antagonistic effects occurred under these conditions. The fact that about 10% of the population survived the 3 month fallow explains why *Meloidogyne* spp. is such difficult pests to manage. The promising cover crops in this study could be allowed to go to seed where seed would be required for future use. Alternatively, the biomass could be ploughed into the soil at flowering time and the economic crop cultivated. Depending upon the nature of the economic crop, a mixed cropping system could be considered. In some cases, however, the cover crop could be planted first and either ploughed in or harvested before the economic crop is planted.

TABLE 4. *Meloidogyne* spp. juveniles in 5,200 cm^3 of rhizosphere soil 3 months after inoculation of pots sown with leguminous cover crops.

Crop	J2/ 5,200 cm^3 soil
M. pruriens	209 (2.3) * b
P. vulgaris	2,620 (3.4) c
C. retusa	153 (2.2) ab
C. spectabilis	145 (2.1) ab
Fallow	104 (2.0) a
LSD (ln(x+1)	(0.19)
P-value	(0.01)

Data are means of four replications. Mean separation was done using Tukey's multiple comparisons at ($P < 0.01$).

* $\sqrt{(x+1)}$ transformed data used in ANOVA in parenthesis

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