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Incorporation of Green Manure Plants into Bean Cropping Systems Contribute to Root-Knot Nematode Suppression

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Abstract: Green manure plants were evaluated to determine their suitability as rotation crops with common bean to suppress root-knot (*Meloidogyne* spp.) nematodes. They were also evaluated as soil amendments in nematode control. The plants were *Calliandra calothyrsus*, *Canavalia ensiformis*, *Chenopodium quinoa*, *Crotalaria juncea*, *Desmodium uncinatum*, *Gliricidia sepium*, *Leucaena leucocephala*, *Mucuna pruriens*, *Tephrosia purpurea*, *Tithonia diversifolia*, *Vicia villosa*, *Sesbania sesban* and *Tagetes minuta*. In the glasshouse, pots were filled with steam-sterilized soil and sown with green manure plants. The rotation experiment entailed growing green manure plants for three months before uprooting them and planting beans in the same pots. The potting medium was infested with 6000 eggs/juveniles of *Meloidogyne javanica*. The field experiments were carried out in microplots infested with a mixture of *M. javanica* and *M. incognita*. Damage to bean roots due to root-knot nematodes was based on galling indices while nematode reproductive potential was based on egg mass index. *Tithonia diversifolia*, *D. uncinatum*, *T. minuta*, *L. leucocephala* and *C. juncea* were among the most effective in root-knot nematode suppression when used in rotation with beans. Their galling indices ranged between 1.0 and 1.5 under field conditions and were thus considered resistant. *Vicia vilosa*, *T. purpurea* and *S. sesban* were susceptible with galling indices ranging between 6.2 and 7.7. The resistant plants reduced the reproductive potential of *Meloidogyne* spp. by up to 80% while the susceptible plants caused an increase of up to 600%. Therefore, *T. diversifolia*, *D. uncinartum*, *T. minuta*, *L. leucocephala* and *C. juncea* can be recommended for use in fields infested with root-knot nematodes.

Key words: *Meloidogyne* spp., rotation, amendment, galling, eggmass index

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is widely grown in Kenya and is the most common and widespread grain legume, serve as the main source of proteins to the majority of small-scale farmers (Karanja *et al*., 2007). In East Africa, common bean is the most important grain legume and has a relatively high commercial value in addition to nutrition (Otipa *et al*., 2006). The crop is grown in pure stands by large-scale farmers but it is generally intercropped with maize, sorghum, coffee and a wide range of other crops in small holdings (Kimenju *et al*., 2004). Among the major factors that constrain bean production are pests and diseases that cause huge losses in yields (Pérez and Lewis, 2004). Among the main pests contributing to these losses are root-knot nematodes (*Meloidogyne* spp.). Bean is highly susceptible to this pest with minimal chances of resistance (Thies *et al*., 2004). For this reason, economic losses can be very high and aggravated especially if the nematode infection is in synergy with other disease causing agents (Karanja *et al*., 2007). The nematodes interact with other soil-borne pathogens causing disease complexes that are devastating to beans. In addition nematode infection is also known to reduce nodulation by rhizobium in beans (Kimenju *et al*., 1999; Karanja *et al*., 2007). Root-knot nematode infections are also known to break host resistance to other pathogens such as fusarium wilt (Abawi and Widmer, 2000). Unfortunately, many of the options that are available for management of the nematodes are unacceptable to the largely resource-constrained small-scale bean grower (Desaeger and Rao, 2000).

Rotating beans with plants that can suppress nematodes offers an alternative to the use of expensive and environmentally unsafe nematicides. Some studies indicate positive results in this direction. For instance two-year rotations with *Mucuna deeringiana* increased kidney bean yields by up to 212% and were effective in controlling of *M. incognita* races 1 and 4 (Roberto *et al*., 2000). Reductions in root-knot nematode levels were reported in interplants of sorghum and sudangrasss (Kratochvil *et al*., 2004). Two-month rotations of *T. erecta* and tomato also reduced populations of

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Meloidogyne spp. (Sturz and Kimpinski, 2004). As green manures, marigolds reduced root-knot nematode populations where they were incorporated into infested soils (Wang *et al*., 2002). It is therefore evident that some green manure plants are likely candidates in mixed and sequential cropping systems for root-knot nematode management. Indeed in cereal-legume rotations and perennial systems investigated in southern Africa, which included improved fallows, interplanting and biomass transfer, the most promising legumes were *Tephrosia* spp. and *Sesbania* spp. (Snapp *et al*., 1998). However, their potential has to be determined under local conditions and populations of the target nematode pest. The main objective of this investigation was to evaluate the effect of alternating and interplanting green manure plants with common beans (*P. vulgaris)* on two root-knot nematode populations (*Meloidogyne javanica* and *M. incognita*).

MATERIALS AND METHODS

Greenhouse and field experiments were carried out in the period between August 2006 and April 2007 at the University of Nairobi farm, Kibwezi. Eleven green manure plants were tested for use as rotation and companion crops with as well as soil amendments to control root-knot nematodes. The plants tested were *Calliandra calothyrsus*, *Canavalia ensiformis*, *Chenopodium quinoa* Var. Narino, *Crotalaria juncea*, *Desmodium uncinatum*, *Leucaena leucocephala*, *Mucuna pruriens*, *Tephrosia purpurea*, *Tithonia diversifolia*, *Vicia villosa* and *Tagetes minuta.*

Greenhouse experiments: About 15 cm-diameter pots were filled with 2 kg of heat sterilized soil and ballast mixed in the ratio 4:1. Diammonium phosphate fertiliser was added at a rate of 5 g/pot. One month-old seedlings of *C. calothyrsus*, *L. leucocephala* and *T. diversifolia* were transplanted from nematode-free nurseries into the pots. The rest of the plants namely *C. ensiformis*, *C. quinoa*, *C. juncea*, *M. pruriens*, *V. villosa* and *T. minuta* were sown directly from seed. One seedling of each of the green manure plant was maintained in the same pot with a bean seedling in the interplanting experiment. Common bean cv GLP-2 alone was included as a control. Root-knot nematodes and juveniles eggs and were obtained from galled roots using the maceration technique described by Hussey and Barker (1973). The potting medium was infested with nematodes ten days after transplanting or emergence of seedlings. Six thousand eggs/juveniles of *M. javanica*, suspended in 10 mL tap water, were pipetted into indentations made around the base of the plants in each pot. The treatments were arranged in a completely randomized design with eight replications. Plants were lightly watered and the experiments were terminated after three months.

The modified Baermann funnel technique was used to extract second-stage juveniles from 200 cm^3 soil (Hooper *et al*., 2005). Galling was quantified using a scale of 1-9 where:

1 = 0 galls/eggmasses; 2 = 1-5 galls/eggmasses;
3 = 6-10 galls/eggmasses; 4 = 11-20 galls/eggmasses;
5 = 21-30 galls/eggmasses; 6 = 31-50 galls/eggmasses;
7 = 51-70 galls/eggmasses; 8 = 71-100 galls/eggmasses;
9 = 100 galls/eggmasses (Sharma, 2001).

For the rotation experiment, the green manure plants were grown for three months, harvested and then beans were grown in the same pots. The experiment was terminated after three months and assessment done on the beans. The modified Baermann funnel technique was used to extract second-stage juveniles from soil (Hooper *et al*., 2005). Galling and eggmass indices were determined as described earlier.

Field experiments: A field that was infested with a mixed population of *M. incognita* and *M. javanica* in the ratio of 1:3 was used. In the interplanting experiment, green manure plants were sown in nematode infested microplots measuring 1×2 m with six plants per row. The green manure plants were alternated with beans in each of the three rows consisting of six plants each. A pure bean stand was included as a control. Diammonium phosphate fertiliser was added at a rate of 5 g per planting hole. Plants were grown for three months after which the experiment was terminated. Treatments were arranged in randomized complete block design with four replications. The initial (P_1) inoculum in the soil was determined by randomly sampling the experimental block before planting. The count for final population (P_2) involved taking five soil samples from within five to 20 cm depth from each plot, mixing it thoroughly and then taking 200 cm^3 sub-samples for juvenile (J_2) extraction as described earlier.

In the rotation experiment, green manure plants were grown for three months, harvested and then beans planted into the same plots. Sampling for final nematode populations was done at termination of the experiment. Sampling for damage by nematodes, EMI and J_2 were determined following the procedures described above. The experimental design was as described earlier.

In the amendment test, green manure plants were grown in the field as described above then harvested after three months. The green manure plants were finely chopped and incorporated into the soil at the rate of 25 g per hole. Beans were then sown into the same holes. The experiment was terminated after three months and assessment done on the beans. GI, EMI and J_2 were determined as described earlier.

RESULTS AND DISCUSSION

Greenhouse experiments: Galling varied significantly (p≤0.05) among beans interplanted with green manure crops (Table 1). Four green manure plants led to a significant (p≤0.05) reduction in galling. These were *D. uncinartum*, *G. sepium*, *T. minuta* and *T. diversifolia*. The highest reduction in galling occurred when *T. minuta* and *T. diversifolia* were interplanted with beans. Two green manure plants, *V. vilosa* and *T. purpurea* led to a significant increase in galling on beans interplanted with them.

The differences in EMI of beans interplanted with green manure plants were significant (p≤0.05). With the exception of *C. quinoa*, *T. purpurea* and *V. vilosa*, all the other plants caused a decrease in EMI on beans (Table 1). The greatest decrease was observed when *M. pruriens* was used. *Mucuna pruriens* had the greatest effect in reducing the population of second-stage *Meloidogyne* juveniles in the soil. Other plants that significantly (p≤0.05) reduced the J_2 numbers were *C. juncea*, *T. minuta* and *T. diversifolia.* An increase in reproductive factor (Rf) was recorded in beans interplanted with green manure plants, with the exception of *D. uncinatum.*

The green manure plants, except *S. sesban*, *T. purpurea* and *V. vilosa* led to significant reduction in galling (Table 2). The highest reduction was recorded when *C. juncea* preceded beans. Significant differences (p≤0.05) were registered for EMI too. The lowest EM indices were recorded on beans grown after *G. sepium*, *T. diversifolia* and *D. uncinatum*. Five green manure plants led to significant reductions in *Meloidogyne* populations. These were *C. juncea*, *D. uncinrtum*, *L. leucocephala*, *T. minuta* and *T. diversifolia.* The highest reduction was under *T. minuta.* With the exception of *G. sepium* all the other plants led to increase in reproductive (Rf) potential of root-knot nematodes. The highest Rf was recorded under *V. vilosa.*

Field experiments: Galling varied significantly (p≤0.05) among beans rotated with different green manure plants (Table 3). With the exception of *C. calothyrsus*, *S. sesban*, *T. purpurea* and *V. vilosa*, the green manure plants reduced galling in succeeding bean crops. The plants that reduced galling most were *C. juncea*, *D. unicartum*, *L. leucocephala* and *T. diversifolia.* The scores for EMI

Table 1: Galling Indices (GI), Eggmass Indices (EMI) and Reproductive Factors (Rf) of *Meloidogyne javanica* on common beans interplanted with green manure plants in a glasshouse

Green manure plant	GI	EMI	Rf
Calliandra calothyrsus	4.1	3.5	1.6
Canavalia ensiformis	4.1	3.0	2.0
Chenopodium quinoa	4.8	6.2	3.0
Crotalaria juncea	3.7	3.8	0.8
Desmodium uncinartum	2.8	3.2	1.0
Gliricidia sepium	3.4	2.7	1.2
Leucaena leucocephala	3.8	3.3	1.2
Mucuna pruriens	5.5	2.2	0.5
Sesbania sesban	4.7	3.8	4.5
Tagetes minuta	2.2	2.5	0.9
Tephrosia purpurea	6.7	5.8	5.2
Tithonia diversifolia	2.7	2.5	0.8
Vicia villosa	8.2	6.7	5.7
Phaseolus vulgaris monocrop	4.8	5.7	3.0
LSD (p ≤ 0.05)	1.4	1.5	0.2
CV (%)	29.9	36.2	9.7

Table 2: Galling Indices (GI), Eggmass Indices (EMI) and Reproductive Factors (Rf) of *Meloidogyne* spp. on beans when planted in rotation with green manure plants in a glasshouse

	GI test		EMI test		
Green manure plant	I	II	I	II	Rf
Calliandra calothyrsus	4.50	3.90	4.70	4.10	1.60
Canavalia ensiformis	2.50	2.20	2.70	2.90	3.40
Chenopodium quinoa	3.70	4.40	5.30	5.70	3.50
Crotalaria juncea	1.00	1.20	1.70	1.20	0.70
Desmodium uncinatum	1.20	1.10	1.50	1.10	0.40
Gliricidia sepium	1.20	1.00	1.20	1.00	1.00
Leucaena leucocephala	1.20	1.00	1.30	1.00	0.30
Mucuna pruriens	2.20	2.10	2.70	2.40	2.30
Sesbania sesban	6.20	5.70	8.50	7.60	5.40
Tagetes minuta	1.30	1.60	1.50	1.70	0.20
Tephrosia purpurea	7.70	7.70	9.00	7.60	5.70
Tithonia diversifolia	1.00	1.40	1.20	1.50	0.60
Vicia villosa	6.20	7.00	9.00	7.90	6.00
Phaseolus vulgaris monocrop	4.50	5.60	9.00	7.40	4.40
LSD (p≤5)	0.25	0.23	0.23	0.21	0.34
CV (%)	8.10	7.40	5.40	5.80	13.50

followed the same trend as GI. With the exception of *C. calothyrsus* and *S. sesban*, all the other plants reduced numbers of second stage juveniles. *Mucuna pruriens* had the highest effect in reducing J_2 numbers.

Galling was significantly (p≤0.05) reduced in plants grown in soils amended with green manures of different species (Table 4). A part from *T. purpurea* and *V. vilosa* which had no significant effect, all the other organic substrates significantly reduced galling in beans. *Crotalaria juncea* had the highest reducing effect. With the exception of *V. vilosa*, all the other amendments significantly reduced the numbers and egg masses with *C. juncea* producing the greatest effect.

This study revealed that most of the green manure plants tested were suppressive to *M. javanica* and *M. incognita.* The main suppressants were *C. juncea*, *D. ncinartum*, *L. leucocephala*, *T. diversifolia* and *T. minuta.* Similar findings were reported by Wang *et al.*

Table 3: Galling Indices (GI), Eggmass Indices (EMI) and juvenile (J_2/200 cm^3) numbers on common bean (*Phaseolus vulgaris*), grown in rotation with green manure plants in a field infested with *Meloidogyne javanica* and *M. incognita*

Green manure plant	GI	EMI	J_2/200 cm^3
Calliandra calothyrsus	6.7	7.0	2683.0
Canavalia ensiformis	1.5	1.7	458.0
Chenopodium quinoa	3.2	3.7	330.0
Crotalaria juncea	1.0	1.0	368.0
Desmodium uncinartum	1.0	1.0	398.0
Leucaena leucocephala	1.2	1.2	353.0
Mucuna pruriens	1.5	1.5	214.0
Sesbania sesban	5.7	7.0	3165.0
Tagetes minuta	1.5	1.5	518.0
Tephrosia purpurea	7.0	8.0	1410.0
Tithonia diversifolia	1.2	1.2	1080.0
Vicia villosa	7.3	8.0	1155.0
Phaseolus vulgaris monocrop	6.8	8.0	2880.0
LSD ($p\leq 0.05$)	1.3	1.4	556.0
CV (%)	29.4	27.8	41.3

Table 4: Galling (GI) and Eggmass Indices (EMI) on beans grown in soils amended with green manure plants

	GI test		EMI test	
Green manure plant	I	II	I	II
Calliandra calothyrsus	3.0	2.0	4.0	3.0
Canavalia ensiformis	3.0	3.0	4.0	4.0
Chenopodium quinoa	2.0	3.0	2.0	4.0
Crotalaria juncea	1.0	2.0	1.0	2.0
Desmodium uncinatum	2.0	4.0	2.0	4.0
Leucaena leucocephala	2.0	4.0	2.0	4.0
Mucuna pruriens	2.0	2.0	2.0	3.0
Sesbania sesban	5.0	2.0	6.0	3.0
Tagetes minuta	2.0	2.0	2.0	2.0
Tephrosia purpurea	5.0	4.0	6.0	6.0
Tithonia diversifolia	2.0	4.0	3.0	4.0
Vicia villosa	4.0	6.0	5.0	8.0
No amendments	5.0	6.0	6.0	8.0
LSD ($p\leq 0.05$)	0.2	0.2	0.2	0.2
CV (%)	7.3	6.3	6.1	5.6

(2002, 2004), Sturz and Kimpinski (2004), Sikora *et al.* (2005) and Otipa *et al.* (2006). *Desmodium* spp. have been reported to be antagonistic to *Meloidogyne* spp. (Sikora *et al.*, 2005) and therefore considered resistant. Previous studies have shown that exudates of *D. ovalifolium* have an immobilizing effect on second-stage juveniles of *M. incognita* (Herrera, 1997).

The suppressive effect of *L. leucocephala* is manifested by inhibiting egg-hatch, infectivity and development of *M. incognita* (Otipa *et al.*, 2006). The leaves of *T. diversifolia* contain a poisonous compound that is a contact poison for most pests (Schösser *et al.*, 2006). *Tagetes minuta* contains compound that are toxic to nematodes. The active compounds isolated so far are thiophenes (Ploeg, 2002). This study showed that *M. pruriens* and *G. sepium* greatly reduced the reproductive potential of root-knot nematodes. *Mucuna pruriens* was noted to be very effective in reducing the population of root-knot nematodes but not as much in reducing damage to beans. *Gliricidia sepium* was also found to have a more mild antagonistic effect on *M. incognita* and *Radopholus similis* compared to *T. minuta* (Wang *et al.*, 2004). These plants have compounds that have insecticidal and rodenticidal properties that are mild compared to the highly resistant ones (Sikora *et al.*, 2005).

The study demonstrated that *S. sesban*, *T. purpurea* and *V. vilosa* should be avoided in nematode infested fields. *Sesbania sesban* is highly susceptible to root-knot nematodes and its continued cultivation has led to a build-up of the nematodes (Desaeger and Rao, 2000). Iwashina *et al.* (2004) showed that *T. purpurea* is susceptible to *Meloidogyne* spp. Vetches (*V. vilosa*) have been shown to be highly susceptible to *M. incognita* race 2 and *M. javanica* (Otipa *et al.*, 2006).

Some green manure plants were found to have intermediate effect in suppressing root-knot nematodes when used as interplant, rotation crops or amendments. These were *C. calothysrsus*, *C. ensiformis* and *C. quinoa*. Previous studies that came to similar conclusions include those of Thomas *et al.* (2005) and Desaeger and Rao (2000).

The green manure plants can be grouped into three categories in relation to their ability to suppress root-knot nematodes in bean cropping systems. The first group comprises of *C. juncea*, *D. uncinartum*, *L. leucocephala*, *T. diversifolia* and *T. minuta* which are resistant. The moderately resistant plants are *M. pruriens*, *G. sepium* and *C. quinoa*. The susceptible category is composed of *T. purpurea*, *S. sesban* and *V. vilosa*. The resistant plants are recommended in soils already infested to reduce nematode populations. The intermediate and susceptible plants may be used in soils not infested by root-knot nematodes. Progress toward sustainable agriculture should benefit on allelopathic nematode control (Sikora *et al.*, 2005). The incorporation of green manure legumes as a management tool for nematodes should be preceded by careful selection of the legumes based on their host status. Based on this study, there is need to evaluate growers acceptance of new crop rotation strategies based on economic considerations as well as efficiency in nematode suppression.

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