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Use of green manure plants in cropping systems to suppress root-knot nematodes

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Abstract: Green manure plants namely *Calliandra calothyrsus, Canavalia ensiformis, Chenopodium quinoa, Crotalaria juncea, Desmodium uncinartum, Gliricidia sepium, Leucaena leucocephala, Mucuna pruriens, Tephrosia purpurea, Tithonia diversifolia* and *Vicia villosa* were evaluated to determine their reaction to *Meloidogyne javanica*. *Sesbania sesban* and *Tagetes minuta* were included as susceptible and resistant checks respectively. In the glasshouse, pots were filled with steam-sterilized soil, sown with the green manure plant, and then infested with 4,000 eggs and juveniles of *M. javanica*. Field experiments were conducted in plots infested with a mixed population of *M. javanica* and *M. incognita. Crotalaria juncea, D. uncinartum, G. sepium, L. leucocephala, M. pruriens, T. diversifolia* and *T. minuta* had galling and eggmass indices lower than 2 and reduced nematode populations by up to 80%. *Calliandra calothyrsus, C. quinoa* and *C. ensiformis* had galling indices lower than 4 and eggmass indices less than 3.2. *Vicia villosa and T. purpurea* were susceptible with galling indices greater than 7 and nematode population buildup of up to 500%. *Chenopodium quinoa, C. juncea, D. uncinartum, G. sepium, L. leucocephala, M. pruriens and T. diversifolia* are suppressive to root-knot nematodes and can therefore be recommended as rotation, fallow or cover crops.

Introduction

Green manure plants play an important role in crop production as short-duration leguminous plants that increase soil fertility and yields of subsequent crops especially in nitrogen depleted soils. These plants are increasingly being adopted and evaluated for soil fertility improvement in Kenya (Szott *et al*., 1999). For example *Desmodium* spp., has been widely accepted as green plants in Western Kenya (Desaeger and Rao, 2000). The crop is highly palatable and makes such excellent forage that it has been termed as "the alfalfa of the tropics" (Skerman, 1977).

Root-knot nematodes are widely distributed in intensively cultivated land and may cause up to 80% losses in heavily infested fields so they have become major limiting factor on many commercial and subsistence crops. The parasites are a major hindrance to the use of green manure plants such as *Sesbania sesban* as cover crops (Kirachi, 1995). This is because some of them are highly susceptible to root-knot nematodes and cause rapid build-up when they are included in the cropping programs (Desaeger and Rao, 2000).

A few of the green manure plants are thought to have resistance or even immunity, which can be utilized in the management of plant parasitic nematodes. Available evidence shows that marigolds are antagonistic to root-knot nematodes (Yen *et al*., 1998). Compounds present in plants such as *Crotalaria juncea* and *T. minuta* are toxic to root-knot nematodes (Janic, 1996).

Many green manure plants and cover crops, however, have not been evaluated for their reaction to root-knot nematodes. Given the relative deficiency of data, farmers take considerable risk when introducing leguminous crops into their farming systems. It is this inadequacy that prompted the investigation with the objective of determining the reaction of green manure plants to *Meloidogyne* spp.

Materials and Methods

Green manure plants were evaluated to determine their reaction to root-knot nematodes under glasshouse and field conditions. The tested plants were, *Calliandra calothyrsus, Canavalia ensiformis, Chenopodium quinoa* var. narino, *Crotalaria juncea, Desmodium uncinartum, Gliricidia sepim, Leucaena leucocephala, Mucuna pruriens, Tephoria purpurea, Tithonia diversifolia* and *Vicia vallosa. Tagetes minuta* and *Sesbania sesban* were included as resistant and susceptible checks, respectively.

Fifteen-cm-diameter pots were filled with heat sterilized soil + ballast (4:1) mixture. Fertiliser (diammonium phosphate) was added at a rate of 5g/pot or into each planting hole for field experiments. One month-old seedlings of *G. sepium*, *C. sepium*, *C. calothyrsus L. leucocephala*, *T. diversifolia* and *S. sesban* were transplanted from nematode-free nurseries into the pots. The rest of the plants namely *C. ensiformis, C. quinoa, C. juncea, M. pruriens, and T. minuta* and *V. villosa* were sown directly from seed. The glasshouse experiments were arranged in a completely randomized design with eight replications while the field micro-plots were arranged in randomized complete block design with four replications.

Galling index (GI) was scored using a scale of 1-9 where: 1 = 0 galls/egg masses; 2 = 1-5; 3 = 6-10; 4 = 11-20; 5 = 21; 6 = 31-50; 7 = 51; 8 = 71-100; 9 = > 100 galls/eggmasses. Eggmass index (EMI) was scored using eggmass numbers (Sharma *et al*, 1994).

Green manure plants were sown in nematode infested micro-plots measuring 1 x 2 m consisting of 3 rows each with six plants. Root-knot nematode infestation in the field comprised of about 80% *M. javanica* and 20% *M. incognita*. Plants were grown for three months after which the experiment was terminated. The initial (Pi) inoculum in the soil was determined by randomly sampling the experimental block. The modified Baermann funnel technique was used to determine the juvenile counts in the soil (Hooper, 1990). The count for final population (P_2) involved taking soil from within 5 to 20 cm depth, mixing it thoroughly and then taking 200 cm^3 sub-samples from each plot for juvenile counts.

Results

Differences in galling were significant (P≤0.05) among the tested green manure plants (Table 1). The highest galling index of 8.9 was recorded on *V. villosa*, followed by *T. purpurea* with a galling index of 7. Moderate galling was observed on *C. calothyrsus* and *C. quinoa* but it was significantly (P≤0.05) higher than in *T. minuta*. The galling index scores of *C. ensiformis, C. juncea, D. uncinartum, G. sepium, L. leucocephala, M. puriens* and *T. diversifolia* were below 2.0. Eggmass index (EMI) scores differed significantly (P≤0.05) among the plants tested and the trend was similar to that of galling (Table 1).

Table 1: Galling indices (GI) and eggmass indices (EMI) on green plants grown under glasshouse conditions.

Green manure plant	GI Test			EMI Test		
	I	II	Mean	I	II	Mean
Calliandra calothyrsus	4.5	2.2	3.4	4.2	2.1	3.2
Canavalia ensiformis	1.0	2.2	1.6	1.0	2.1	1.6
Chenopodium quinoa	1.7	2.7	2.2	1.5	2.5	2.0
Crotalaria juncea	1.0	2.7	1.8	1.0	2.3	1.6
Desmodium uncinartum	2.2	1.0	1.6	2.2	1.0	1.6
Gliricidia sepium	2.0	1.0	1.5	1.5	1.0	1.2
Leucaena leucocphala	1.2	1.0	1.1	1.0	1.0	1.2
Mucuna pruriens	1.0	2.2.	1.6	1.0	1.7	1.3
Sesbania sesban	8.7	7.5	8.1	8.7	8.1	8.4
Tagetes minuta	1.0	1.0	1.0	1.0	1.0	1.0
Tephrosia purpurea	6.2	7.7	7.0	5.4	7.4	6.4
Tithonia diversifolia	3.0	1.0	2.0	1.5	1.0	1.2
Vicia villosa	8.9	8.9	8.9	8.9	9.0	8.9
LSD (P≤0.05)	0.3	0.2	0.9	0.2	0.2	0.8
CV %	7.8	5.0	27.9	6.4	7.4	26.6

Variable numbers of *Meladogyne* juveniles (J_2) were recovered from pots where the green manure plants were grown (Table 2). The juvenile numbers were greater than 1000 in soils grown with *C. ensiformis, T. purpurea* and *V. villosa*. The highest and lowest juvenile numbers were associated with *V. villosa* and *T. diversifolia*, respectively. An increase in nematode numbers, Rf > 1, was recorded in the rhizosheres of *C. ensiformis, T .purpurea* and *V. villosa*. The highest increase in nematode population was recorded in the rhizosphere of *V. villosa* (Rf = 5.4 times). With the exception of *C. quinoa*, all other plants caused a decrease in juvenile numbers exemplified by reproductive factors of less than one.

Table 2. Numbers of *Meloidogyne* juveniles (J_2) and reproductive factors (Rf) on green manure plants grown for three months under glasshouse conditions.

Green manure plant	J_2 Test		Mean	Rf
	I	II		
Calliandra calothyrsus	300	510	405	0.9
Canavalia ensiformis	1248	1020	1134	1.1
Chenopodium quinoa	780	525	652	1.0
Crotalaria juncea	765	268	516	0.5
Desmodium uncinartum	1050	488	769	0.7
Gliricidia sepium	525	150	337	0.3
Leucaena leucocephala	130	238	184	0.2
Mucuna pruriens	675	150	412	0.4
Sesbania sesban	850	525	687	4.0
Tagetes minuta	300	120	210	0.2
Tephrosia purpurea	1137	1120	1128	3.2
Tithonia diversifolia	168	120	144	0.2
Vicia villosa	600	2897	1748	5.4
LSD (P≤0.05)	40.64	29.9	257.1	0.16

The variability in the damage caused by the nematodes was significant among the green manure plants tested under field conditions (Table 3). Severe galling of over 7 on a scale of 1-9 was observed on *V. Villosa* and *T. purpurea*. Minimal damage was noted on *M. pruriens*, and the GI scores were lower than on any of the three aforementioned.

Table 3. Galling indices (GI), eggmass indices (EMI) and second-stage juvenile (J_2) numbers on green manure plants grown in a field infested with *Meloidogyne javanica* and *M. incognita*.

Green manure plants	GI	EMI	J_2	Rf
Calliandra calothyrsus	4.0	4.0	926	1.6
Canavalia ensiformis	1.2	1.2	195	0.2
Chenopodium quinoa	1.0	1.0	1705	1.4
Crotalaria juncea	1.2	1.2	214	0.8
Desmodium uncinartum	1.2	1.2	720	0.6
Leucaena leucocephala	1.0	1.0	319	0.2
Mucuna pruriens	1.7	1.7	1403	1.2
Sesbania sesban	7.7	7.5	1181	3.7
Tagetes minuta	1.0	1.0	461	0.8
Tephrosia purpurea	8.5	8.0	3740	6.3
Tithonia diversifolia	1.0	1.0	780	0.6
Vicia villosa	7.7	8.0	1743	4.7
LSD (P≤0.05)	1.6	1.5	644	0.4
CV %	38.6	35.9	37.1	16

Differences in eggmass indices (EMI) were significant among the tested plants. Scores above 7.5 were observed on *V. Villosa*, *T. purpurea* and *S. sesban.* Intermediate eggmass index score (4) was recorded on *C. calothyrsus.* There were few eggmasses on *C. calothysus* with an average of 4 and *M. pruriens* with a low of 1.7. The other EMI scores were not significantly different from *T. minuta.*

Signifigant (P≤0.05) differences in juvenile numbers were observed. High (> 1400) J_2 counts were associated with *V. Villosa*, *Tephrosia purpurea*, *M. pruriens*, and *C. quinoa.* (Table 3). Minimal nematode population increase was recorded on both *C. quinoa* and *M. pruriens* with reproductive factors ranging from 1.2-1.4. Nematode population reductions ranging from 20 to 80% were associated with *C. ensiformis, C. juncea, D. uncinartum, L. leucocephala, T. minuta* and *T. diversifolia*.

Discussion

This study has established that seven green manure plants namely *C. ensiformis, Crotalaria juncea, D. uncinartum, G. sepium, L. leucocephala, M. prupiens,* and *T. diversifolia* were not significantly damaged compared to *T. minuta,* the resistant check. They were also found to have a negative effect on reproduction of root-knot nematodes. It is therefore evident that the seven are resistant to root-knot nematodes *M. javanica* and *M. incognita.* The findings are consistent with earlier studies which demonstrated that these plants are resistant to root-knot nematodes (Herrera, 1997; Asmus and Ferraz, 1998). The root extracts of *C. ensiformis, C. quinoa, C. juncea, D. uncinartum, L. leucocephala, T. minuta and T. diversifolia* caused paralysis in the second-stage juveniles of the nematodes. This confirms earlier reports that the low nematode populations associated with these plants was a result of the active ingredients that they release (Herrera, 1997; Asmus and Ferraz, 1998).

Minimal damage was noted on *C. calothyrsus* and *C. quinoa*, indicating that they were poor hosts of *M. javanica* and *M. incognita* when compared to either *V. villosa* or *T. purpurea.* High juvenile counts were also associated with *C. ensiformis* meaning that though it may not be adversely affected, its effect on overall nematode population was minimal. It may not be a favourable choice for nematode management as an interplant or in rotations. According to McSorley *et al*. (1994), *C. ensiformis* was an intermediate host compared to tomato (cv Rutgers). Thus *C. calothyrsus, C. quinoa* and *C. ensiformis* may be classed together as intermediate hosts, because even their root extracts showed low levels of juvenile immobilization. However, with proper management, they could be used as trap crops but they must be uprooted before nematodes complete their life cycles resulting in population buildup.

Severe damage by nematodes was recorded on roots of *V. villosa* and *T. purpurea*. This clearly shows that the plants are highly susceptible to the root-knot nematodes *M. javanica* and *M. incognita.* The findings confirm earlier reports indicating these plants are good hosts of root-knot nematodes (Saka, 1991; Guertal *et al*, 1998). The plants should be avoided in cropping systems where root-knot nematodes are potential threats.

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