EFFECT OF GREEN MANURE PLANTS ON ROOT KNOT NEMATODES INFECTING COMMON BEANS

Kagundu Abed Mathagu

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DECLARATION

This thesis is my original research work and has not been presented for a degree in any other university

Kagundu, A. M.__________ **Date** 23-9-2002

This thesis has been submitted for examination with our approval as university supervisors

Dr. J. W Kimenju__________ **Date**__________

Dr. J. H Nderitu__________ **Date** 2/10/2002

DEDICATION

To my dear friend Sarah Ruth

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF APPENDICES

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ABSTRACT

Green manure plants are increasingly being adopted for soil fertility management especially in low-input agriculture. Information on their reaction to plant parasitic nematodes is, however, scanty. Green manure plants (*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* and *M. incognita.*

Their effects were evaluated in interplant, rotation and as amendments, under glasshouse and field conditions.

Sesbania sesban and *Tagetes minuta* were included as susceptible and resistant checks, respectively. In the glasshouse, pots filled with steam sterilised soil was infested with 10,000 eggs and juveniles (J_2) of *M. javanica.* Completely randomised design with eight replications was used. The field experiments were conducted in a plot infested mainly with *M. javanica* and *M. incognita* and treatments were arranged in a randomised complete block design with four replications. Ninety days after planting, galling and egg masses were assessed on a scale of 1-9. Second stage juveniles were extracted from the soil using the modified Baermann funnel technique.

Differences in galling indices, egg mass indices and juvenile populations were significant among the green manure plants evaluated. The green manure plants were grouped into three categories with *V. villosa, S. sesban* and *T. purpurea* being rated as susceptible and having little or no effect on nematode populations and damage when used as soil amendments. *Calliandra calothyrsus, C quinoa* and *C. ensiformis* had galling indices lower than 3 and low reproductive factors and were placed in an intermediate category. They also resulted in intermediate effect on nematodes when used in rotation or interplanted with beans. All the other green manure plants (*C. juncea, D. uncinartum, G. sepium, L. leucocephala* and *T. diversifolia*) had low galling indices and were not significantly different from *T. minuta*. They also reduced nematode damage and reproduction in rotation and interplant systems and when incorporated in the soil as green manures. Damage by nematodes and *Meloidogyne* juvenile numbers were highest on *V. villosa* and lowest on *T. diversifolia*, whereas high reduction in populations and damage on beans were observed where *C. juncea* was grown. *Crotalaria juncea*, and *T. diversifolia* can therefore be recommended for incorporation in cropping systems for root-knot nematode management in fields with high infestation.

CHAPTER 1
INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is widely grown in Kenya and is the most common and widespread grain legume (Stoetzer, 1981). It is second to maize in production (MOA, 1986; Allen *et al.*, 1996). The most common varieties include GLP-2 (Rosecoco), adapted to between 750mm and 1000mm of rainfall, and GLP-24 (Canadian Wonder) adapted to 500 – 750 mm (Muigai and Ndegwa, 1991). GLP-2 is among the most popular cultivars because of its wide adaptability and good yield (Kimani *et al.*, 1990).

Per capita bean consumption and its contribution to nutrition is highest in the African "great lakes" region where it provides one third of the total protein intake and one eighth of the total calories (Redhead *et al.*, 1983; CIAT, 1989). Beans have a relatively high commercial value in addition to nutrition (Kirkby, 1998).

Beans are highly susceptible to root knot nematodes (*Meloidogyne spp.*) and economic losses can be immense especially if nematode infection is in synergy with other disease causing agents (Ngundo and Tayor, 1975). Its susceptibility has been equated to that of squash, *Cucurbita pepo* (McSorley *et al.*, 1994) and overall losses of up to 60% have been reported (Allen *et al.*, 1996). The most common species of root knot nematodes in Kenya are *Meloidogyne incognita* (Kofold and White) Chitwood, *M. javanica* (Treub) Chitwood and *M. hapla* Chitwood (Gichure and Ondieki, 1984; Njuguna and Bridge, 1998).

They are important not only as independent plant disease agents but they are also associated with fungal, bacterial and viral pathogens, aggravating damage caused by diseases such as Fusarium wilt, caused by *Fusarium oxysporum f.sp. phaseoli* (Allen *et al.*, 1996), and bean rust, caused by *Uromyces appendiculatus* (Bookbinder and Bloom, 1980). In such disease complexes, nematodes act as initiators or synergists of fungal and bacterial diseases (Mace *et al.*, 1981). Despite such problems caused by root knot nematodes, chemical control is difficult in many agricultural systems especially because, many small scale farmers cannot afford nematicides (Desaeger and Rao, 2000).

Green manure plants such as *Leucaena leucocephala* (Lam.) de Wit., *Sesbania sesban* L., *Mucuna pruriens* (L.) DC and *Canavalia ensiformis* (L) DC. play an important role in crop production as short-duration leguminous plants that increase soil fertility and subsequent crop yields in N-depleted soils (Szott *et al.*,1999). Other legumes with potential for soil fertility improvement are gaining popularity among scientists and farmers (Rao *et al.*, 1998). *Canavalia ensiformis,* for instance, has been shown to increase yields of sweet potato and to reduce root knot nematode populations (Espindola *et al.*, 1998).

However, information on the reaction of green manure plants to root knot nematodes which are a major limiting factor on many commercial and subsistence crops in Kenya and the dynamics of the *Meloidogyne spp.* in cropping systems where the green manure plants have been grown is inadequate (MOA, 1986).

Root knot nematodes are a major hinderance to the use of plants such as *Sesbania sesban* as cover crops (Karachi, 1995). Nematode populations indeed build up rapidly when such a crop is included in the cropping program (Desaeger and Rao, 2000). Nitrogen fixing (leguminous) plants are very susceptible to root knot nematodes (Cadet *et al.*, 1996) and given the relative deficiency of data, farmers assume a significant amount of risk when introducing leguminous crops into their farming systems (Wolf, 1994). Many green manure crops and cover crops, however, have not been evaluated for their reaction to root knot nematodes (Wolf, 1994). *Chenopodium quinoa,* for example, may act as a trap crop for *Globodera spp.* (Mian *et al.*, 1999). Compounds present in such plants as *Crotalaria juncea* L. are also toxic to root knot nematodes (Janick, 1996), whereas exudates from *Desmodium ovalifolium* immobilise juveniles of *Meloidogyne incognita* (Herrera, 1997). Green manure plants are also increasingly being adopted and monitored for soil nutrient improvement in Kenya (Szott *et al.*, 1999).

Information about the dynamics of soil-borne diseases and nematodes in cropping systems where green manure plants have been incorporated is inadequate and the reaction of these green manure plants to *Meloidogyne spp.* remains little known.

Overall objective

This study was undertaken with the aim of incorporating green manure plants in cropping systems for root knot nematode management.

Specific objectives of the study were:

- To determine the reaction of green manure plants to root knot nematodes (*Meloidogyne javanica* and *M. incognita*).
- To determine the effect of rhizosphere interactions of green manure plants and common beans on root knot nematodes.
- To determine the effect of rhizosphere interactions of green manure plants and common beans (*P. vulgaris*) on root knot nematodes (*Meloidogyne javanica* and *M. incognita*).

CHAPTER 2
LITERATURE REVIEW

2.1 Common bean, *Phaseolus vulgaris* L.

2.1.1 History

Common bean, *P. vulgaris* L. belongs to the class, Dicotyledonae, subclass Rosidae, order Fabales, family Fabaceae or Leguminoseae-Papilionaseae and genus *Phaseolus* (Holmes, 1986). The genus includes about 30 species of which about ten are cultivated crops (Martin, 1984).

Sanchez (1996) traced back and authenticated the centres of origin and domestication of beans to the Andes (Bolivia, Colombia, Ecuador, Peru and Venezuela) and Meso-America (Argentina and Mexico). In a collection of 112 bean populations, character cluster analyses also revealed correlations to the two centers of origin (Sanchez, 1996). Gutierrez *et al.* (1999) used biochemical markers and found the same relationship, confirming the origin of the beans beyond doubt.

It is now grown primarily in tropical low-income countries with Sub-Saharan Africa producing 24% of the world production (Kirkby, 1998). This is only second to Latin America where the bean originated. The major production areas include, Brazil, Mexico and the eastern African highlands (Kirkby, 1998). In Kenya beans are grown mostly in association with other crops (Muthamia *et al.*, 1990, Wortman and Allen, 1994).

2.1.2 Importance of beans

Common bean is sown in pure stands by large-scale farmers, but it is generally intercropped with maize in small holdings (Redhead *et al.*, 1983, Allen *et al.*, 1996). In Kenya it is grown in association with crops such as maize, sorghum and coffee (Muthamia *et al.*, 1990; Wortmann and Allen, 1994). It is the main source of proteins to a majority of Kenyans especially small-scale farmers (Wortmann and Allen, 1994) and it is the most important grain legume in E Africa (Redhead *et al.*, 1983).

Per capita bean consumption and its contribution to nutrition is highest in the African "great lakes" region where it provides one third of the total protein intake and one eighth of the total calories (CIAT, 1989). Beans have a relatively high commercial value in addition to nutrition (Kirkby, 1998). In Kenya it is the most common and widespread grain legume, being second to maize in consumption and production (Stoetzer, 1983; MOA, 1986)

2.1.3 Constraints to bean production

Pests and diseases are major constraints in bean production and cause immense yield reductions (Muigai and Ndegwa, 1991; Wortman and Allen, 1994). The most economically important diseases include, angular leaf spot (*Phaeoisariopsis griseola*), anthracnose (*Colletotrichum lindemuthianum*), halo blight (*Pseudomonas syringae pv. phaseolicola*) and bean rust, *Uromyces appendiculatus* (Stoetzer, 1981; Gathuru and Mwangi, 1991). The most common pests on the other hand, include bean stem maggot, *Ophiomyia phaseoli* (bean fly), bruchids (*Acanthoscelides obtectus*), aphids (*Myzus spp.*) and mites, *Tetranychus spp.* (Stoetzer, 1981; Nderitu *et al.*, 1990).

Low soil fertility has been identified as one of the factors limiting bean production in Kenya (Muigai and Ndegwa, 199). However, most of these varieties (including GLP – X – 27a, Kat. B1 and F4 and F5 lines that are resistant to diseases, pests and nematodes) have not been tested for farmer acceptability or are not readily available to them (Muigai and Ndegwa, 1991; Rao *et al.*, 1998).

Common bean is highly susceptible to nematode attack (Kirkby, 1998). However, of the numerous plant-parasitic nematodes associated with bean roots and soils, only the species of *Meloidogyne* and *Pratylenchus* genera are frequently and consistently found on beans in relatively high densities (Abawi and Jacobsen, 1984; Allen *et al.*, 1996; Kimenju *et al.*, 1999).

Differential hosts and cytogenetic analyses have shown four races of *Meloidogyne incognita,* two races of *M. arenaria*, and one each of *M. javanica* and *M. hapla*, to be commonly associated with common bean roots (Mai and Lyon, 1975). In the tropics and subtropics, the most common are, *M. incognita* and *M. javanica* (Taylor and Sasser, 1978; Sasser and Kirkby, 1979).

In a study of 20 breeding lines, all of them were found to be highly susceptible to *M. javanica* with leaf and stem galls being observed on common bean(*P. vulgaris)* cv. Rico 23 (Sharma and Guazelli, 1982). Sixteen cultivars evaluated for resistance to *M. javanica* and *M. incognita*, showed that none of the cultivars was resistant or immune (Moura *et al.*, 1997).

In southern Africa, beans and other legumes were evaluated to determine their reaction to *Meloidogyne spp.* and most selections were good hosts of *M. hapla, M. javanica* and races 1 and 3 of *M. incognita* (Saka, 1991). In western Africa, Lamberti *et al.* (1998) found *M. arenaria, M. incognita* and *M. javanica* associated with *Phaseolus* beans in a survey in the region. Campos and Campos (1996) confirmed that *M. incognita* race 2 was a pest on common bean.

In eastern Africa, a survey of nine districts, considered *Meloidogyne spp.* to be the most important parasite among other nematodes in bean fields (Bafokuzara, 1996). In similar studies, 23 local and imported bean cultivars were evaluated for resistance to *M. incognita* and *M. javanica*. None of the cultivars was immune (Ngundo, 1977).

Besides, it is known that interaction between the host crop and the nematodes reduces nodulation by rhizobium in leguminous plants (Chahal and Chahal, 1991; Saka, 1991; Kimenju *et al.*, 1999). Root knot nematode infections also break host resistance to other pathogens such as Fusarium wilt, caused by *Fusarium spp.* (Ribeiro and Feraz, 1983; Singh and Reddy, 1981), rhizoctonia root rots and bean rust, caused by *Uromyces appendiculatus* (Bookbinder and Bloom, 1980).

2.2 Root knot nematodes, *Meloidogyne spp.*

Root knot nematodes belong to the phylum Nematoda, order, Tylenchida, suborder, Tylenchina, Subfamily, Heteroderoidea, family, Heteroderidae and genus *Meloidogyne*. "Root knot nematodes" is the common name for *Meloidogyne spp.* which cause galls on many plant roots and sometimes leaves (Louis, 1982). They are endoparasitic and mature females are sedentary (Louis, 1982).

The adult male and female root knot nematode are easily distinguishable morphologically. The males are wormlike and about 1.2 -1.5mm long by 30 - 36μm in diameter while the females are pear - shaped and 0.4 - 1.3 mm long by 0.27 - 0.75mm wide (Sherf and Macnab, 1986). Each female lays approximately 300-500 eggs in a gelatinous substance produced by the nematode (Agrios, 1988).

The first - stage larva develops inside each egg and after undergoing the first molt within the egg, it becomes second - stage larva (Taylor and Sasser, 1978). The latter emerges from the egg into the soil, where it moves until it finds a susceptible root (Taylor and Sasser, 1978). A pair of cephalic sense organs, the amphids are the perceptors, which the nematodes use for the detection of the presence of root exudates or excretions and other chemicals in the soil (Melakeberhan *et al.*, 1985).

The second stage juveniles of *Meloidogyne spp.* are the only infective stage (Agrios, 1988). Inside the plant tissue, the second stage juveniles molt into third stage juveniles which undergo a final molt into fourth stage juveniles, which can be distinguished as either male or female (Taylor and Sasser, 1978). A male fourth stage juvenile becomes wormlike and is free - living in the soil while, the fourth stage female juvenile continues to swell and with or without fertilisation by males, it produdes eggs (Taylor and Sasser, 1978). Males are not essential for reproduction; indeed *M. incognita, M. javanica* and *M. arenaria* have been found to be exclusively parthenogenetic (Bichir and Dalmasso, 1979). A life cycle is completed within 25 days at 27^0C, but may take longer or shorter at other temperature levels (Agrios, 1988; Sherf and MacNab, 1986).

2.2.1 Disease development

The ability of root knot nematodes to move on their own is limited, but they can be spread by water or by soil adhering onto farm equipment or otherwise transported into uninfested areas (Allen *et al.*, 1996). Infective second stage larvae enter roots behind the root tips by inserting their stylet and secreting saliva into the plant cells (Agrios, 1988). They push their way between the cells until they reach positions behind the growing tip. There they become sedentary and cells around the head of the nematode begin to enlarge (Sherf and Macnab, 1986).

Cell nuclei divide but no cell walls are laid and the existing walls between some of the cells break down and disappear (Ngundo and Taylor, 1975). The protoplasmic contents of several cells coalesce, giving rise to giant cells (Agrios, 1988). The enlargement of the cells seems to be brought about by substances contained in the saliva secreted by the nematode to plant cells during feeding (Wilcox and Loria, 1986). The giant cells degenerate when nematodes cease to feed or die. Xylem element may be crushed by the mechanical pressure exerted by the enlarging cells. Swelling of the roots also results from hypertrophy and hyperplasia of the vascular parenchyma, pericycle and the epidermal cells surrounding the giant cells (Wilcox and Loria, 1986).

2.2.2 Symptoms caused by *Meloidogyne spp.*

Root knot nematodes damage plants by devitalising root tips and either stopping their growth or causing excessive root production, but the presence of galls on the root system is the primary symptom associated with *Meloidogyne spp.* (Wilcox and Loria, 1986). The above-ground symptoms are however similar to those caused by many other root diseases and environmental factors that result in decreased efficiency in the absorption of water and nutrients by the plant (Agrios, 1988). Severely infected plants show chlorosis, stunting, necrosis of leaf margins and excessive wilting during periods of moisture stress (Melakeberhan *et al.*, 1985). Blossoms and fruits are either lacking or are dwarfed and of poor quality (Agrios, 1988).

2.3 Mechanisms of nematode resistance in antagonistic plants

Nematode antagonistic plants act in various ways to suppress infection by nematodes (Allen *et al.*, 1996). Pre-infectional mechanisms are effected through structural characteristics that act as physical barriers (Agrios, 1988). Xylem bundle sheaths and sclerenchyma cells of the leaf veins have been shown to effectively block the spread of some nematodes as well as fungal and bacterial pathogens (Agrios, 1988).

Biochemical reactions that take place in the cells and tissues of the plants also produce substances that are toxic to the nematodes and create conditions that inhibit their growth (Sequeira, 1983). Antagonistic plants also exude a variety of substances, through the surfaces of their roots and other parts (Topp *et al.*, 1998). This is the case with *Tagetes spp.* that contain thiophenes in their roots, which are toxic to root knot nematodes (Topp *et al.*, 1998).

Post-infectional mechanisms include production of toxic compounds in response to attack by the nematodes, majority of which are phenols (Bridge, 1996). In this way some plants such as *Crotalaria spp.* allow invasion by the nematodes but the juveniles do not grow to maturity (Esparrago *et al.*, 1999).

Resistance may also depend upon the fact that not every plant contains substances necessary for the development and reproduction of certain nematodes species or contain them in insufficient amounts (Giebel, 1982). This form of resistance is expressed by the failure of females to reach maturity as reported by Valle *et al.* (1997).

2.4 Responses of green manure plants to plant parasitic nematodes

Leguminous plants are generally susceptible to root knot nematodes (Cadet and Sengor, 1996) and given the relative deficiency of data, farmers assume a significant amount of risk when introducing leguminous fallow crops in their farming systems (Wolf, 1994).

Calliandra calothyrsus Meissn. is used as a forage crop and in soil improvement. It contains up to 22% protein, hence its suitability for forage (Halliday and Nakao, 1983). It has been reported to harbour root knot nematodes (Halliday and Nakao, 1983). In a more recent study, *C. calothyrsus* exhibited a degree of root galls similar to that on stylo (*Stylosanthes guianensis* and *Desmodium spp.*, but it did not contribute to build-up of nematode populations (Desaeger and Rao, 1999).

Jack bean (*Canavalia ensiformis*) is useful as a green manure as it fixes nitrogen (Uemura *et al.*, 1997) and as medicine in Asia (Kathiravan and Ignacimuthu, 1999). The pod is used as human food and animal feed substitute (Costa *et al.*, 1995). Its use as human food is limited by the presence of a toxic principle, con - canavalin A (Con-A) in its seed (Belmar *et al.*, 1999). Con- A is confirmed to be a plant antibody (lectin) that protects the plant from diseases and pests (Costa *et al.*, 1995; Boscan and Godoy, 1998; Gatehouse *et al.*, 1999; Vieira *et al.*, 1999).

When used as a green manure, jack bean significantly increased yields of sweet potato and reduced root knot nematode populations (Espindola *et al.*, 1998). McSorley *et al.* (1994) reported that jack bean exhibited an intermediate level of galling and egg mass production compared to tomato (cv. Rutgers) and okra (cv. Clemson Spineless) in response to *M. javanica*, *M. incognita* races 1 and 3 and *M. arenaria* race 1.

Chenopodium quinoa is a tropical flowering herb, common in the tropics and sub-tropics (Vieira *et al.*, 1999). The seed is used as human food while the foliage is used as forage (Vieira *et al.*, 1999). Studies on medicinal plants showed that *C. album* var. Centrorubrum was susceptible to *Meloidogyne spp.* (Gray *et al.*, 1997, Kim-HyeongHwan *et al.*, 1998). Holz *et al.* (1998) also recovered second stage juveniles from soils supporting *C. quinoa*.

Leaf extracts of *C. anthelminthicum* among other plants, resulted in high mortality of the root knot nematode, *M. incognita* (Mahmood *et al.*, 1982). Four lines of *C. quinoa* tested also acted as trap crops for *Globodera spp.* (Mian *et al.*, 1999). *Chenopodium ambrisioides* on the other hand showed higher antagonistic effect against *M. javanica* in the field compared to *Mucuna spp.* (Asmus and Ferraz, 1998).

Crotalaria juncea is a plant that is most commonly grown in ordinary tropical soils (Singh and Reddy, 1981). Resins and steroids were extracted from *C. juncea* (Costa *et al.*, 1995). Seeds of *C. juncea* also contain pyrrolizidine alkaloids which are toxic to *M. javanica, M. arenaria* and *M. incognita* (Janick, 1996). In an isolated case, Kibani and Msabaha (1995) reported that sunnhemp (*Crotalaria spp.*) and maize increased the populations of *Meloidogyne spp.* among other nematodes in rotation experiments. *Meloidogyne spp.* larvae indeed freely entered the roots of resistant plants like *Crotalaria spp.* but failed to multiply (Sabadin, 1984; Desaeger and Rao, 1999). In a comparative study of *Crotalaria spp.* with *Chenopodium ambroisioides* and *Stizolobium aterrima, Crotalaria spp.* showed the highest antagonistic effect against *M. javanica* in the field (Jasy and Koshy, 1994; Asmus and Ferraz, 1998).

In rotation experiments, showy *Crotalaria* and hairy indigo, when planted in rotations, reduced populations of root knot and lesion nematodes in all the succeeding crops (Reddy *et al.*, 1986; Bunte and Muller, 1996; Robinson *et al.*, 1998). In screening experiments, Esparrago *et al.* (1999) reported that *C. spectabilis* and *C. juncea* allowed invasion by *Meloidogyne javanica* and *M. arenaria* but the nematodes failed to reproduce on the plants.

Desmodium spp. have been widely accepted as green plants in western Kenya (Desaeger and Rao, 2000). They are also so palatable and make such excellent forage that they have been termed as "the alfalfa of the tropics" (Skerman, 1977). The plant is reportedly resistant to insect pests and nematodes (Cameron, 1984). *Desmodium uncinartum* and *D. tortuosum* were reported to be antagonistic to *M. arenaria, M. incognita, M. javanica* and *Xiphinema americanum* in greenhouse and field experiments (Kretschmer *et al.*, 1980). Herrera (1997) also reported that exudates of *D. ovalifolium* gave greater immobilisation of second stage juveniles (J_2) of *M. incognita* than *Mucuna spp.* Ten accessions of *Desmodium spp.* tested for reaction to *M. javanica* were also found to be resistant (Lenne, 1981).

In some screening experiments, *D. gangeticus* was reported as a new host of *M. javanica* (Haseeb *et al.*, 1985). Desaeger and Rao (1999) also reported that *D. distortum* supported high root knot nematode populations in soil compared to those for *Sesbania spp.* but exhibited fewer galls on their roots.

Gliricidia sepium (Jacq) Walp., a common tropical tree, is attractive to farmers because of the ease with which it can be propagated through cuttings (Horne *et al.*, 1989). It is reported to contain insecticidal and rodenticidal properties in all parts of the plant (Horne *et al.*, 1989). Leaves of *G. sepium* were effective in reducing the populations of *M. incognita* and *Radopholus similis*, but the effectiveness was lower than carbofuran (Mustika *et al.*, 1994). Extracts of the plant were also found to be lethal to *Radopholus similis* (Jasy and Koshy, 1995) and also reduced fungal diseases (leaf spots and rust) of groundnuts (Schroth *et al.*, 1995). Alley cropping with *G. sepium* however showed no significant effect on the populations of parasitic nematodes (Kang *et al.*, 1999).

Leucaena leucocephala is used throughout the tropics as a cover crop, for fuel, as human food (seed) and animal feeds as well as in soil fertility improvement (Azmi, 1989). Alley cropping with *Leucaena spp.* showed no effect on the populations of parasitic nematodes (Kang *et al.*, 1999). Vicente *et al.* (1986) also found no galls on the roots of *L. leucocephala* and concluded that it was resistant to *M. incognita* and *M. javanica*. Lenne (1981) had ealier reported that *L. leucocephala* was resistant to *M. javanica*.

Root extracts of *L. leucocephala* completely inhibited egg hatching of *M. incognita* and the infectivity and development of the extract-treated juveniles on tomato plants was significantly reduced (Le-Thi-Hoan and David, 1979). Chabra *et al.* (1998) did efficacy tests for some plant extracts on *M. incognita* and reported that juveniles (J_2) were killed by extracts of *Leucaena spp.* Planting of *Leucaena spp.* as a fallow also dramatically reduced spiral and root lesion nematode populations in the soil (Caveness, 1985).

Velvet bean, *Mucuna pruriens* is used as an animal feed and as a green manure crop (Reddy *et al.*, 1986). Its use as human food is, however, limited by the presence of a cyanogenic glucoside in the leaves (Wedhastri, 1992). The active substance in the plant was identified as L-3,4-dihydroxyphenylalanine (L-DOPA), a well known substance that is biologically active in animals. It is an intermediate of many alkaloids (Fujii *et al.*, 1992).

When tested alongside tomato cv. Rutgers and okra cv. Clemson Spineless, *M. deeringiana* had only a few galls and egg masses of *M. arenaria* and *M. javanica* but none from either races of *M. incognita* (McSorley *et al.*, 1994). In an earlier screening experiment, *Mucuna spp.* was considered an unfavourable host of *M. incognita* race 2 and only 2% of the 5000 eggs used as inoculum were recovered after 28 days (Hu and Tsai, 1983). When compared with cotton, groundnut and *C. juncea*, which were highly resistant, *M. pruriens* was moderately resistant to *M. javanica* (Hu and Tsai, 1983).

In crop rotation studies, black velvet bean (*M. aterrima*) followed by a groundnut crop had no effect on nematode populations, with the exceptions of free living nematodes and *Trichodorus spp.* whose populations increased (Moura *et al.*, 1997). Two-year rotations with *M. deeringina* increased kidney bean yields by up to 212% and were effective in control of *M. incognita* races 1 and 4 (Acosta *et al.*, 1995). *Mucuna spp.* was, however, only beneficial to one crop of maize grown immediately after the velvet bean fallow (Rodriguez-Kabana and Canullo, 1992; Acosta *et al.*, 1995).

Exudates from *Mucuna deeringiana* also significantly reduced the population of *M. incognita* in coffee fields (Herrera, 1997) and leachates stimulated hatching of *Heterodera glycines*, but the nematode did not multiply in the bean, forming only males after 15 days (Valle *et al.*, 1997). It was concluded that velvet bean acts as a trap plant for *H. glycines* (Valle *et al.*, 1997). Crude extracts from *M. aterrima* leaves and stems also had inhibitory effects on egg hatching in *M. incognita* race 3 (Nogueira *et al.*, 1994; Queneherve *et al.*, 1998)

Tephrosia purpurea (Roxb.) DC is used as a green manure plant for soil productivity improvement (Pallaniappan and Srinivasul, 1990). It contains alkaloids in its stem, branches and leaves that are toxic to caterpillars (Pallaniappan and Srinivasul, 1990) and rotenoid (4.25%) in its leaves (rotenone) but it is susceptible to *Meloidogyne spp.* (Minton and Adamson, 1970). *Tephrosia cinerea* was identified as a host of root knot nematodes, *Meloidogyne hapla*, *M. incognita* and *M. javanica* (Ponte *et al.*, 1982). The reniform nematode (*Rotylenchulus reniformis)* also showed a host preference for *Tephrosia spp.* rather than cacao when planted together (Yuen, 1979).

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).

Tithonia diversifolia (Hemsl.) A. Gray, is a perennial shrub, that is used for fodder and as a green manure (Drechsel and Reck, 1997). Increases in bean yields when interplanted with *Tithonia spp.* have been reported (Ascher *et al*, 1980). Leaves contain a poisonous compound, which is a contact poison for most pests (Ascher *et al.*, 1980). Information on response of *Tithonia spp.* to nematodes is, however, not readily available.

Vicia villosa Roth, also known as purple vetch is a valuable winter green manure crop in temperate climates (Bin, 1983). Milk vetches in China (Bin, 1983) for example have been shown to accumulate well over 100kg/ha N, without interfering with the scheduling of summer grain crops. *Vicia spp.* however, were shown to be highly susceptible to the stem and bulb nematode, *Ditylenchus dipsaci* (Canbel *et al.*, 1998). *Vicia villosa* and *V. sativa* cv. Canaba White, were shown to be highly susceptible to *M. arenaria* and the reniform nematode, *Rotylenchus reniformis* (Guertal *et al.*, 1998). In studies on effect of nematodes on green manures, vetches still showed great susceptibility to *Meloidogyne incognita* race 2 and *M. javanica* (Santos and Ruano, 1987).

Sesbania sesban, a tropical perennial tree/shrub, is used as a green manure plant, animal feed and in nitrogen fixation (Singh and Reddy, 1981). The plant is fast growing with extraordinarily prolific nodulation (Singh and Reddy, 1981). It is highly susceptible to root knot nematodes (Balasubramanian and Sekayange, 1988; Saka, 1991) and its continued cultivation has led to build-up of the nematodes (Desaeger and Rao, 1999). When compared with *Crotalaria spp.* and *Mucuna spp.*, *Sesbania spp.* was found to be highly susceptible to *M. javanica* (Hu and Tsai, 1983).

When planted as a rotation fallow, *Sesbania spp.* increased the populations of *M. javanica* and *M. incognita* and the yields of french beans following this fallow were lower compared to a rotation incorporating sorghum (Rhodes, 1976). In alley cropping using *Sesbania spp.*, there were significantly more nematodes at between 0 and 2.5 m from the hedgerow than 5m away from the hedge (Yamoah and Getahun, 1989).

Tagetes minuta L. has been used for drug provision and has ornamental value especially in India (Sethi and Gaur, 1986; Bridge, 1996). It contains compounds, in its leaves and other parts, which are toxic to *M. arenaria, M. incognita, M. javanica* and other nematodes (Topp *et al.*, 1998). Thiophenes (α-terthienyl and bithienyls such as BBTOAc and BBT) are the active compounds isolated from *Tagetes spp.* (Jacobs *et al.*, 1994; Kanagy and Kaya, 1996). But other compounds may be present (Kyo *et al.*, 1990).

Screening experiments have shown marigolds to be highly resistant to root knot nematodes (Yen *et al.*, 1998). Extracts from marigolds too have been reported to have high efficacy against *M. incognita, M. arenaria, M. hapla* and *M. javanica* (Sasnelli, 1995; Parada and Guzman, 1997). When interplanted with beans and cowpea (*Vigna unguinculata*), there was no effect on galling by *M. incognita* on the bean or the cowpeas (El-Hanawi and Mohamed, 1990).

Reductions were reported in interplants with tomato on *Nacobbus aberrans* (Zavaleta-Mejia *et al.*,, 1993) and *M. incognita* on aubergine (Dhangar *et al.*, 1995). Two-month rotations of *T. erecta* and tomato also reduced populations of *Meloidogyne spp.* (Sellami and Cheifa, 1997). Rotations of marigolds also resulted in reduced soil populations of root knot and lesion nematodes in all succeeding crops (Reddy *et al.*,1986). As green manures, marigolds reduced root knot nematode populations when they were incorporated into infested soil (Odubr-Owino, 1993; Zavaleta-Mejia *et al.*, 1993).

CHAPTER 3
MATERIALS AND METHODS

3.1 Experimental sites

Glasshouse experiments were carried out at Kabete campus, which lies at approximately 1°15°S to 36°52°E and 1°17°S to 36°48°E (Macmillan Atlas, 1995). The elevation is 1700m a s.l while the mean annual rainfall is 1000mm and the soil type is predominantly nitosol (Macmillan Atlas, 1995). Field experiments were carried out at Kibwezi, located in a region with loamy soils and the temperature ranges from 25 - 30°C (28°C). The mean annual rainfall is 600mm (Orion, 1996) and the altitude is700m. The area lies at latitude, 2°21.5′S and longitude, 38°025′E (Macmillan Atlas, 1995). The experiments were done from October 1999 to July 2000

3.2 Reaction of green manure plants to *Meloidogyne javanica* and *M. incognita*

The green manure plants used in this study were, *Calliandra calothyrsus, Canavalia ensiformis, Chenopodium quinoa* var. narino, *Crotalaria juncea, Desmodium uncinartum, Gliricidia sepium, Leucaena leucocephala, Mucuna pruriens, Tephrosia purpurea, Tithonia diversifolia and Vicia villosa.* The plants were evaluated to determine their reaction to root knot nematodes under glasshouse and field conditions. *Tagetes minuta* and *Sesbania sesban* were included as resistant and suceptible checks, respectively. Seeds were obtained from the Legume Screening Network (at the University of Nairobi and at Kenya Agricultural Research Institute, KARI), Kenya Forestry Research Institute (KEFRI) or Kenya Seed Company.

Fifteen-cm-diameter pots were filled with 2 kg of heat sterilised soil+ballast (4:1) mixture. Fertiliser (DAP) was added at a rate of 5g per pot or planting hole (Chindo and Khan, 1990). One month-old seedlings of *G. sepium*, *C. calothyrsus*, *L. leucocephala*, *T. diversifolia* and *S. sesban* were transplanted from nematode-free seed beds into pots and field microplots. The rest of the green manure plants (*C. ensiformis*, *C. quinoa*, *C. juncea*, *M. pruriens*, *T. minuta* and *V. villosa*) were sowed directly from seed. The glasshouse experiments were arranged in a completely randomised design with eight replications while the field microplots were arranged in randomised complete block design with four replications. Bean cv. GLP-2 was used.

3.2.1 *Meloidogyne spp.* identification

Globose females were extracted from galled tomato roots and the sharp tip of a small scapel was used to cut the body at a point slightly posterior to its widest part. A dissecting needle was used to steady the nematodes. The specimens were then lifted using a fine scapel and transferred onto a microscope slide. The more or less hemi-spherical posterior portion of the body was then placed with the cut surface in contact with the slide. A cover slip was gently lowered over the specimen and a drop of lactophenol was placed to flow between the cover slip and the specimen. Light pressure and gentle movement of the cover slip ensured that the specimen was cleared of the internal contents.

The key developed by Taylor *et al.* (1955) with modifications and illustrations by Eisenback *et al.* (1981) was used in identification of *Meloidogyne spp.*

Key to typical perineal patterns of *Meloidogyne spp.*

1. Arch low and rounded or flattened dorsally..2

Arch high, pattern roughly oval or roughly rectangular...6

2 Lateral lines, distinct incisors often extending beyond edge of pattern. Few or no striae extending unbroken from dorsal to ventral sector........*Meloidogyne javanica*

Lateral lines not distinct incisors, but marked only by irregularities or discontinuities in striae, or with striae meeting at an angle along lateral lines......................................3

3. Arch more or less rounded...4

Arch flattened...5

4. Often with stippled or punctate area near tail tip.

Without irregular striae near lateral lines..*M. hapla*

With irregular striae near lateral lines. Never with stippled or punctate area near tail tip...*M. arenaria*

5. Striae folded near lateral lines...*M. exigua*

Lateral lines bordered by numerous short striae near tail tip............................*M. themesi*

6. Patterns roughly oval...7

Patterns roughly rectangular..*Meloidogyne brevicauda*

7. Dorsal striae closely spaced, wavy to zigzag, arch often ill-formed. *Meloidogyne incognita*

Dorsal striae smooth to wavy, arch usually well-formed.......................*Meloidogyne acrita*

3.2.2 Inoculum preparation

Meloidogyne eggs and juveniles were obtained from galled roots by the maceration extraction method described by Hussey and Barker (1973) and modified by Sikora and Greco (1990). Galled roots of tomato, *Lycopersicon esculentum* var. Moneymaker, were washed free of adhering soil particles using tap water. The roots were then cut into segments of about 1-cm length and macerated using a warring blender for fifteen seconds at high speed. The macerate was vigorously shaken in 0.5% sodium hypochlorite solution for three minutes and then poured into a bucket containing about ten litres of water and passed through 2mm sieves to remove plant tissues. The egg/juvenile suspension was then filtered using 0.25mm-aperture sieves. The eggs were rinsed free of sodium hypochlorite and then transferred into a 1000ml conical flask to which 500ml of sterile water was added. The egg suspension was continuously aerated using an aquarium pump for 10 days. The second stage juveniles obtained were used as inoculum. Inoculation was done ten days after transplanting or emergence of seedlings. Ten thousand eggs/juveniles (Montalvo and Esnard, 1994) suspended in 10ml of water was pipetted into indentations made around the base of the plants in each pot.

3.2.3 Damage assessment

After three months the plants were uprooted and washed free of adhering soil. Galling index (GI) and egg mass index (EMI) were scored using gall and egg mass numbers (Sharma *et al.*, 1994). Staining for egg masses was done using phloxine B. EMI and GI were rated on a scale of 1 to 9 where: 1 = no galls/egg masses; 2 = 1-5 galls /egg masses; 3 = 6-10 galls/egg masses; 4 = 11-20 galls/ egg masses; 5 = 21-30 galls/ egg masses; 6 = 31-50 galls/egg masses; 7 = 51-70 galls/ egg masses; 8 = 71-100 galls/ egg masses; 9 = > 100 galls/egg masses (Sharma *et al.*, 1994).

Second stage juveniles (J_2) were extracted from 250cm^3 soil by the modified Baermann funnel technique, using extraction dishes (Hooper, 1990). The soil was spread on a double layer of milk filters supported by a sieve. The sieves were then placed in a shallow dish and water added to a level where it just touched the soil so that the soil layer looked wet. After 24 hours, the sieves were carefully removed and the nematode suspension concentrated by passing through a series of 45μm - aperture sieves and the juveniles collected from each of the sieves. One ml aliquots of a well-agitated nematode suspension was then pipetted into a counting slide and observed under a light microscope. Counting was repeated for four aliquots and the average calculated.

3.2.4 Field experiments

In the field, the method described in section 3.2.1 above was used to identify the species of *Meloidogyne* present in the experimental plot. Green manure plants were sown in microplots measuring 1 by 2m. Each plot had 3 rows each with six plants. Sowing and transplanting was as described in section 3.2 and the plants were grown for 3 months and then uprooted.

The initial inoculum (Pi) in the soil was determined by randomly sampling the experimental block. The modified Baermann funnel technique described in section 3.2.3 was used to determine the juvenile counts in the soil. The count for final population involved taking soil from within 5 to 20 cm depth, mixing it thoroughly and then taking 250cm^3 sub-samples from each plot for juvenile counts. Ten bean plants were randomly taken from the middle of each plot. Galling and egg mass indices were determined as described in section 3.2.3, above.

3.2.5 Effect of green manure root extracts on the mobility of *Meloidogyne* juveniles

Root extracts from the green manure plants were obtained from the rooting zones using drippers at the bottom of the growing pots (Salisbury and Ross, 1986). Every pot was fitted with a slow releasing perforation that ensured that water with root exudates from the plants oozed into collection dishes beneath. Juveniles were extracted using the procedure described in section 3.2.2 and then placed in 10ml of the extracts, for 24 hours. The number of immobile juveniles was recorded out of every 100 juveniles observed. This was repeated four times. A control where juveniles were placed in distilled water was included.

3.3 Effect of rhizosphere interactions of beans and green manure plants on root knot nematodes

The green manure seedlings were raised on sterile soil as described in section 3.2. A seedling of each of the green manure plants was sown in the same pot with a bean seedling. Inoculation was done ten days after transplanting or emergence of seedlings. Ten thousand eggs/juveniles, suspended in 10ml - water, were pipetted into indents made around the base of the plants in each pot. Plants were lightly watered and the experiments were terminated after 3 months. The modified Baerman funnel technique described in section 3.2.3 was used for juvenile extraction and counts. Galling indices (GI) and egg mass indices (EMI) were also scored on both the green manure plants and the beans, using the scales described in section 3.2.3.

3.4 Effect of growing beans in rotation with green manure plants on root knot nematodes

Identification of the *Meloidogyne spp.* present in the field was done using the method described in section 3.2.1. One-month-old seedlings of *G. sepium*, *C. calothyrsus*, *L.*

leucocephala, *T. diversifolia* and *S. sesban* were transplanted from nematode-free seedbeds into pots and field microplots. The rest of the green manure plants (*C. ensiformis*, *C. quinoa*, *C. juncea*, *M. pruriens*, *T. minuta* and *V. villosa*) were planted directly from seed. A control where beans were planted as a monoculture was included. Inoculation was done as described above (Section 3.2.3). Plants were lightly watered and the experiments were terminated after 3 months.

In the field, green manure plants were sown in microplots of three rows per plot, each with six plants per row. The green manure plants were grown for 3 months, harvested and then beans planted into the same plots. Sampling for final nematode populations at the time of termination of green manure plants and at the time of termination of beans was done. Samples were taken from within 5 to 20 cm depth, mixed thoroughly and $250cm^3$ sub-samples from each plot obtained for juvenile counts. Galling indices and egg mass indices were scored on the bean roots using the keys described in section 3.2.3. The number of juveniles (Pi) in the soil was determined by taking randomly samples from the experimental plot. The modified Baermann funnel technique described by Hooper (1990) was used.

Population (J_2 and eggs) changes were monitored throughout the rotation period as Pi (initial population), Pm (population at removal of green manure plants) and Pf (population at harvesting of the bean crop).

3.4.1 Effect of amending soil with green manures on root knot nematodes in beans

Green manure plants were raised in the field as described in section 3.4. They were harvested after three months. The green manures were incorporated in the soil in a field infested mainly with *Meloidogyne javanica* and *M. incognita*. The layout was as described above (section 3.4) for other field experiments. Finely chopped plant tissues of green manures were incorporated by applying 250g into planting holes. Beans were then planted on the same holes. The experiment was terminated after 3 months and assessment done on the beans. Galling indices (GI) and egg mass indices (EMI) were scored on a scale of 1-9 as described in section 3.2.3 while juveniles were extracted from $250cm^3$ soil using the method described in the same section (Hooper, 1990).

3.5 Data analysis

Analysis of variance of the data was done using GENSTAT 5 Release 3.2 (appendix 1-29). The least significant difference (LSD) test was used to separate the means (Ott, 1988).

CHAPTER 4

RESULTS

The *Meloidogyne* species used in the study were identified as *M. incognita* and *M. javanica* (Plate 1 and 2). Out of 25 samples taken 17 were identified as *M. javanica*, while 8 were identified as *M. incognita*, a proportion of about 2:1, confirming that *Meloidogyne javanica* predominate the higher and intermediate altitudes of East Africa sporadically mixed with low numbers of *M. incognita* (Whitehead, 1986).

4.1 Reaction of green manure plants to root knot nematodes under glasshouse conditions

Galling indices were significantly ($P \leq 0.05$) different among the green manure plants tested (Table 1). The highest galling was recorded on *V. villosa*, with an index of 8.9. *Sesbania sesban* and *T. purpurea,* also, had gall index scores above 7. Their scores were significantly ($P \leq 0.05$) higher than those of *T. minuta* on which no galling (GI = 1.0) was observed. Minimal damage was observed on *C. calothyrsus* and *C. quinoa.* But galling in *C. calothyrsus* was significantly ($P \leq 0.05$) higher than in *T. minuta*. The other green manure plants (*C. ensiformis, C. juncea, D. uncinartum, G. sepium, L. leucocephala, M. pruriens* and *T. diversifolia*) had galling index scores below 2.0, indicating that no significant ($P \leq 0.05$) damage was caused.

Egg mass index (EMI) scores also differed significantly ($P \leq 0.05$) among the green manure plants (Table 1). The scores were highest on *V. villosa.* A similar trend as observed with galling index also followed in this case. *V. villosa, Sesbania sesban* and *T. purpurea* had EMI scores above 6.0. Egg mass index scores in all the three green manure plants were significantly ($P \leq 0.05$) higher than those of *T. minuta. Calliandra calothyrsus* and *C. quinoa* had EMI scores of

Table 1. Galling indices (GI) and egg mass indices (EMI), observed on 13 green manure plants grown under glasshouse

	Test I	II	Mean	Test I	II	Mean
Green manure plant	GI	GI	GI	EMI	EMI	EMI
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 leucocephala	1.2	1.0	1.1	1.0	1.0	1.0
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 ($P \leq 0.05$)	0.7	1.7	0.9	0.8	1.2	0.8
Cv %	23.0	46.2	27.9	28.1	37.6	26.6

3.2 and 2.0, respectively, which were also significantly ($P \leq 0.05$) higher than those of *T. minuta*. This indicated presence of few egg masses on these green manure plants. All the other green manure plants (*C. ensiformis, C. juncea, D. uncinartum, G. sepium, L. leucocephala, M. pruriens* and *T. diversifolia*) had EMI scores that were not significantly ($P \leq 0.05$) different from those on *T. minuta*.

Nematode juveniles (J_2) were recovered from all soils where the green manure plants were grown but there were significant ($P \leq 0.05$) differences among the plants (Table 2). However, only in soils grown with *C. ensiformis, T. purpurea* and *V. villosa* were the counts over 1000 and were significantly ($P \leq 0.05$) higher than the number of juveniles recovered from soils where *T. minuta* was grown.

Juvenile counts in soil where *C. calothyrsus, C. quinoa, C. juncea, D. uncinartum, G. sepium, L. leucocephala, M. pruriens, S. sesban* and *T. diversifolia* were grown, were between 144 and 769. Of these however, counts from soils where *T. diversifolia, M. pruriens, L. leucocephala, G. sepium, C. juncea* and *C. calothyrsus* were grown, were not significantly ($P \leq 0.05$) different from those recovered from soils grown with *T. minuta*. The lowest count was from soils where *T. diversifolia* was grown, while the highest number of juveniles were recovered from soils where *V. villosa* was grown.

Nematode populations increased in rhizosperes of *V. villosa, T. purpurea* and *S. sesban* by ($Rf = 3.2$ to $Rf = 5.4$). Only minimal change in nematode population was noted in the rhizospheres of *C. quinoa*. Where *C. calothyrsus, C. juncea, D. uncinartum, G. sepium, L. leucocephala, M. pruriens, T. diversifolia* and *T. minuta* were grown, nematode populations were reduced by between 10 and 90% of the initial inoculum (Table 2).

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

Green manure plant	Test I J_2	II J_2	Mean J_2	Test I Rf	II Rf	Mean Rf
Calliandra calothyrsus	300	510	405	1.1	0.7	0.9
Canavalia ensiformis	1248	1020	1134	0.9	1.4	1.1
Chenopodium quinoa	780	525	652	0.7	1.4	1.0
Crotalaria juncea	765	268	516	0.6	0.4	0.5
Desmodium uncinartum	1050	488	769	1.1	0.4	0.7
Gliricidia sepium	525	150	337	0.5	0.1	0.3
Leucaena leucocephala	130	238	184	0.2	0.2	0.2
Mucuna pruriens	675	150	412	0.5	0.3	0.4
Sesbania sesban	850	525	687	4.2	3.8	4.0
Tagetes minuta	300	120	210	0.2	0.2	0.2
Tephrosia purpurea	1137	1120	1128	2.8	3.7	3.2
Tithonia diversifolia	168	120	144	0.2	0.1	0.2
Vicia villosa	600	2897	1748	4.5	6.3	5.4
LSD ($P \leq 0.05$)	310	574	401	0.4	0.4	0.5
Cv %	54.1	73.7	52.3	19.7	16.9	20.2

Rf = (Final egg numbers + J_2 in soil) + Inoculum (10,000).

The biggest increase in nematode population was recorded in the rhizosphere of *V. villosa* (Rf =5.4 times), while the biggest reduction in nematode populations was noted from the rhizosphere of *T. minuta*, *T. diversifolia* and *L. leucocephala*. These represented 80% reduction. The least reduction was recorded in the rhizosphere of *C. calothyrsus*, representing 10% reduction.

4.1.2 Effect of green manure plants on root knot nematodes under field conditions

Significant ($P \leq 0.05$) differences in the damage by the nematodes were observed on different green manure plants under field conditions (Table 3). Severe galling was observed on *V. villosa*, *T. purpurea* and *S. sesban*, which had GI scores above 7. The GI scores on the three plants were not significantly ($P \leq 0.05$) different. Minimal damage was noted on *C. calothyrsus* and *M. pruriens*, but the GI scores were significantly ($P \leq 0.05$) lower than on any of the three green manure plant scores stated above. Galling on *M. pruriens* was also not significantly ($P \leq 0.05$) different from that on *T. minuta*. Differences in galling on *C. ensiformis*, *C. quinoa*, *C. juncea*, *D. uncinartum*, *L. leucocephala* and *T. diversifolia*, were not significant ($P \leq 0.05$).

Differences in egg mass indices were also significantly ($P \leq 0.05$) different among the green manure plants. EMI scores higher than 7.5 were observed on *V. villosa*, *T. purpurea* and *S. sesban*. There were few egg masses on *C. calothyrsus* with an average index of 4 and *M. pruriens* with a low score of 1.7. In all the other green manure plants, *C. ensiformis*, *C. quinoa*, *C. juncea*, *D. uncinartum*, *L. leucocephala* and *T. diversifolia*, the EMI scores were not significantly ($P \leq 0.05$) higher than in *T. minuta*.

Second stage juvenile (J_2) populations and overall nematode reproduction followed a similar trend. Significant ($P \leq 0.05$) differences in juvenile numbers were observed. Upto 1100 J_2 counts were associated with *V. villosa*, *T. purpurea*, *S. sesban*, *C. quinoa* and

Table 3. Galling indices (GI), egg mass indices (EMI) and 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	0.4
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 ($P \leq 0.05$)	1.6	1.5	644	0.6
Cv %	38.6	35.9	37.1	21.6

Rf = (Final egg numbers + J_2 in soil) ÷ Inoculum (10,000).

M. pruriens. The lowest J_2 count was in *C. ensiformis* (Table 3). The highest overall nematode population increase was noted in *T. purpurea* with a Rf of 6.3 followed by *Vicia villosa* and *S. sesban* with Rf of 4.7 and 3.7, respectively. On both *C. quinoa* and *M. pruriens*, there were minimal nematode population increases (Rf = 1.4 and 1.2, respectively). Nematode population reductions ranging from 20 and 80% were associated with the other green manure plants, *C. ensiformis, C. juncea, D. uncinartum, L. leucocephala, T. minuta* and *T. diversifolia* (Table 3).

4.1.3 Effect of root extracts of green manure plants on the mobility of *Meloidogyne* juveniles

Extracts from roots of green manure plants had variable ($P \leq 0.05$) effects on mobility of *Meloidogyne* juveniles (Table 4). Extracts of *L. leucocephala, T. diversifolia* and *T. minuta* immobilised at least more than 90%, of the nematode juveniles treated. This percentage was significantly ($P \leq 0.05$) higher than the controls (sterile distilled water and extracts of *P. vulgaris)*. Extracts from *C. calothyrsus, C. ensiformis, C. quinoa, C. juncea* and *D. uncinartum* immobilised between 50% and 82% of the juveniles observed. This level was also significantly ($P \leq 0.05$) higher than the controls. Extracts of *M. pruriens, S. sesban, T. purpurea, V. villosa* and *P. vulgaris* immobilised less than 50% of the juveniles treated. Of these five extracts, however, only the extract from *M. pruriens* immobilised significantly ($P \leq 0.05$) more juveniles than the extract from *P. vulgaris* and the sterile water.

Table 4. Percentage of *Meloidogyne* juveniles (J_2) immobilised by extracts of green manure plants after 24 hours of exposure

Sources of plant extracts	Test I J_2	Test II J_2	Mean J_2
Calliandra calothyrsus	53	49	51
Canavalia ensiformis	83	78	80
Chenopodium quinoa	80	77	78
Crotalaria juncea	78	84	81
Desmodium uncinartum	84	80	82
Leucaena leucocephala	96	90	93
Mucuna pruriens	42	55	48
Sesbania sesban	24	28	26
Tagetes minuta	90	89	90
Tephrosia purpurea	15	22	18
Tithonia diversifolia	95	92	93
Vicia villosa	24	18	21
Control *(Phaseolus vulgaris)*	28	24	26
Control (Sterile water)	24	25	24
LSD ($P \leq 0.05$)	24	12	11.4
Cv%	24.6	25.0	11.8

4.2 Effect of interplanting beans with green manure plants on root knot nematodes

Differences in galling on beans interplanted with green manure plants were significant ($P \leq 0.05$). The highest galling (GI) was recorded in beans interplanted with *V. villosa* (Table 5). Where beans were interplanted with *T. purpurea*, the galling index was also significantly ($P \leq 0.05$) higher than on beans that were planted alone. When interplanted with *C. calothyrsus*, *C. ensiformis*, *C. quinoa*, *M. pruriens* and *S. sesban*, the beans had damage index scores between 4.1 and 5.5 while those planted alone had a galling index of 4.8. Beans interplanted with *C. juncea*, *G. sepium* and *L. leucocephala* had damage scores between 3.4 and 3.8. The lowest damage was recorded in beans interplanted with *T. minuta*. Root galling on beans interplanted with *D. uncinartum* and *T. diversifolia* were 2.8 and 2.7, respectively.

Egg masses were also observed on all beans interplanted with the green manure plants, but the scores varied ($P \leq 0.05$) significantly. The highest egg mass score was recorded in beans interplanted with *V. villosa* (Table 5). EMI scores on beans interplanted with *C. quinoa* and *T. purpurea* were also above 5.0, being 6.2 and 5.8, respectively. Beans planted alone also had an EMI score of 5.7. When interplanted with *C. calothyrsus*, *C. ensiformis*, *C. juncea*, *D. uncinartum*, *L. leucocephala* and *S. sesban* the beans had EMI scores ranging from 3.2 to 3.8, indicating an intermediate level of nematode reproduction.

Where beans were interplanted with *G. sepium*, *M. pruriens*, *T. minuta* and *T. diversifolia*, the EMI scores remained less than 2.7. These scores were significantly ($P \leq 0.05$) lower than scores from beans planted alone.

Table 5. Galling indices (GI) and egg mass indices (EMI) on common beans (*Phaseolus vulgaris*) interplanted with green manure plants in a glasshouse

Bean/green manure plant combinations	Test I GI	II GI	Mean GI	Test I EMI	II EMI	Mean EMI
Calliandra calothyrsus / beans	5.2	3.0	4.1	4.0	3.0	3.5
Canavalia ensiformis / beans	5.3	3.0	4.1	3.6	3.0	3.3
Chenopodium quinoa / beans	5.7	4.0	4.8	6.2	6.2	6.2
Crotalaria juncea/ beans	5.2	2.2	3.7	5.0	2.7	3.8
Desmodium uncinartum/ beans	4.7	1.0	2.8	5.5	1.0	3.2
Gliricidia sepium/ beans	5.7	1.2	3.4	4.2	1.2	2.7
Leucaena leucocephala/ beans	6.2	1.5	3.8	4.2	2.5	3.3
Mucuna pruriens/ beans	3.5	2.0	2.6	2.2	2.2	2.2
Sesbania sesban/ beans	6.5	3.0	4.7	3.0	4.7	3.8
Tagetes minuta/ beans	3.5	1.0	2.2	4.0	1.0	2.5
Tephrosia purpurea/ beans	5.2	8.2	6.7	3.5	8.2	5.8
Tithonia diversifolia/ beans	4.5	1.0	2.7	4.0	1 0	2.5
Vicia villosa/ beans	7.5	8.9	8.2	4.5	9.0	6.7
Control *(Phaseolus vulgaris* monocrop*)*	6.5	3.2	4.8	6.2	5.2	5.7
LSD ($P \leq 0.05$)	1.3	1.0	1.4	2.1	1.3	1.5
Cv %	24.3	30.4	29.9	47.9	34.4	36.2

Varying ($P \leq 0.05$) numbers of second stage juveniles were recovered from the soils where beans were grown together with green manure plants and where it was grown alone (Table 6). Counts of over 1000 were obtained in the rhizospheres where beans were interplanted with *T. purpurea, S. sesban, V. villosa* and where beans were planted alone (Table 6). Beans interplanted with, *C. calothyrsus, C. ensiformis, C. quinoa, G. sepium, L. leucocephala* and *T. diversifolia* resulted in counts less than 1000 but more than 500. The counts were significantly ($P \leq 0.05$) lower than in pots where beans were planted alone. On the other hand, counts in soil where beans were interplanted with *C. juncea, M. pruriens* and *T. minuta* were all lower than 500 and also significantly ($P \leq 0.05$) lower than when the beans were planted as a sole crop.

The reproductive factor of *Meloidogyne spp.* under a sole bean crop was 3.0 but ≥ 4.5 when beans were interplanted with *V. villosa, S. seban* or *T. purpurea.* Population increases were also observed on beans interplanted with *C. calothyrsus, C. quinoa, C. ensiformis, G. sepium, L. leucocephala* and *D. uncinartum.* Population reduction was on the other hand noted on beans interplanted with *M. pruriens, C. juncea, T. minuta* and *T. diversifolia.* Where beans were interplanted with *G. sepium* and *L. leucocephala,* there was no significant ($P \leq 0.05$) change in nematode population. The highest population increase was recorded where beans were interplanted with *V. villosa.*

Table 6. Juvenile (J_2) numbers and reproductive factors (Rf) of *Meloidogyne spp.* on beans interplanted with green manure plants in a glasshouse

Bean-green manure Interplant combination	Test I J_2	II J_2	Mean J_2	Test I Rf	II Rf	Mean Rf
Calliandra calothyrsus / beans	880	175	527	2.1	1.1	1.6
Canavalia ensiformis / beans	882	320	601	1.9	2.2	2.0
Chenopodium quinoa / beans	625	550	587	2.7	3.3	3.0
Crotalaria juncea / beans	400	425	412	1.4	0.2	0.8
Desmodium uncinartum/ beans	2549	144	1346	3.8	0.1	1.9
Gliricidia sepium / beans	1700	140	920	2.4	0.1	1.2
Leucaena leucocephala/ beans	750	234	492	2.2	0.2	1.2
Mucuna pruriens / beans	325	505	415	0.7	0.5	0.6
Sesbania sesban / beans	1350	1342	1346	2.6	6.3	4.5
Tagetes minuta / beans	1100	295	947	1.8	0.6	0.9
Tephrosia purpurea / beans	1400	3749	2574	1.9	8.5	5.2
Tithonia diversifolia / beans	1250	263	756	1.5	0.2	0.8
Vicia villosa / beans	625	1086	855	2.6	8.9	5.7
Control *(Phaseolus vulgaris)* monocrop	1620	1450	1535	3.8	2.2	3.0
LSD ($P \leq 0.05$)	730	383	400	0.7	1.0	0.5
Cv %	65.0	52.5	39.2	20.9	24.3	12.7

Rf = (Final egg numbers + J_2 in soil) ÷Inoculum (10,000), calculated from both the green manure plants and the beans

4.3 Effect of growing beans in rotation with green manure plants on root knot nematodes

4.3.1 Glasshouse experiment

Significantly (P ≤0.05) variable damage was observed when beans were grown in rotation with green manure plants (Table 7). Galling index (GI) scores on beans grown in rotation with *V. villosa, T. purpurea* and *S. sesban* were all above 5.8 and were significantly (P ≤0.05) higher than the bean monoculture scores. The highest damage was observed on beans grown in rotation with *T. purpurea* (GI =7.7). Rotations with *C. calothyrsus, C. ensiformis, C. quinoa* and *M. pruriens* resulted in intermediate galling on the beans with galling indices ranging from 2.1 to 4.0. No significant (P ≤0.05) damage was observed on beans grown in rotation with *C. juncea, D. uncinartum, G. sepium, L. leucocephala, T. minuta* and *T. diversifolia.*

There were also significant (P ≤0.05) differences in egg masses on the beans and the EMI scores followed a trend similar to the one observed on galling. Beans grown in rotation with *V. villosa, T. purpurea* and *S. sesban* had EMI scores higher than 8 (Table 7). These scores were, however, not significantly (P ≤0.05) different from the bean monoculture scores. Where beans were grown in rotation with *C. calothyrsus, C. ensiformis, C. quinoa* and *M. pruriens*, intermediate numbers of egg masses were recorded. These ranged from 2.8 to 5.1 and were significantly (P ≤0.05) lower than in the bean monoculture. In rotations with *C. juncea, G. sepium, L. leucocephala, T. minuta* and *T. diversifolia*, no significant (P ≤0.05) differences in the egg masses were observed on the beans. The highest EMI score was on beans grown in rotation with *V. villosa* while the lowest was on beans grown in rotation with *G. sepium.*

Table 7. Galling indices (GI) and egg mass indices (EMI) on common beans (*Phaseolus vulgaris*) planted in rotation with green manure plants in a glasshouse

Bean-green manure plant rotations	Test I GI	Test II GI	Mean GI	Test I EMI	Test II EMI	Mean EMI
Calliandra calothyrsus - beans	4.5	1.9	3.2	4.7	2.1	3.4
Canavalia ensiformis - beans	2.5	2.2	2.3	2.7	2.9	2.8
Chenopodium quinoa - beans	3.7	4.4	4.0	4.5	5.7	5.1
Crotalaria juncea - beans	1.0	1.2	1.1	1.7	1.2	1.4
Desmodium uncinartum - beans	1.2	1.1	1.1	1.5	1.1	1.3
Gliricidia sepium - beans	1.2	1.0	1.1	1.2	1.0	1.1
Leucaena leucocephala - beans	1.2	1.0	1.1	2.0	1.0	1.5
Mucuna pruriens - beans	2.2	2.1	2.1	4.7	2.4	3.5
Sesbania sesban - beans	6.2	5.4	5.8	8.5	7.6	8.0
Tagetes minuta - beans	1.2	1.6	1.4	1.5	1.7	1.6
Tephrosia purpurea - beans	7.7	7.7	7.7	9.0	7.6	8.3
Tithonia diversifolia - beans	1.0	1.4	1.2	1.0	1.5	1.2
Vicia villosa - beans	6.2	7.0	6.6	9.0	7.9	8.4
Control (*Phaseolus vulgaris* monoculture)	4.5	5.6	5.0	9.0	7.4	8.2
LSD ($P \leq 0.05$)	0.9	2.0	1.3	1.3	2.0	1.5
Cv %	31.2	64.0	36.9	33.1	55.4	37.3

Nematode juveniles were recovered from all the rhizospheres where beans were grown in rotation with the green manure plants. The counts however differed significantly ($P \leq 0.05$) among different treatments (Table 8). Counts of above 2000 were made from the rhizospheres of beans grown in rotation with *S. sesban*, *T. purpurea*, *V. villosa* and the bean monoculture. These counts were significantly ($P \leq 0.05$) higher than scores from beans grown in rotation with *T. minuta*.

Where beans were grown in rotation with C. *calothyrsus*, *C. ensiformis*, *C. quinoa* and *M. pruriens*, the juvenile counts were intermediate, ranging between 1188 and 1812. In the rhizosphere of beans grown in rotation with *C. juncea*, *D. uncinartum*, *L. leucocephala*, *T. minuta* and *T. diversifolia*, the juvenile counts ranged from 294 to 912.

Table 8. Numbers of *Meloidogyne* juveniles (J_2) extracted from soils where beans (*Phaseolus vulgaris*) were planted in rotation with green manure plants in a glasshouse

Green manure - bean rotations	Test I J_2	Test II J_2	Mean J_2
Calliandra calothyrsus - beans	1527	2002	1764
Canavalia ensiformis - beans	2876	1732	1438
Chenopodium quinoa - beans	1825	1800	1812
Crotalaria juncea - beans	1468	150	809
Desmodium uncinartum - beans	552	360	912
Gliricidia sepium - beans	1213	360	787
Leucaena leucocephala - beans	432	157	294
Mucuna pruriens - beans	1995	382	1188
Sesbania sesban - beans	3240	2633	2936
Tagetes minuta - beans	1392	110	751
Tephrosia purpurea- beans	2025	3183	2604
Tithonia diversifolia - beans	805	585	695
Vicia villosa - beans	4412	2300	3356
Contol (*Phaseolus vulgaris* monoculture)	2100	2010	2055
LSD ($P \leq 0.05$)	1037	397	423
Cv %	34.4	29.4	27.3

As shown in table 9, nematode populations showed a general decline where beans were grown in rotation with, *C. juncea, D. uncinartum, G. sepium, L. leucocephala, M. pruriens, T. minuta* and *T. diversifolia,* after the removal of the green manure plants. However, at harvest of the bean crop, intermediate population increase was observed in pots grown with *C. ensiformis* (Rf = 3.4), *C. quinoa* (Rf = 3.5) and *C. calothyrsus* (Rf = 1.6). In the rotation with *D. uncinartum*, the nematode populations continued to decline even after the bean crop was introduced. In rotations with *L. leucocephala, C. juncea, T. diversifolia* and *T. minuta,* marginal increases were observed under the bean crop but the population did not reach the initial population level.

Rotations using *V. villosa*, *T. purpurea* and *S. sesban* resulted in increased nematode population both, at harvest of the green manure plants and at the harvest of the bean crops that followed. The same trend was observed on the bean monocrop. The reproductive factors ranged from 4.4 in the bean monocrop to more than 5.7 in bean rotation with *T. pupurea.*

The highest nematode population increase was noted in *T. pupurea* while the lowest was observed on *C. calothyrsus.* The highest reduction in nematode population was noted in rotatons using *L. leucocephala.* There was no notable change in population where beans were grown in rotation with *G. sepium.*

Table 9. Nematode population changes on beans (*Phaseolus vulgaris*) grown in rotation with green manure plants in a glasshouse

Green manure – bean rotations	Pi	Pm	Pf	Rf
Calliandra calothyrsus - beans	10,000	9295	16005	1.6
Canavalia ensiformis - beans	10,000	12240	34300	3.4
Chenopodium quinoa - beans	10,000	10785	35600	3.5
Crotalaria juncea - beans	10,000	4270	7100	0.7
Desmodium uncinartum - beans	10,000	7650	4500	0.4
Gliricidia sepium - beans	10,000	2925	10800	1.0
Leucaena leucocephala - beans	10,000	1,895	3000	0.3
Mucuna pruriens - beans	10,000	4050	23800	2.3
Sesbania sesban - beans	10,000	40000	53950	5.4
Tagetes minuta - beans	10,000	2,100	2600	0.2
Tephrosia purpurea - beans	10,000	32245	57350	5.7
Tithonia diversifolia - beans	10,000	1500	6200	0.6
Vicia villosa - beans	10,000	53950	60200	6.0
C ontrol (*Phaseolus vulgaris* monoculture)	10,000	34568	44,000	4.4
LSD		2966	3272	1.0
CV%		11.4	7.6	23.7

Pi = Initial population

Pm = Population after removal of green manure plants

Pf = Population at time of removal of beans

Rf = Population change = Pf ÷ Pi = (Final egg numbers + J_2 in soil) ÷ Inoculum

4.3.2 Field experiment

In the field, galling by nematodes was significantly ($P \leq 0.05$) different among the green manure plants tested. Severe galling was observed on beans grown in rotation with *V. villosa, T. purpurea, S. sesban,* and *C. calothyrsus* (Table 10). Their GI scores were, however, not significantly different ($P \leq 0.05$) from the bean-monocrop. The highest damage by nematodes was observed where beans were grown in rotation with *V. villosa,* while the lowest was observed in rotations incorporating *C. juncea* and *D. uncinartum.* No significant ($P \leq 0.05$) damage was observed on beans grown in rotation with *C. juncea, D. uncinartum L. leucocephala, M. pruriens, T. diversifolia* or *T. minuta.*

Juvenile counts and EMI scores followed a similar trend as the galling, being high on *V. villosa. T. purpurea, S. sesban* and *C. calothyrsus.* The EMI scores on beans grown in rotation with *V. villosa, T. purpurea, S. sesban* and *C. calothyrsus* were above 7.0 (Table 10). The EMI scores were, however, not significantly ($P \leq 0.05$) different from the bean monocrop but juvenile counts were lower in rotations with *T. purpurea* and *V. villosa* than the bean monocrop. There were few egg masses on beans grown in rotation with *C. ensiformis* and *C. quinoa* but the overall nematode population was reduced in the rotation where *C. ensiformis* was used. Nematode population did not change in the *C. quinoa*-bean rotation. Juvenile counts for *C. ensiformis* and *C. quinoa* rotations were however significantly ($P \leq 0.05$) lower than the bean monoculture. Egg mass indices on beans grown after *C. juncea, D. uncinartum, L. leucocephala, M. pruriens,* and *T. diversifolia* were not significantly ($P \leq 0.05$) different from those of beans grown after *T. minuta.*

Table 10. Galling indices (GI), egg mass indices (EMI) and juvenile (J_2) numbers on common beans (*Phaseolus vulgaris*), grown in rotation with green manure plants in a field infested with *Meloidogyne javanica* and *M. incognita*

Green manure-bean rotations	GI	EMI	J_2
Calliandra calothyrsus - beans	6.7	7.0	2683
Canavalia ensiformis - beans	1.5	1.7	458
Chenopodium quinoa - beans	3.2	3.7	330
Crotalaria juncea - beans	1.0	1.0	368
Desmodium uncinartum - beans	1.0	1.0	398
Leucaena leucocephala - beans	1.2	1.2	353
Mucuna pruriens - beans	1.5	1.5	214
Sesbania sesban - beans	5.7	7.0	3165
Tagetes minuta - beans	1.5	1.5	518
Tephrosia purpurea - beans	7.0	8.0	1410
Tithonia diversifolia - beans	1.2	1.2	1080
Vicia villosa - beans	7.3	8.0	1155
Control (*Phaseolus vulgaris* monoculture)	6.8	8.0	2880
LSD ($P \leq 0.05$)	1.3	1.4	556
Cv %	29.4	27.8	41.3

4.4 Effect of amending soil with green manures on root knot nematodes

Soil amendment with the green manures resulted in significant ($P \leq 0.05$) reductions of galling by nematodes on beans when compared with an unamended plot. However, in plots amended with *S. sesban*, *T. purpurea* and *V. villosa* there were no significant ($P \leq 0.05$) differences in damage compared to the unamended plot (Table 11). All these had GI scores higher than 3.5. Incorporation of *C. calothyrsus*, *C. ensiformis*, *C. quinoa*, *L. leucocephala*, *M. pruriens*, *T. minuta* and *T. diversifola* into the soil resulted in significantly ($P \leq 0.05$) lower galling on the beans than the unamended plots. There was no significant ($P \leq 0.05$) galling on beans grown in soils amended with *C. juncea*.

The highest egg mass index was recorded in beans where soils were not amended with any of the green manures. Amending soil with *V. villosa*, *T. purpurea* and *S. sesban*, however, did not reduce the number of egg masses on beans significantly ($P \leq 0.05$). Amendments with the other green manures (*C. calothyrsus*, *C. ensiformis*, *C. quinoa*, *L. leucocephala*, *M. pruriens*, *T. minuta* and *T. diversifolia*) reduced the number of egg masses significantly ($P \leq 0.05$). Very few masses were observed on beans grown in soils amended with *C. juncea.*

Application of *V. villosa* as a green manure reduced the number of juveniles significantly ($P \leq 0.05$) but not galling caused by the nematodes when compared to the unamended plots. All the other green manures (*T. purpurea*, *S. sesban*, *T. diversifolia* *C. calothyrsus*, *C. ensiformis*, *C. quinoa*, *C. juncea*, *D. uncinartum*, *L. leucocephala*, *M. pruriens* and *T. minuta)* reduced the number of juveniles as well as the galling by the nematodes.

Table 11. Galling indices (GI), egg mass indices (EMI) and juvenile (J_2) numbers on beans grown in soils amended with green manures

Green manures	Test I GI	II GI	Mean GI	Test I EMI	II EMI	Mean EMI	Test I J_2	II J_2	Mean J_2
Calliandra calothyrsus	3	2	2.5	4	3	3.5	149	460	304
Canavalia ensiformis	3	3	3.0	4	4	4.0	875	781	828
Chenopodium quinoa	2	3	2.5	2	4	3.0	495	338	416
Crotalaria juncea	1	2	1.5	1	2	1.5	49	114	81
Desmodium uncinartum	2	4	3.0	2	4	3.0	335	298	316
Leucaena leucocephala	2	4	3.0	2	4	3.0	376	277	326
Mucuna pruriens	2	2	2.0	2	3	2.5	1095	867	981
Sesbania sesban	5	2	3.5	6	3	4.5	1486	1040	1263
Tagetes minuta	2	2	2.0	2	2	2.0	187	158	172
Tephrosia purpurea	5	4	4.5	6	6	6.0	630	810	720
Tithonia diversifolia	2	4	3.0	3	4	3.5	750	843	796
Vicia villosa	4	6	5.0	5	8	6.5	1185	922	1053
Control (No amendments)	5	6	5.5	6	8	7.0	4553	2433	3493
LSD ($P \leq 0.05$)	2	2.1	1.1	2.4	2.2	1.3	343	216	228
Cv %	51.5	52.3	30.1	56.3	52.1	29.0	24.2	28.7	19.4

Data are means of four replications

Plate 1. Perineal patterns of *Meloidogyne javanica*
[B - Perineum, C - lateral line, D - tail end]

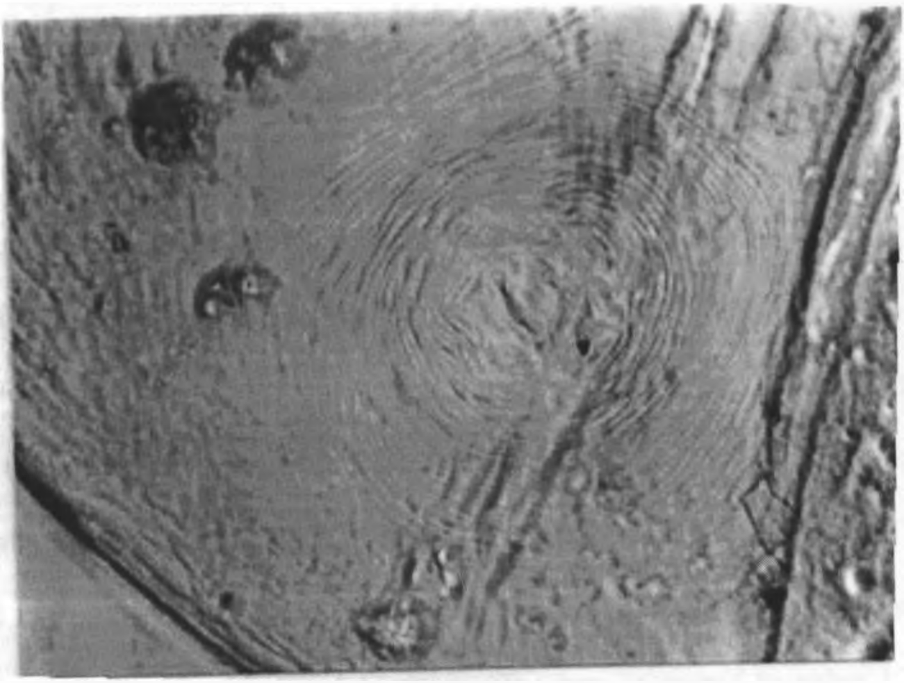

Plate 2. Perineal patterns of *Meloidogyne javanica*
(A – conspicuous perineum)

CHAPTER 5

DISCUSSION

5.1 Reaction of green manure plants to *Meloidogyne javanica* and *M. incognita*

The high nematode damage on roots of *V. villosa, T. purpurea* and *S. sesban* confirms earlier reports on the three plants (Minton and Adamson, 1970, Saka, 1991, Guertal *et al*., 1998) implicating them as hosts of *Meloidogyne spp*. The high galling indicates that the plants were good hosts and should only be introduced with care not to be followed in the same fields with other susceptible crops such as beans. However, with proper management, they could be used as trap crops if they could be uprooted before nematodes reach maturity and if economic considerations allow.

Minimal damage was noted on *C. calothyrsus* and *C. quinoa*, indicating that they were poor hosts of *Meloidogyne javanica* and *M. incognita* when compared to either *V. villosa, T. purpurea* or *S. sesban*. The findings were in agreement with those of other authors (Halliday and Nakao, 1983; McSorley *et al*., 1994; Holtz *et al*., 1998) who reported that these green manure plants were moderately susceptible to root knot nematodes. *Calliandra calothyrsus* was reported to harbor nematodes (Desaeger and Rao, 1999) and this study confirms the earlier findings.

Damage by root knot nematodes on *Crotalaria juncea, D. uncinartum, G. sepium, L. leucocephala, M. pruriens* and *T. diversifolia* was not significantly different from *T. minuta* which was used as a control. Most of these plants have been reported to be antagonistic to root knot nematodes (Antonio and Naumaier, 1986, Sabari *et al*., 1992, Mustika *et al*., 1994, Herrera, 1997; Asmus and Ferraz, 1998). Some of these plants are favorable options that could be used either in rotations, fallows or in other cropping patterns such as alley cropping or as cover crops since they have many other advantages such as nitrogen fixation. *Tithonia* *diversifolia* especially showed a high level of resistance to attack by the nematodes and because it has been shown to increase the amount of nitrogen available to other crops, it is recommended for fallows and even cover cropping.

The high nematode reproduction associated with *V. villosa, T. purpurea, S. sesban* renders them unsuitable as companion or rotation crops in fields harboring high populations of root knot nematodes. *Sesbania spp* were reported to increase populations of *M. javanica* and *M. incognita* (Desaeger and Rao, 1999), while *Tephrosia sp.* and *Vicia spp.* were identified as hosts for root knot nematodes (Ponte *et al.*, 1982; Guertal *et al.*, 1998). The findings in this study were therefore consistent with those of ealier studies.

This study also established that, *C. ensiformis, C. quinoa C. juncea, D. uncinartum, G. sepium, L. leucocephala, M. pruriens* and *T. diversifolia* had a negative effect on reproduction by root knot nematodes. They can therefore be recommended as rotation, fallow or interplants in fields where *Meloidogyne* population have build up beyond economic thresholds. Other authors (Antonio and Naumaier, 1986; Sabari *et al.*, 1992, Mustika *et al.*, 1994, Herrera, 1997, Asmus and Ferraz, 1998) have reported that these green manure plants reduce nematode populations. The only exception was *C. ensiformis* which showed unusually high juvenile counts. This indicated that though it may not itself be adversely affected, its effect on overall nematode population was minimal as an interplant or in rotations it may not be a favourable choice for nematode management. According to McSorley *et al.* (1994), *C. ensiformis* was an intermediate host compared to tomato (cv Rutgers).

Two other green manure plants, *C. calothyrsus* and *C. quinoa*, did not affect nematode populations, indicating that they were probably not obviously antagonistic but rather could be unsuitable hosts or trap plants which arrest nematode multiplication at some some stage(s).

Desaeger and Rao (1999) found galls on *C. calothyrsus* but as in this study no build-up of nematodes was observed. *Chenopodium quinoa* was also reported to support *Meloidogyne* juveniles (Holz *et al.*, 1998), yet acting as a trap crop for *Globodera spp.* (Mian *et al.*, 1999). It also showed higher antagonistic effects than *Mucuna spp.* (Asmus and Ferraz, 1998). The findings on these plants were therefore consistent with those of other authors.

In root extract tests, *C. calothyrsus* and *M. pruriens* showed low levels of juvenile immobilisation while C. *ensiformis, C. quinoa, C. juncea, D. uncinartum, L. leucocephala, T. minuta* and *T. diversifolia* showed high juvenile immobilisation. This indicated to some extent that the low nematode populations associated with these green manure plants was a resort of the active principles reported in them (Antonio and Naumaier, 1986; Sabari *et al.*, 1992; Mustika *et al.*, 1994; Herrera, 1997; Asmus and Ferraz, 1998). There was no significant immobilisation by *S. Sesban, T. purpurea* or *S. sesban*. At one case there were more immobile juveniles in water than in the extracts of these plants. This could be explained by osmotic differences since the extracts did not have any effect on the juveniles.

5.2 Effect of interplanting beans with green manure plants on root knot nematodes

When beans were interplanted with *V. villosa, S. sesban* and *T. purpurea*, there was high nematode galling, indicating that these plants had no effect on the nematodes. Damage by nematodes was also noted on these green manure plants indicating that they were not suitable for interplanting with beans when nematode control is the aim of such an interplant.

Tephrosia purpurea was preferred by the reniform nematode *(Rotylenchulus reniformis)* when interplanted with cacao. *Vicia villosa* and *T. purpurea* are also known to be susceptible to root knot nematodes as reported by Minton and Adamson (1970), Canbel *et al.* (1998) and

Guertal *et al.* (1998) and therefore this explains why there was high build-up of nematodes when they were interplanted with beans. *Sesbania sesban* is also reported to increase nematode populations in alley cropping (Yamoah and Getahun, 1989). Nematode preference for *S. sesban* to beans may have resulted in more damage to the plant than to beans. In the field, *S. sesban* interplants resulted in nematode damage on beans as high as *V. villosa* and *T. purpurea* all which were higher than the non-interplanted plots. This was also noted in the pot experiment and meant that it would be better to plant beans as monocrops than to interplant them with any of the above suscepts.

Calliandra calothyrsus interplants resulted in bean damage which was significantly different from the bean monocrop but the scores were intermediate between *T. minuta* and the highest (*V. villosa* interplant), indicating to some degree that the plant supported nematode populations. Desaenger and Rao (1999) did not consider *Calliandra calothyrsus* as a boundary plant to pose a major threat of nematode multiplication. These results confirm their findings.

Canavalia ensiformis and *C. quinoa* resulted in considerable damage when interplanted with beans. It was reported earlier that *Canavalia ensiformis* and *C. quinoa* supported nematodes for some time or favored their build-up (Vieira *et al.*, 1999; Holtz *et al.*, 1998). *Canavalia ensiformis* was reported to reduce root knot nematode populations when interplanted with sweet potato (Espindola *et al.*, 1998).

Minimal damage by nematodes was observed on beans interplanted with *Crotalaria juncea, D. uncinartum*, *G. sepium*, *L. leucocephala, M. pruriens*, *T. minuta* and *T. diversifolia*. The only exception in this study was probably *L. leucocephala* and *M. pruriens*. Desaeger and Rao (1999) concluded that *L. leucocephala* as a boundary plant did not pose a serious problem of nematode multiplication. A number of authors (Azmi, 1989; Fernadez *et al.*, 1991; Kang *et al.*,

1999) reported *L. leucocephala* to be susceptible or to have no effect on root knot nematodes. Other workers (Caveness, 1985; Vicente *et al.*, 1986; Sabari *et al.*, 1992; Chabra *et al.*, 1998) reported that *L. leucocephala* suppressed *Meloidogyne spp*. So the findings in this study support the latter group.

In interplants with coffee, *Mucuna spp*. reduced populations of *M. incognita* (Herrera, 1997). Variable results have also been reported on *M. pruriens* with some authors indicating that it is susceptible (Moura *et al.*, 1997) and some, such as McSorley *et al.* (1994) reporting only a few galls and others reporting good control of root knot nematodes by the velvet bean (Acosta *et al.*, 1995). The findings in this study therefore are more in line with those of McSorley *et al* (1994) since there was limited damage on the beans. It may however not be advisable to interplant the velvet bean and common bean. This is because of the sprawling nature of its growth which overshadows the common bean and because also their nutritional requirements are nearly the same, being leguminous plants. It may be worth considering interplanting the velvet bean with other crops such as maize.

Though the nematodes did not cause substantial damage in beans interplanted with *C. quinoa* and *D. uncinartum*, there are indications from the egg mass scores that they still multiplied despite the presence of the green manure plants. These plants therefore reduced damage but not nematode reproduction. Mahmood *et al.* (1982) and Kretschmer *et al.* (1980) reported that extracts of the two plants suppresed nematodes. Interestingly also, *T. purpurea* and *S. sesban* resulted in lower egg mass production on the beans than the control indicating a probable preference for these green manure plants by the nematodes when they grew together with common beans. Nematode population build-up and bean yield reduction had been reported in *Sesbania*-bean interplants (Rhodes, 1976; Desaeger and Rao, 1999)

In the beans interplanted with *M. pruriens*, although there was intermediate damage, there were still fewer egg masses than in interplants with *L. leucocephala*. The same was noted with *C. ensiformis*, meaning that probably their antagonistic effects was in, inhibition of egg formation rather than strongly antifeedant factors. The same phenomenon was noted when juvenile counts and reproductive factors were considered. Both *M. pruriens* and *L. leucocephala* were reported to inhibit egg hatching or immobilise juveniles (Nogueira *et al*., 1994; Chabra *et al*., 1998).

The effects of the green manure plant - bean interplants persisted in the experiments indicating that consistently, *V. villosa* and *T. purpurea* resulted in nematode damage higher than the bean monocrop. *Sesbania sesban* and *C. calothyrsus* did not lower the damage caused by the nematodes compared to the bean monocrop, probably because the nematodes could reproduce in the green manure plants themselves and therefore no high increase was realised on the bean roots.

When reproductive factors, were considered *V. villosa, T. purpurea* and *S. sesban* interplants resulted in higher nematode populations than beans grown alone. Build-up of the nematodes was reported in *Sesbania* (Desaeger and Rao, 1999). This explains the above assertion that overall nematode populations in the rhizosphere increased when the above suscepts were considered. On one side, then, these green manure plants could be used as trap crops if they can be removed before nematodes complete a life cycle. On the other hand, they can lead to high nematode increases even though this may not be immediately seen on the first crop. This would however, be disastrous for the succeeding crops. Therefore the advantages of using the plants with a susceptible crop like common beans may be probably fewer than disadvantages in nematode infested fields, meaning an intercrop would not be advisable in such areas even as a means to check nematodes

Of the green manure plants (*C. juncea*, *D. uncinartum*, *G. sepium*, *L. leucocephala*, *M. pruriens*, *T. minuta* and *T. diversifolia*) that resulted in nematode population reduction, there are obvious advantages mentioned by various authors (Skerman, 1977, Le Thi-Hoan and David, 1979, Sethi and Gaur, 1986, Horne *et al.*, 1989). These include nitrogen fixation and quality forage and may be recommended for farming systems. An exception here is *M. pruriens* which may be better used as a green manure or in rotations and fallows rather than being interplanted with common beans as mentioned above.

5.3 Effect of growing beans in rotation with green manure plants on root knot nematodes

Beans grown in rotation with green manure plants that were susceptible (*V. villosa*, *T. purpurea* and *S. sesban*) to root knot nematodes (*Meloidogyne javanica* and *M. incognita*) had higher damage than under continuous beans (monoculture) system. This indicated that these green manure plants favoured rapid nematode multiplication. Similar trends tended to consistently follow even when juvenile numbers were considered. The counts showed that *V. villosa*, *T. purpurea* and *S. sesban* rotations could result in up to six-fold increase in nematode populations on the succeeding bean crop.

Sesbania sesban has been reported to be susceptible to root knot nematodes (Desaeger, 1999) and they pose a serious threat when grown in rotation with susceptible crops like beans. The findings were also consistent with those of Rhodes (1976) which showed that *V. villosa* and

T. purpurea are susceptible to *Meloidogyne spp.* and therefore unsuitable rotation crops where beans are involved.

Some level of galling was observed on *C. calothyrsus*, but the change in nematode population was minimal. Desaeger and Rao (1999) also reported that *C. calothyrsus* was a good host of *Meloidogyne spp.* but did not contribute to nematode build-up. This is a plant that has attracted much attention in agroforestry systems and it is worth noting that in rotation with a susceptible crop such as common bean, it resulted in some increase in nematode populations. Attempts should therefore be made to avoid having susceptible hosts in fields or alleys where *C. calothyrsus* is growing.

When compared with the bean monoculture, *C. quinoa* and *C. ensiformis* rotations did not favour rapid nematode multiplication. Damage on the succeeding bean crop was also not extensive. *Chenopodium quinoa* has been reported to be strongly antagonistic to *Meloidogyne spp.* (Mahmood, *et al.*, 1982) but that strong antagonism was not observed in this study and the benefit to the succeeding bean crop was not so dramatic. However, when compared with *S. sesban* and the bean monoculture, there was much lower damage on the succeeding beans.

Canavalia ensiformis also was reported to produce a lectin that protects it from pests (Costa *et al.*, 1995). The effect of this lectin however was not profound on the nematodes as the findings were more in agreement with those by McSorley *et al.* (1994) who reported that the plant is an intermediate host when compared with susceptible tomato cv. Rutgers. *Canavalia ensiformis* has tremendous potential as human food and as a green manure and would be suitable in rotation or fallow systems since it only allowed minimal damage on the succeeding bean crop and reduced nematode populations.

Other rotations with *C. juncea, D. uncinartum, G. sepium, L. leucocephala, T. diversifolia* and *T. minuta* resulted in reduced nematode populations and no damage on the succeeding bean crop. All these have attracted tremendous attention as fallow crops and therefore can be recommended for use in fields that are heavily infested with root knot nematodes. The findings were in agreement with those of other authors (Caveness, 1985; Reddy *et al.*, 1986; Rodriguez-Kabana and Canullo, 1992; Acosta *et al.*, 1995, Jasy and Koshy, 1994, Janick, 1996; Herrera, 1997). *Tithonia diversifolia* reduced nematode population, and provides one of the most promising choices in nematode management based upon cropping systems

Nematode population in rotations with *D. uncinartum* consistently continued to decline even after beans were introduced, indicating that probably there were residual antagonistic effects that continued to suppress nematode reproduction for some time after removal of the green manure plants. Strong resistance to root knot nematodes by this plant had been reported by Cameron (1984). Herrera (1997) reported that exudates of *Desmodium spp* immobilised more juveniles than *Mucuna spp*. Other authors reported reduced nematode populations using the above green manure plant (Rodriguez-Kabana and Canullo, 1992). On the other hand, in the bean monoculture, there was continued rise in nematode population as would be expected when a susceptible crop is grown as a monoculture.

5.4 Effect of green manure soil amendments on root knot nematodes

All amendments resulted in reductions in both galling and build-up of nematode populations. However, it was notable that *S. sesban, T. purpurea* and *V. villosa* as soil amendments did not have a significant effect on nematodes. So, besides the advantage of fertility improvement, the three were not found to be good in root knot nematode (*M. incognita* and *M.*

javanica) management Morgan and Rodriguez - Kabana (1987) suggested that microbial agents could be encouraged to proliferate in the soil by the use of amendments by plants There was however no profound effect on nematode populations when either *V. viollosa, T. purpurea* or *S. sesban* were used as amendments, showing reduction only on the juvenile numbers They may be useful where nematode pressure is not very high since they also have soil improvement qualities, as mentioned by Smith *et al.* (1987).

Calliandra calothyrsus and *C. ensiformis* amendments had only minimal reduction in nematode galling indicating that they were not strongly suppressive Reduction in nematodes when *C. calothyrsus* was used as an amendment was reported by Halliday and Nakao (1983). *Canavalia ensiformis* was also reported to reduce nematode populations and increase potato tuber yields (Espindola *et al.*, 1998). But in this study the reductions were not profound

The two may therefore, serve as double advantage when used for root knot nematode control Amendments with *Chenopodium quinoa, C. juncea, L. leucocephala, M. pruriens, T. minuta* and *T. diversifolia* resulted in reduced nematode populations and damage on beans *Chenopodium quinoa* has been reported to be antagonistic to *M. javanica* (Asmus and Ferraz, 1988) Therefore, this study confirmed earlier findings Soil amendments with *C. juncea* was also reported to reduce infection by *M. incognita* and *M. javanica* on mungbean (Jasy and Koshy, 1994; Haque *et al.*, 1997).

Significant reductions of *M. incognita* and *M. javanica* populations were also reported in soils amended with *L. leucocephala* (Verma *et al.*, 1998). Complex modes of action, including the proliferation of biological agents that suppress nematodes occur in soils amended with organic materials (Chavarria and Rodrigues-Kabana, 1998) An increase in nematode parasitism was indeed observed in soils amended with *Mucuna pruriens* (Chavarria and Rodriguez-Kabana, 1998).

Tagetes minuta as a soil amendment has been shown to reduce *M. javanica* populations (Oduor-Owino, 1993). *Tithonia diversifolia* was reported to increase bean yield when incorporated in bean plots (ICRAF, 1997) and therefore provides an excellent choice for green manuring. The green manures tested in this study provide valuable and viable alternatives for nematode management.

Numbers of *Meloidogyne* juveniles recovered from soil amended with green manures were significantly lower compared to the control. This suggests that application of soil amendments had substantial effect on juvenile survival, probably by enhanced parasitism on the juveniles more than suppression of reproduction as suggestd by Noguera *et al.* (1994). This is because there were low juvenile counts in soils amended with green manures of the plants that were susceptible to root knot nematodes. Such was the case with *S. sesban*, *T. purpurea* and *V. villosa* amendments.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

The green manure plants tested in this study showed three rather distinct categories in their reaction to root knot nematodes. The same tendency was noted in the interplant and in the rotation experiments.

Vicia villosa, T. purpurea and *S. sesban* were found to be highly susceptible to *Meloidogne javanica* and *M. incognita,* showing both high damage and nematode reproduction. *Canavalia ensiformis, C. quinoa* and *C. calothyrsus* were intermediate or unsuitable hosts that supported limited nematode multiplication or maintained nematode populations at initial inoculum levels. The rest of the green manure plants (*C. juncea, D. uncinartum, G. sepium, L. leucocephala, M. pruriens, T. diversifolia* and *T. minuta)* were rated as resistant.

Tephrosia purpurea, Vicia villosa and *Sesbania sesban*, resulted in high nematode build - up and damage when interplanted with beans. They therefore pose a serious problem in farmlands where *M. incognita* and *M. javanica* are predominant. Interplanting beans with *D. uncinartum*, *G. sepium*, *L. leucocephala, M. pruriens*, *T. minuta* and *T. diversifolia* resulted in nematode population reduction. These have advantages such as nitrogen fixation and supply of quality forage. An exception here is *M. pruriens*, which tends to overshadow beans when the two are grown together.

Since they had higher damage by root knot nematodes (*Meloidogyne javanica* and *M. incognita*) than under bean monoculture, growing of beans in rotation with *V. villosa, T. purpurea* and *S. sesban* should be avoided in nematode infested fields. Bean rotations with *C. calothyrsus*, *C. quinoa* and *C. ensiformis* did not favour rapid nematode multiplication compared with the bean monoculture. Their effect was considered intermediate. These plants could be used in rotations but with caution since they may serve as sources of infestation for susceptible crops such as beans. Rotations with *C. juncea, D. uncinartum, G. sepium, L. leucocephala, T. diversifolia* and *T. minuta* resulted in reduced nematode populations and no damage on succeeding bean crop. These have attracted tremendous attention as fallow crops and therefore can be recommended for use in fields that are heavily infested with root knot nematodes.

Incorporation of *Chenopodium quinoa, C. calothyrsus, C. ensiformis, C. juncea, L. leucocephala, M. pruriens, T. minuta* and *T. diversifolia* as green manures reduced damage by the nematodes significantly and resulted in low juvenile numbers in the soil. They could be recommended for nematode management in areas with high infestation.

More research is, however, needed on the reaction of these green manure plants to the other potentially demanding nematodes on beans. The green manure plants should be also investigated under other cropping systems. The effect on yield of beans and on the green manure plants biomas should also be investigated.

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APPENDICES

Appendix 1. Analysis of variance for nematode egg mass indices for green manure plants grown in a glasshouse

Variate: EMI

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
treatment	12	789.9607	65.8301	92.73	<.001
Residual	39(52)	27.6875	0.7099		
Total	51(52)	422.8606			

Table	treatment
rep.	8
d.f.	39
s.e.d.	0.4213
l.s.d.	0.8521

s.e.	cv%
0.8426	26.6

Appendix 2. Analysis of variance for nematode galling indices for green manure plants grown in a glasshouse

Variate: GI

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
treatment	12	736.5445	61.3787	66.73	<.001
Residual	39(52)	35.8750	0.9199		
Total	51(52)	404.3269			

Table	treatment
rep.	8
d.f.	39
s.e.d.	0.4796
d.f.	39
l.s.d.	0.9700

s.e.	cv%
0.9591	27.9

Appendix 3. Analysis of variance for juvenile counts from soils grown with green manure plants in a glasshouse

Variate: Juv

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
treatment	12	22201550.	1850129.	11.74	<.001
Residual	39(52)	6146493.	157602.		
Total	51(52)	17259905.			

Table	treatment
rep.	8
d.f.	39
s.e.d.	198.5
l.s.d.	401.5

s.e.	cv%
397.0	52.3

Appendix 4. Analysis of variance for percentage of juveniles immobilised by extracts of green manure plants

Variate: J2imob

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatment	13	35125.14	2701.93	57.75	<.001
Residual	28	1310.00	46.79		
Total	41	36435.14			

Table	treatment
rep.	3
d.f.	28
s.e.d.	5.58
l.s.d.	11.44

s.e.	cv%
6.84	11.8

Appendix 5. Analysis of variance for nematode egg mass indices for green manure plants in the field

Variate: Emi

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	2.519	0.840	0.75	
block.*Units* stratum					
treatment	11	404.077	33.673	0.13	<.001
Residual	36	40.231	1.118		
Total	50	446.827			

Appendix 6. Analysis of variance for nematode galling indices for green manure plants in the field

Variate: Gi

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	2.519	0.840	0.63	
block.*Units* stratum					
treatment	11	424.731	35.394	26.70	<.001
Residual	36	47.731	1.326		
Total	50	474.981			

Appendix 7. Analysis of variance for juvenile counts from soils grown with green manure plants in the field

Variate: Juv

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	326682.	108894.	0.54	
block.*Units* stratum					
treatment	11	45482506.	3790209.	18.76	<.001
Residual	36	7271787.	201994.		
Total	50	53080975.			

Appendix 8. Analysis of variance for nematode galling indices for beans interplanted with green manure plants in a glasshouse

Variate: Gii

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
treatment	13	215.442	16.572	8.90	<.001
Residual	42(56)	78.188	1.862		
Total	55(56)	186.031			

Table	treatment
rep.	8
d.f.	42
s.e.d.	0.682
l.s.d.	1.377

s.e.	cv%
1.364	29.9

Appendix 9. Analysis of variance for nematode egg mass indices for beans interplanted with green manure plants in a glasshouse

Variate: Emii

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
treatment	13	254.175	19.552	9.25	<.001
Residual	42(56)	88.750	2.113		
Total	55(56)	215.982			

Table	treatment
rep.	8
d.f.	42
s.e.d.	0.727
l.s.d.	1.467

s.e.	cv%
1.454	36.2

Appendix 10. Analysis of variance for juvenile counts from soils where beans were interplanted with green manure plants in a glasshouse

Variate: Juvi

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
treatment	13	38348357.	2949874.	18.74	<.001
Residual	42(56)	6610049.	157382.		
Total	55(56)	25806056.			

Table	treatment
rep.	8
d.f.	42
s.e.d.	198.4
l.s.d.	400.3

s.e.	cv%
396.7	39.2

Appendix 11. Analysis of variance for egg mass indices for beans grown in rotation with green manure plants in a glasshouse

Variate: EMIr

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
treatment	13	873.476	67.190	29.49	<.001
Residual	42(56)	95.688	2.278		
Total	55(56)	532.638			

Table	treatment
rep.	8
d.f.	42
s.e.d.	0.755
l.s.d.	1.523

s.e.	cv%
1.509	37.3

Appendix 12. Analysis of variance for nematode galling indices for beans grown in rotation with green manure plants in a glasshouse

Variate: GIr

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr
treatment	13	576.469	44.344	28.54	<.001
Residual	42(56)	65.250	1.554		
Total	55(56)	353.625			

Table	treatment
rep.	8
d.f.	42
s.e.d.	0.623
d.f.	42
l.s.d.	1.258

s.e.	cv%
1.246	36.9

Appendix 13. Analysis of variance for juvenile counts from soils where beans were grown in rotation with green manure plants in a glasshouse

Variate: Juv_r

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr
treatment	13	90075242.	6928865.	39.46	<.001
Residual	42(56)	7374123.	175574.		
Total	55(56)	52433714.			

Table	treatment
rep.	8
d.f.	42
s.e.d.	209.5
l.s.d.	422.8

s.e.	cv%
419.0	27.3

Appendix 14. Analysis of variance for nematode egg mass indices for beans grown in rotation with green manure plants the field

Variate: Emi

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	1.3077	0.4359	0.48	
block.*Units* stratum					
treatment	12	410.6923	34.2244	37.69	<.001
Residual	36	32.6923	0.9081		
Total	51	444.6923			

Appendix 15. Analysis of variance for nematode galling indices for beans grown in rotation with green manure plants in the field

Variate: Gi

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	0.3846	0.1282	0.15	
block.*Units* stratum					
treatment	12	314.8077	26.2340	31.36	<.001
Residual	36	30.1154	0.8365		
Total	51	345.3077			

Appendix 16. Analysis of variance for nematode juvenile counts from soils where beans were grown in rotation with green manure plants in field

Variate: Juv

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	3	154793.	51598.	0.32	
block.*Units* stratum					
treatment	12	44881944.	3740162.	23.42	<.001
Residual	36	5749076.	159697.		
Total	51	50785813.			

Appendix 17. Analysis of variance for nematode egg mass indices for beans grown in soils amended with green manures

Variate: EMIa

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	5.1923	1.7308	1.93	
rep.*Units* stratum					
treatment	12	125.2308	10.4359	11.63	<.001
Residual	36	32.3077	0.8974		
Total	51	162.7308			

Table	treatment
rep.	4
d.f.	36
s.e.d.	0.670
l.s.d.	1.359

Stratum	d.f.	s.e.	cv%
rep	3	0.365	11.2
rep.*Units*	36	0.947	29.0

Appendix 18. Analysis of variance for nematode galling indices for beans grown in soils amended with green manures

Variate: GIa

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	2.5529	0.8510	1.36	
rep.*Units* stratum					
treatments	12	77.3750	6.4479	10.31	<.001
Residual	36	22.5096	0.6253		
Total	51	102.4375			

Table	treatments
rep.	4
d.f.	36
s.e.d.	0.559
l.s.d.	1.134

Stratum	d.f.	s.e.	cv%
rep	3	0.256	9.7
rep.*Units*	36	0.791	30.1

Appendix 19. Analysis of variance for nematode juvenile counts from where beans were grown in soils amended with green manures

Variate: Juva

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	42495.	14165.	0.56	
rep.*Units* stratum					
treatment	12	37563940.	3130328.	123.80	<.001
Residual	36	910241.	25284.		
Total	51	38516677.			

Table	treatment
rep.	4
d.f.	36
s.e.d.	112.4
l.s.d.	228.0

Stratum	d.f.	s.e.	cv%
rep	3	33.0	4.0
rep.*Units*	36	159.0	19.4

Appendix 20. Analysis of variance for nematode reproductive factors on green manure plants grown in a glasshouse

Variate: Test1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatmen	12	81.35231	6.77936	98.65	<.001
Residual	26	1.78667	0.06872		
Total	38	83.13897			

Appendix 21. Analysis of variance for nematode reproductive factors on green manure plants grown in a glasshouse

Variate: Test2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatmen	12	134.22769	11.18564	181.01	<.001
Residual	26	1.60667	0.06179		
Total	38	135.83436			

Appendix 22. Analysis of variance for nematode reproductive factors on green manure plants grown in a glasshouse

Variate: means

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatmen	12	99.27188	8.27266	105.07	<.001
Residual	26	2.04707	0.07873		
Total	38	101.31894			

Appendix 23. Analysis of variance for nematode reproductive factors on green manure plants grown in a field infested with root knot nematodes

Variate: rf

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatmen	11	132.0000	12.0000	79.12	<.001
Residual	24	3.6400	0.1517		
Total	35	135.6400			

Apppendix 24. Analysis of variance for nematode populations on beans grown in rotation with green manure plants in a glasshouse

Variate: Pf

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatmen	13	1.807E+10	1.390E+09	363.07	<.001
Residual	28	1.072E+08	3.829E+06		
Total	41	1.818E+10			

Appendix 25. Analysis of variance for nematode populations on beans grown in rotation with green manure plants in a glasshouse

Variate: Pm

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
Treatmen	13	1.149E+10	8.842E+08	281.05	<.001
Residual	28	8.809E+07	3.146E+06		
Total	41	1.158E+10			

Appendix 26. Analysis of variance for nematode reproductive factors on beans grown in rotation with green manure plants in a glasshouse

Variate: RF

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatmen	13	181.4829	13.9602	38.55	<.001
Residual	28	10.1400	0.3621		
Total	41	191.6229			

Variate: Test1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatmen	13	35.0374	2.6952	13.41	<.001
Residual	28	5.6267	0.2010		
Total	41	40.6640			

Appendix 27. Analysis of variance for nematode reproductive factors on beans interplanted with green manure plants in a glasshouse

Variate: Test2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatmen	13	384.3707	29.5670	81.81	<.001
Residual	28	10.1200	0.3614		
Total	41	394.4907			

Appendix 28. Analysis of variance for nematode reproductive factors on beans interplanted with green manure plants in a glasshouse

Variate: means

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
treatmen	13	109.55244	8.42711	100.84	<.001
Residual	28	2.34000	0.08357		
Total	41	111.89244			