DIVERSITY OF PLANT PARASITIC NEMATODES ASSOCIATED WITH MAIZE (*Zea mays* L.) AND INTEGRATED CONTROL OF LESION NEMATODES (*Pratylenchus* spp.)

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A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE A WARD OF A DEGREE OF MASTER OF SCIENCE (M.SC.) IN PLANT PATHOLOGY OF KENYATTA UNIVERSITY

2005

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Diversity of plant parasitic nematodes
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DECLARATION

This thesis is my original work except where acknowledgement is in the text and has not been presented for a degree in any other university or any other award.

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DEDICATION

This thesis is dedicated to my beloved Dad
Gabriel A. Ogolla
In memory of my dear mum
Agnes A. Ogolla

ACKNOWLEDGEMENT

I would like to express my deference to Dr. Waceke Wanjohi who was the principal investigator in this Rockefeller Foundation funded project for considering me as one of the student trainees. Without her encouragement and guidance during the entire research period this work might have been impossible. She thoroughly reviewed my papers and contributed tremendously in my art of writing papers, I greatly feel indebted to her.

Much appreciation to Dr. John Kimenju for training me in identification of lesion nematodes and his immense contribution during preliminary survey. His valuable comments on the proposal, thesis and manuscripts are highly acknowledged.

Prof. Stanley Waudo contributed tremendously to this study, especially during the write-up of the proposal and manuscripts. Although he was an extremely busy person (Dean of the school of Pure and Applied Science) he always found time to go through my work and promptly made invaluable suggestions, for which I am very thankful.

I also wish to recognize Mrs. Jogoo on whose farm the field trials were located. With due regard I extend my appreciation to all farmers who allowed me to take samples from their farms during the preliminary survey. Thanks to Mr. Alfred Micheni and other KARI staff (Embu) who were involved in the survey.

Special thanks to Rockefeller Foundation for funding this study, Botany department of Kenyatta university for the technical support and the Ministry of Agriculture (K) for granting me the study leave.

ABSTRACT

Up to 50% maize (*Zea mays* L.) yield losses in Kenya have been attributed to phytonematodes. Efforts to mitigate maize yield losses caused by nematodes have been hampered by inadequate knowledge on impact of nematodes on maize and lack of affordable nematode management strategies. The objectives of this study, therefore, was to determine diversity and density of phytonematodes associated with maize in smallholder farms in Kirinyaga and Embu districts, evaluate response of maize varieties (Pan5195, Pan5355, HB614, H627, H625, H513, Emap11 and Phb) and legumes (*Canavalia ensiformis* L., *Mucuna pruriens* L., *Lablab purpureus* L. and *Crotalaria ochroleuca* G.) to lesion nematodes (*Pratylenchus* spp. Filipjev), to investigate the effect of inter-cropping maize with non-host legumes and the efficacy of organic soil amendments (*C. ensiformis*, *M. pruriens* and cattle-manure) against lesion nematodes. In addition, the study investigated the damage potential of varying lesion nematodes densities (1000, 2000 and 4000 nematodes per 2000 cm^3 of soil) on the above maize varieties. All experiments on the above objectives except the later were inoculated at a rate of 1 nematode/g of soil. The nematode inoculum was obtained from farmers' field and multiplied on susceptible maize varieties in the greenhouse. In the greenhouse tests, maize and legume seeds were planted in pots containing autoclaved laterite soil, sandy loam mixed with 690 mg N kg^{-1} of soil. In addition, for efficacy test, amendments were applied at 135 g kg^{-1} of soil and 5.0 tonnes ha^{-1} for greenhouse and on-farm tests, respectively. Treatments were replicated 6 times and laid in Randomised Complete Block Design (RCBD). Twelve nematode genera (*Pratylenchus, Paratylenchus*, *Meloidogyne*, *Tylenchus*, *Ditylenchus, Scutellonema*, *Tylenchorhynchus, Hemicycliophora, Tetylenchus, Heterodera, Trichotylenchus* and *Helicotylenchus*) were found associated with maize in the study area. *Pratylenchus* was dominant genus both in soil and maize roots and caused up to 50% root necrosis (disease severity). Greenhouse test revealed that Pan5195 and H627 were tolerant to lesion nematodes. Conversely, the highest nematode inoculum density, 4000 significantly suppressed Phb, HB614, 513, Emap11, Pan5355 and H625 plant growth by up to 40%. The study indicated that *C. ensiformis* and *M. pruriens* were non-hosts while *L. purpureus* was tolerant to lesion nematodes. However, *C. ochroleuca* was highly susceptible and its growth was suppressed by up to 36%. Inter-cropping maize with *C. ensiformis* was more effective than *M. pruriens* in suppressing nematodes and improving crop performance. *Canavalia ensiformis* significantly improved plant growth by up to 34%, reduced nematode soil infestation and disease severity by up to 70% and 26%, respectively. On-farm test revealed that inter-cropping Emap11, Pan5195 and H627 with *C. ensiformis* significantly, increased maize grain yield by 190%, 29% and 22%, respectively. *Mucuna pruriens* significantly increased H627 grain yield by 12% but suppressed Pan5195 and Emap11 grain yield by 79% and 40%, respectively. Efficacy test revealed that *M. pruriens* and cattle-manure were equally effective against the nematodes. Results of greenhouse test, indicate that cattle-manure, *C. ensiformis* and *M. pruriens* significantly improved plant growth by up to 106%, 90% and 81%, respectively, and reduced nematode disease severity by up to 46%. In the on-farm experiment, cattle-manure and *M. pruriens* significantly improved maize grain yield by up to 88% and 53%, respectively, and suppressed nematode population in soil by up to 68%. In addition, amending soil with cattle-manure, *C. ensiformis* and *M. pruriens* significantly reduced nematode disease severity by up to 70%.

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CHAPTER 1

1.0 INTRODUCTION

Maize is a staple food for 28.7 million Kenyans and is cultivated on approximately 1.2 million hectares of land annually. The total annual production is estimated at 3.4 millions tonnes with production of 2.8 tonnes per hectare. However, the production gap between expected and actual yields has remained enormous. The gap ranges from 2.0 tonnes/ha in the lowlands to 5.0 tonnes/ha in the highlands annually (Onyango, 1995).

Low maize yield has been partly attributed to low soil fertility, poor seed quality and poor cropping practices (Muriuki, 1998). Phytonematodes as production constraints were largely ignored until their wide distribution and impact on maize production in Kenya was documented by Desaeger & Rao, (2001) and Kimenju *et al.*, (1999, 1998). Lesion nematodes (*Pratylenchus* spp. Filipjev) are important pests of maize worldwide (Luc *et al.*, 1993). In Kenya, the nematodes have been associated with up to 50% maize yield losses in heavily infested fields (Bridge, 1994; Adeniji *et al.*, 1979). The heavy nematode impact on maize is aggravated by poor cropping practices such as continuous monocropping and failure to apply nematode management practices such as crop rotation, fallowing and use of nematicides (DALEO, 1999a & b; Bridge, 1987).

Small land size makes fallow farming and crop rotation impractical for nematode management. Most of the rotations practiced by subsistence farmers are random and not necessarily appropriate for nematode control (Luc *et al.*, 1993). High costs of nematicides, make it impractical to use them on low value crops such as maize (Sikora, 1992; Stirling, 1991). Use of resistant varieties in minimizing maize yield losses

associated with phytonematodes is a viable alternative within the socio-economic status of farmers in Kenya (Trudgill, 1991). A previous study by Kimenju *et al.*, (1998) revealed that there were no nematode resistant local maize varieties. However, since the liberalization of the seed industry in Kenya, the high number of new maize hybrids/varieties introduced into the market (DeVries, 1998) has increased the chances of identifying nematode resistant germplasm. It is therefore, prudent that efforts are renewed towards the search for lesion nematode resistant germplasm within the available germplasm as this would increase the chances of adoption by farmers.

Breakdown of resistance due to the development of more virulent pathological races remains a great challenge to the use of host resistance (Welsh, 1981). In addition, existence of nematode races restricts adoption of resistant varieties over a wide area (Martin, 1973). This necessitates exploitation of other unequivocal nematode management strategies, viz intercropping of maize with non-host legumes and use of soil amendments such as animal and plant organic manure. Some inter-cropping (diversified) systems especially those involving legumes are often suppressive to nematodes and can lead to yield amelioration besides playing a role in nitrogen fixation (McIntyre *et al.*, 2001). Therefore, there is need to develop maize intercropping systems that are compatible with nematode management strategies.

While regular use of cattle manure for soil fertility improvement was reported to suppress lesion nematodes in Kenya (Kimenju *et al.*, 1999), the poor quality and availability leading to use of low quantities limit its efficacy. Use of leguminous green manure is a feasible alternative for suppressing nematodes and improving soil fertility and texture (Wortmann *et al.*, 2000). Although efforts to improve soil fertility in Kenya

have focused on incorporation of green manure and/or crop residue in crop land (Njarui & Mureithi, 1999; Gachene *et al.*, 1999), literature on the impact of this practice on phytonematodes in maize cropping systems is scanty. It's therefore, imperative that the impact of the green manure on nematodes be assessed and considered among other criteria when selecting green manure for incorporation into the cropland.

Pratylenchus spp. are polyphagus and can also hibernate in roots or soil during adverse conditions (Agrios, 1997) hence cannot be effectively managed through single control strategies. For instance, during a rotation programme, *Pratylenchus zeae* Graham was reported to survive in soil during the entire season of cropping resistant varieties of cowpea and mungbean (Tin & Jean, 1990). Intercropping susceptible tomato varieties with resistant *Tagetes patula* L. did not mitigate *Pratylenchus alleni* Ferris damage on tomato (Hackney and Dickerson, 1975). In addition, compost treatments don't guarantee suppression of *Pratylenchus* spp. (McSorley & Gallaher, 1995). It's therefore, prudent that an integrated rather than a single nematode control strategies are evaluated for effectiveness in mitigating nematode damage on maize. Studies on pest and pathogen densities and diversity are a prerequisite for development of effective integrated management strategies.

1.1 Hypotheses

(a) Cropping systems do not affect the diversity and density of plant parasitic nematodes.

(b) Lesion nematodes do not reduce yield of Maize

(c) Maize yield is not reduced by varying nematodes inoculum densities.

(d) Lesion nematodes do not reduce yield of leguminous crops

(e) Intercropping maize varieties with legumes do not reduce lesion nematodes population and disease severity on maize.

(f) Incorporation of leguminous crop residue into the soil do not reduce lesion nematodes population and associated disease severity on maize.

1.2 Objectives of the study

1.2.1 General objective

Increase maize yields in Kirinyaga and Embu districts by reducing lesion nematode disease severity by use of resistant maize varieties, soil amendments and through intercropping of maize with the non-host leguminous cover crops.

1.2.2 Specific objectives

(a) To determine diversity of plant parasitic nematodes associated with maize in the study areas.

(b) To evaluate the responses of different maize genotypes to lesion nematodes.

(c) To evaluate the effect of different inoculum densities of lesion nematodes on maize.

(d) To evaluate the responses of different leguminous cover crops to lesion nematodes.

(e) To investigate effects of inter-cropping maize with selected legumes on lesion nematodes' population and disease severity.

(f) To assess the efficacy of organic soil amendments against lesion nematodes.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Maize production in Kenya

Up to 80% of the Kenyan population depend on agriculture (Anonymous, 2004). Twenty five percent of this population is involved in maize production. Maize is cultivated on about 1.4 million hectares, which yield an estimated 2.8 million tonnes of grain annually (Anonymous, 1992). This is approximately 20% of the total agricultural produce. Maize is cultivated from 0-2100 M above the sea level. Besides low input, productivity is limited by unreliable rainfall and low soil fertility. In addition, an estimated 20-40% of the grain is lost due to pest and disease (Kedera *et al.*, 1999). It's estimated that nematodes cause up to 50% maize yield losses in heavily infested fields (Bridge, 1994; Adeniji *et al.*, 1979).

2.2 Lesion nematodes (*Pratylenchus* spp.)

Lesion nematodes belong to the family *Pratylenchidae*. The major species in sub-tropical and tropical include *P. brachyurus* Filipjev & Schuurmans Steckhoven, *P. zeae* Graham and *P. penetrans* Chitwood & Oteifa . Other species are *P. coffeae* Filipjev & Schuurmans Steckhoven, *P. delattrei* Luc, *P. goodeyi* Sher & Allen, *P. hexincisus* Taylor & Jenkins, *P. minyus* Sher & Allen, *P. pratensis* Filipjev, *P. sefaensis* Fortuner and *P. thornei* Sher & Allen. *Pratylenchus* spp. are migratory endoparasites and a wide host range including vegetables, fruits trees and ornamentals and so forth (Luc *et al.*, 1993). Some species reproduce sexually whereas others are parthenogenetic. The life cycle of *Pratylenchus* spp. is completed within 45 to 65 days. The females lay eggs either singly or in groups inside infected roots. The eggs then hatch either within the roots or in the

soil when released after a root tissue breaks down. The emerging second-stage juvenile (J-2) develops into J-3 which in turn develops into J-4 which finally becomes an adult either in the soil or after it enters the roots. Temperature and plant species greatly influence the development and reproduction of *Pratylenchus* spp. *Pratylenchus* spp. survive in a wide range of soil but reproduce best in sandy soil. During unfavorable weather condition, the nematodes hibernate in roots or soil as eggs, juvelines, or adults, except for the egg-producing females, which are unable to survive adverse conditions (Agrios, 1997; Swarup & Sosa-moss, 1990).

2.2.1 Morphology of *Pratylenchus* spp.

Both male and female nematodes are vermiform. Adult nematodes range from 500-800 μm in length and 20-25 μm in diameter (Plate 1). The head is flat with the lip region having 2, 3 or 4 annules which are continuous with the body contour. Like other parasitic nematodes, they possess a stylet, which measures 20μm or less in length, and is moderately sclerotized compared to the body. Both sexes have equally developed oesophagus with definite medium bulb and oesophagus gland lobes that overlap the intensive ventrally (Filipjev, 1936). *Pratylenchus* spp. show no marked sexual dimorphism in the anterior region. In female, the vulva is more to the posterior at 70-80% of the body length. The genital system has a single anteriorly directed reproductive tract and a variable post vulva section that may show some differentiation but is never functional. The female genital system is more prodelphic with an oval or round spermatheca that is usually filled with sperm in bisexual species. The female has a sub-cylindrical or more less conoid tail with a broad to narrowly round or truncated terminus

which may be smooth or annulated. In males, the tail is short and dorsally convex-conoid with the bursa extending to tail tip (Filipjev, 1936).

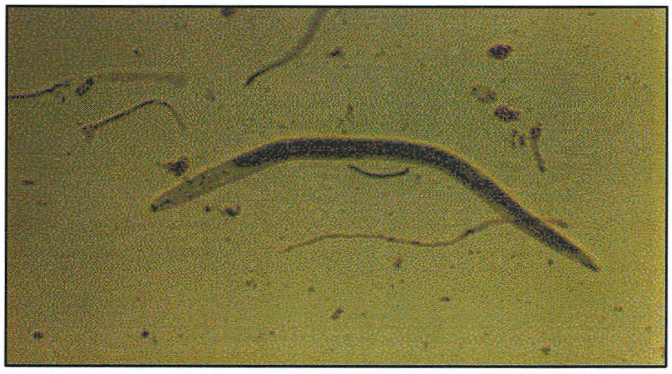


Plate 1. Adult lesion nematode under light microscope (×40) (Photo Waceke, J. W)

2.3 Disease development and symptoms

2.3.1 Disease development

Juveniles and adult nematodes enter the roots usually in a radial direction by a persistent thrusting of the stylet and head, which soften and break the cell wall. After penetration, nematodes move longitudinally into the cortex, where they feed and reproduce. Necrosis of the cortical cells follow the path of nematodes. As the feeding of the nematode on cortical cells continues, cell walls break down and cavities appear in the cortex. The cavities are usually occupied by more than one nematode and are lined with brown deposits. In severe infection, the cavities coalesce and cell walls break down leading to death of the root ends which gives the roots a pruned appearance (Luc *et al.*, 1993). Secondary infection of nematode infected roots by fungi and bacteria, cause

extensive root rot and damage adversely affecting the plant's physiological activities such as respiration, photosynthesis, mineral uptake and translocation, water uptake and phytohormones balance (Khan & Dasgupta, 1993). Phytohormones are produced by root tissue (Scott and Horgan, 1984) and the amount produced is proportional to root size (Carmi and Heuer, 1981). Thus root tips damaged by the nematodes results in smaller root biomass and a decrease in levels of cytokinins and gibberellins. Phytohormone imbalance eventually leads to stunting and chlorosis.

2.3.2 Disease symptoms

Plants growing in fields heavily infested with nematode appear uneven in growth, usually with distinct patches of stunted chlorotic plants (Plate 2). Primary symptoms include combinations of the following: - stunted plants with chlorosis or other discoloration that are due to mineral deficiency, wilting of plants that often slowly recover in the evening. On roots, the symptoms include excessive branching, pruned appearance of root tips, necrotic lesions and root rotting due to secondary invaders. The entire plant is normally weak, produce smaller and fewer ear and kernels and less forage (Malcolm, 1986).



Plate 2. Uneven maize plant growth characteristic of nematode infestation in the study area (Photo Waceke, J. W)

2.4 Management and Control of nematodes

Nematode management and control is usually through cultural practices such as crop rotation, fallowing and use of cover crops; through biological control with resistant varieties and use of antagonistic bacteria and fungi; through physical agents such as heat and flooding; and/or through use of nematicides. Lesion nematodes can be controlled effectively by nematicides. However, use of nematicides can lead to build up of toxic residues in soil and the ground water/ negative environmental impact (Scarascia, 1992). In addition, high cost associated with nematicides make their use unattractive (Scarascia *et al.*, 1997; Giuseppe, 1993).

In hot and dry climates, effective control of lesion nematodes can be achieved by dry fallowing, which reduces the nematode populations through heat, desiccation and elimination of host plants. In addition, effective control of lesion nematodes can also be achieved flood fallowing, which reduces the nematode populations through suffocation. Control through crop rotation is rather unsuccessful due to the wide host range of lesion nematodes (Agrios, 1997; Luc *et al.,* 1993; Malcolm, 1986). Significant control of *Pratylenchus thornei* on potatoes and *P. penetrans* on cowpea has been obtained by soil

solarization (Grinstein *et al.*, 1979). Although soil solarization is effective in nematode control, it adversely affects soil rhizobia leading to reduced nodulation in beans (Cartia, 1985; Rahim *et al.,* 1988). In addition its expensive and denies the resource-poor farmers the opportunity to maximize the use of small land parcels. Several fungi and bacteria that parasitize and kill lesion nematodes are known, but none are effective as biological control agents under field conditions (Agrios, 1997; Kerry *et al.,* 1991; Heuber *et al.,* 1983; Mian & Rodriguez-kabana, 1982; Walker, 1971).

2.4.1 Soil amendments

Nematode suppression through incorporation of organic material in crop land has been attributed to nematicidal effects of metabolites such as ammonia, nitrites, hydrogen sulphide, organic acids and other chemicals produced during decomposition (Akhtar & Malik, 2000; Waceke *et al.*, 1993). For example, decomposing plant residues from rye and timothy have nematicidal effect on *Pratylenchus* (Patrick *et al.,* 1965; Sayre *et al.,* 1965). Sawdust from *Shorea robusta* L. and *Dalbergia sissoo* L. control *Pratylenchus penetrans* while municipal refuse is suppressive to *Pratylenchus* spp. (D'Errico and Maio, 1980). In addition, *Mucuna* spp. has nematicidal bioactive natural products such as triacontanol and tricontyl tetracosanate which reduce mobility of the juveniles and or cause their paralysis and death (Marisa *et al.*, 1996). The potential of organic amendment to suppress nematodes has also been attributed to increase in nematode antagonists (Mateille *et al.*, 1995) such as nematode trapping or predacious fungi (Stirling, 1991), phytonematode obligate parasitic bacteria (Hewlett *et al.*, 1994) and increase in predatory nematodes (Lal *et al.*, 1983) and omnivorous arthropods (Van de Bund, 1972). In

addition, high temperatures produced during the initial stages of decomposition have the potential of lowering nematode density and the inoculum potential (Stapleton *et al.*, 1985; Katan, 1987). This in turn reduces capacity of the nematodes to reproduce and produce enzymes and consequently make the nematodes highly vulnerable to antagonism. Besides improved soil fertility, nutrient supply through mineralization of leguminous crops enhances activities of nematode antagonists, which have a great potential for biological control of phytonematodes (Mateille *et al.*, 1995). In addition, release of nematoxic gases such as ammonia from decomposing nitrogenous material has been implicated in suppression of *Pratylenchus* spp. (Heubner *et al.*, 1983; Walker, 1971). Contrary to perceptions that incorporation of majority of organic material in croplands reduces nematode population, the converse for some material has also been reported (Waceke *et al.*, 2003; Zoon *et al.*, 1993; Sikora, 1992). It is therefore, imperative that the impact of green manure on nematodes be assessed and considered among other criteria when selecting green manure.

2.4.2 Intercropping

The potential of leguminous crops to increase N_2 supply is fundamental in increasing crop tolerance and/or resistance to nematodes (Sharma *et al.*, 1997; Ko *et al.*, 1984; Wallace, 1973). In addition, legumes such as *C. ensiformis and M. pruriens* contain alkaloids which have nematicidal effects (Bisby *et al.*, 1994). The alkaloids mode of action on nematodes is scantly documented. However, alkaloids are known to cause feeding deterency in insect pests (Grewal *et al.*, 1995). Inter-cropping by alternating single row produces maximum contact between crop species (RAFR, 1991) and can

interfere with nematode locating the susceptible crop (Risch *et al.*, 1983). In addition, inter-cropping maize with other leguminous crops such as soybeans increases bacterial population in the rhizosphere of maize (Keswani *et al.*, 1977). Naturally occurring rhizobacteria can have significant antagonistic effects on plant parasitic nematodes (Sikora, 1992) and can lead to decline in nematode population. Whilst it's generally accepted that nematodes' movement in the soil is limited, reduced nematode immigration and increased emigration from a maize row between rows of non-host crops is likely to occur (Altiera and Letournean, 1982; Risch *et al.*, 1983) leading to reduced nematode crop damage on maize. However, increase in lesion nematode population under *Crotalaria* spp. as reported by Kandji, (2001) validate Desaeger & Rao, (2000 & 1999) and Yassin, (1973) findings that certain leguminous crops increase lesion nematode population in the soil. Therefore, there is need to evaluate the response of leguminous crops inter-cropped with maize to lesion nematodes so that their inclusion in maize-cropping system is not counter productive.

2.4.3 Resistant cultivars

Host resistance is important for production of maximum yields in the presence of phytopathogens such as lesion nematodes (Welsh, 1981). It is also important as it's economically and environmentally friendly (Welsh, 1981; Ouwueme, 1978). Resistant varieties prevent nematode damage as a result of reducing population densities hence extending protection to a susceptible crop in the next season (Khan & Dasgupta, 1993). Horizontal and Vertical resistance occur in plants. Vertical resistance is preferred due to the ease with which it can be bred in plants. However, due to gene mutation and

recombination in phytopathogens, vertical resistance is short-lived, except in a few cases (Welsh, 1981; Nelson, 1975 & 1973; Hayes, 1973; Van der plank, 1968). Screening plant materials (both wild and cultivated) is usually necessary in identifying resistant genes against pathogens such as lesion nematodes. Lordell *et al.,* (1985) identified several maize genotypes that are resistant to *P. zeae* and *P. brachyurus* while Norton *et al.*, (1985) identified two wild species; *Zea diploperennis* and *Z. mexicana* as being resistant to *P. scribneri*. Maize cultivars, Seneca 110, Seneca Explorer, Pioneer 309B, Composite 108 and sweet maize cultivars among others, have been demonstrated to be resistant to a number of maize parasitic nematodes (Oteifa and Elgindi, 1982; Hutton, 1981; Sasser and Kirby, 1979; Johnson, 1975). However, response of crops to nematodes depends on nematode species and race (Rodrigruez-Kabana *et al.*, 1992). Moreover, nematode populations differ in pathogenecity (Sidhu & Webster, 1981) and no two spatially separated populations are identical (Rumbaugh & Townsend, 1985). Assessing the response of crops to nematodes in a specific location is therefore, a prerequisite for effective utilization of resistant crops in nematode management.

2.4.4 Integrated nematode control

Although nematodes can be controlled through cultural practices, biological and physical agents and use of nematicides, in practice, a combination of two or more methods is usually employed for effective and economical nematode management. Soil solarization for instance has effectively been combined with resistant or tolerant cultivars to provide significant control of root galling of tomato caused by *Meloidogyne incognita* (Fiume *et al.*, 1994; Chellemi *et al.*, 1993). In addition, solarization in combination with

nematicide enhances control of *Meloidogyne* spp. and *Rotylenchulus reniformis* (Chellemi *et al.*, 1997). Use of *Tagetes erecta* L. as a cover crop for 7 months reduced population of *Pratylenchus* spp. by 75%. Since longer periods of cover cropping is not feasible for small holder farmers, integrating soil amendments with cover cropping for further nematode suppression is an economical and a viable alternative (Gabrielle, 2000). *Pratylenchus zeae* population can survive in the soil during the entire cultivation period of the resistant varieties (Tin and Jean, 1990). Integrating use of resistant varieties with cover crops that suppress weeds some of which can also be hosts for the nematodes is therefore, vital for effective nematode control (Gabrielle, 2000). In addition, inter-cropping resistant varieties with leguminous crops that have repellent, nematicidal or insecticidal properties has enhanced nematode suppression (McIntyre *et al.*, 2001). Research is therefore, needed to explore more sustainable approach to nematode control involving integrating several strategies, including use of resistant varieties, suppressive organic material and inter-cropping systems.

CHAPTER 3

3.0 Materials and methods

3.1 General materials and methods

A survey was conducted in farmer's maize fields in Kirinyaga and Embu districts between December 2000 and February 2001 to determine the diversity and density of plant parasitic nematodes associated with maize. Soil and maize root samples were obtained from five locations (Baricho, Kagumo, Kagio, Kibirigwi and Mwea) in Kirinyaga and three locations (Nembure, Manyatta and Karurina) in Embu (Figure 1). In addition, greenhouse and on-farm tests were conducted at Kenyatta University, Nairobi and in Kibingo'ti location (0^0 34'S, 37^0 11'E, 1354 m asl) Kirinyaga district, respectively, between April, 2001 and December, 2002 to investigate the objectives (section 1.1.2) of the study.

3.1.1 Description of study areas: Kirinyaga and Embu districts

Kirinyaga district lies to the South of Mt. Kenya. Maize is cultivated in three Agro-ecological zones namely Upper Midland Zone 2 (UM2), UM3 and UM4. Upper midland 2 is mainly dominated by deeply weathered andosols while UM3 and UM4 have nitosols. Upper midland 4 is the main maize growing zone. It lies between 1280 and 1340 m above sea level and has an annual average rainfall of 950-1200 mm. Average land holding is 0.4 ha/family with subsistence farming of maize and beans being the major farm activity through out the year (DALEO, 1999a; Jaetzold & Schmidt, 1983a).

Embu district lies to the south-east of Mt Kenya. Areas with altitude of 2500 m and above receive average annual rainfall of >2200 mm while those at 700 m have rainfall of less than 650 mm per year. Maize is mainly cultivated in UM3, which has an average annual rainfall ranging from 980-1100 and 960-980 mm for the long and short rainy seasons, respectively. A third of the district has ideal climate and fertile soils for farming with the rest (2/3) having marginal agricultural potential. Histosols, Lithosols, Andosols, Regosols, Cambisols, Vertisols, Ferralsols, Acrisols and Luvisols are the major soil types. Average land holding is 0.92 ha/family with subsistence farming of maize and beans being the major farm activity throughout the year (DALEO, 1999b; Jaetzold & Schmidt, 1983b).

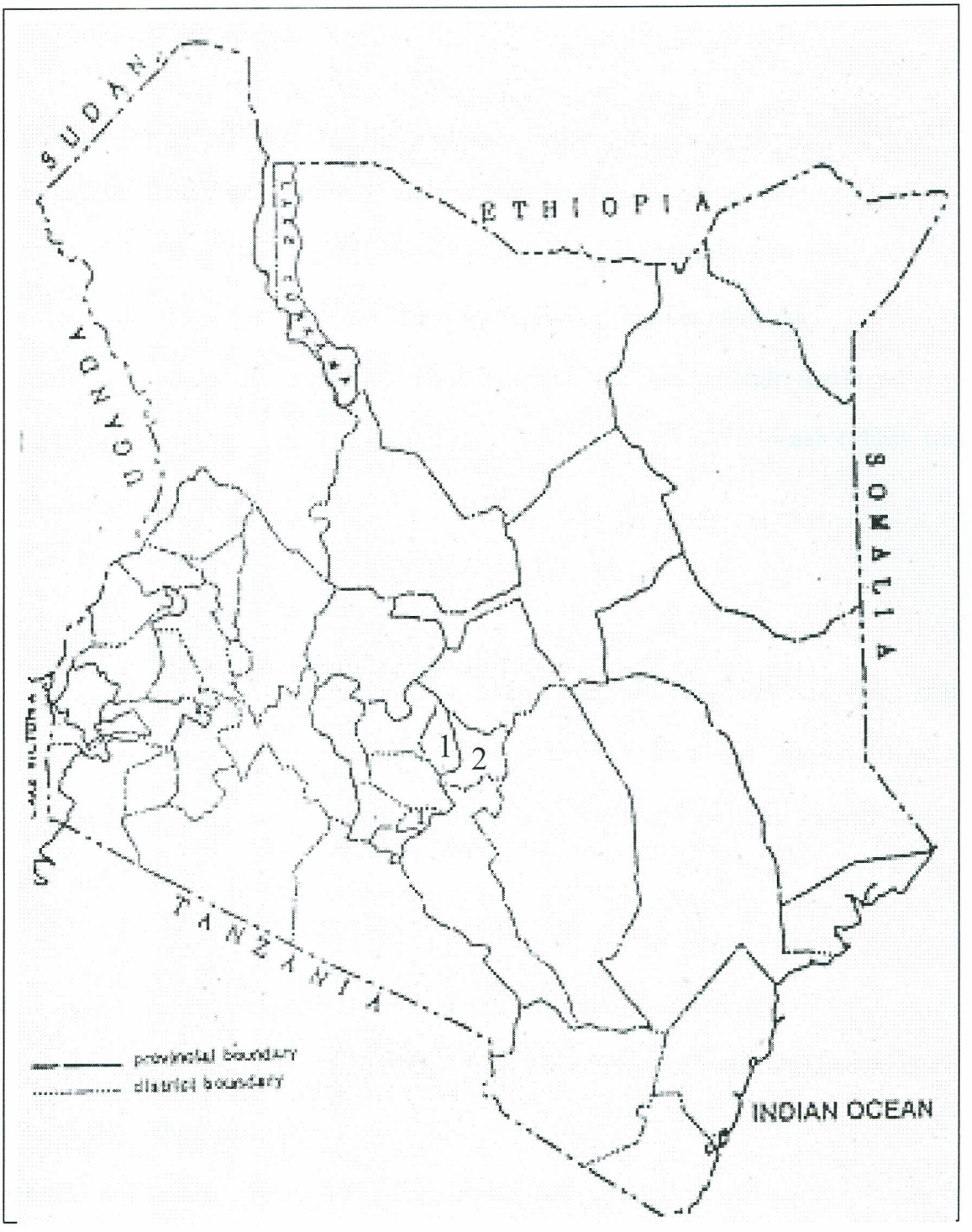


Fig 1. A map of Kenya showing Kirinyaga and Embu Districts where samples were obtained

1. Kirinyaga

2. Embu

3.1.2 Test plants

Six maize varieties (Pan5195, Pan5355, Emap11, HB614, H625 and H627) and four leguminous cover crops (*Canavalia ensiformis* L. (Jack bean), *Crotalaria ochroleuca* G. Don (Sunnhemp), *Lablab purpureus* L. (Hyacinth bean) and *Mucuna pruriens* L. (velvetbean)) were evaluated for response to lesion nematodes. In addition, the damage potential of varying nematode inoculum densities on eight maize varieties (H513, HB614, H625, H627, Emap11, Pan5195, Pan5355 and Pioneer (Phb)) was evaluated.

3.1.3 Growth media

Soil (laterite soil, sandy loam) was obtained from the Kenyatta university farm and sieved to remove the large particles and plant debris. The soil was then autoclaved at 121^0 C and 151b pressure for an hour. After cooling the soil was mixed with 690 mg N kg^{-1} of soil.

3.1.4 Preparation of nematode inoculum and inoculation procedure

Nematode inoculum was obtained from infected maize roots obtained from the farmer's fields during the survey. Nematodes were recovered from maize roots using the maceration filtration technique (Fallis, 1943) and multiplied on susceptible maize varieties (Pan5355 & H625) in the greenhouse. At inoculation, a 6 cm deep depression was made around the rhizosphere of the seedling (s) and the nematode suspension dispensed into the depression and covered with soil.

3.1.5 Nematode extraction and Disease assessment

3.1.5.1 Extraction of nematodes from soil

Soil samples were thoroughly mixed before taking a 200 cm^3 sub-sample per sample for nematode extraction using the extraction tray method by Thomas (1959). Nematodes were collected from the tray at 24 hour interval for 3 days. The nematode suspension was then passed through a 38 µm aperture sieve and nematodes on the sieve were back washed into vials and concentrated in 27 ml of water.

3.1.5.2 Extraction of nematodes from roots

Washed roots were cut into 5 cm long segments. Segments were then thoroughly mixed before taking a known sub-sample and macerating it in a blender for 15 seconds (Fallis, 1943). Nematodes were extracted from the macerated roots and concentrated as described in section 3.1.5.1.

3.1.5.3 Assessment of root necrosis

Clean roots were cut into 5 cm long segments and thoroughly mixed before taking 20 g and 10 g of maize and legumes, respectively, for root necrosis assessment. Root necrosis index was determined as a measure of disease severity. The root necrosis was based on a modification of Bridge and Gowen (1993) root necrosis index as shown below.

0 no root damage = (0% epidermis necrosis)

1 slight root damage = (<25% epidermis necrosis)

2 moderate root damage = (26-50% epidermis necrosis)

3 severe root damage = (51-75% epidermis necrosis)

4 very severe root damage = (>76% epidermis necrosis)

3.1.5.4 Nematode reproductive factor (Rf)

Nematode reproductive factor (Rf) by Wallace, (1973) was used to determine the host status of maize and legumes to lesion nematodes. The Rf's were determined by expressing final nematode population (P_f) as a ratio of initial population (P_i). The P_f for each crop was determined from nematodes recovered from the roots and the soil.

3.1.6 Crop loss assessment

Effects of different treatments on maize growth was assessed by measuring shoot height, obtaining weights of fresh shoot, dry shoot, fresh root and dry grain. Shoot height was determined from the first leaf node to the shoot apex. Plants were then harvested at soil line and fresh shoot weights determined before being oven dried at 60^0C for 7 days and the dry shoot weights determined. Soil was carefully shaken off from the roots to minimize damage of fine roots. The roots were then washed, blotted dry and fresh weights determined. Maize grain from each treatment was dried, shelled and yield determined 150 days after planting.

3.1.7 Data analysis

Survey data were subjected to analysis of variance (ANOVA) and treatment means were compared using Tukey's Honestly Significant Difference (Instat +v1.5). Data on plant growth, maize grain yield and disease were also subjected to a two way

ANOVA, student *t*-test statistics were used to test for significant differences between growth of uninoculated and inoculated plants while treatment means in all experiments were compared using Fisher's Least Significant Difference (LSD) at 5% ($p<0.05$) probability level. Linear tests (correlations) were also used to determine relationships between different variables.

3.2 Specific materials and methods

3.2.1 Nematode diversity and density

3.2.1.1 Roots and soil sampling

Farms under continuous maize cultivation (both irrigated and rain fed) were selected with the help of Research Liaison Officers from the Kenya Agricultural Research Institute (KARI), Embu. Maize was at the tasseling stage at the time of sampling. Samples were obtained from a total of 18 farms at 3 locations (Nembure, Manyatta and Karurina) and 32 farms at 5 locations (Baricho, Kagumo, Kagio, Kibirigwi and Mwea) of Embu and Kirinyaga districts, respectively. At each farm, one composite sample of soil and maize roots each consisting of ten cores or root systems, respectively, were collected at random. Soil samples were obtained from the rhizosphere of maize after removing the top 5 cm of soil (Barker & Campbell, 1981). The soil sample was thoroughly mixed before taking a final 500 g for nematode assay. Likewise roots from ten maize plants per farm were collected, thoroughly mixed and assessed for nematode counts and disease severity.

3.2.1.2 Nematode identification and enumeration

Plant parasitic nematodes were identified to the genus using Mai & Lyon (1975) identification manual and counted using a Hawksley slide under a compound microscope (×40).

3.2.1.3 Analysis of nematode community

Absolute frequency, Absolute density and Prominence of the various genera of plant parasitic nematodes recovered from both soil and root samples were calculated based on methods of community analysis (Norton, 1978).

(a) Absolute frequency = $\frac{\text{number of samples containing a species} \times 100}{\text{number of samples collected}}$

(b) Absolute density = number of a species in a given soil sample.

(c) Prominence of species = Absolute density$\sqrt{}$Absolute frequency (Beals, 1960).

3.2.2 Greenhouse test 1: Response of maize to lesion nematodes

A greenhouse test was carried out between April and August, 2001 to evaluate response of different maize germplasm to lesion nematodes. Six maize varieties (Pan5195, Pan5355, Emap11, HB614, H625 and H627) were evaluated for response to 0 (D0) and 1 (D1)-nematode/cm^3 of soil. The treatments were Pan5195 + D0, Pan5195 + D1, Pan5355 + D0, Pan5355 + D1, Emap11 + D0, Emap11 + D1, HB614 + D0, HB614 + D1, H625 + D0, H625 + D1, H627 + D0, H627 + D1. Each treatment was replicated six times in a RCBD. Maize seeds were pre-germinated on petri-dishes lined with moistened filter papers and planted immediately after emergence into 15-cm diameter plastic pots

containing 2.0 kg of autoclaved laterite soil, sandy loam mixed with 690 mg N kg^{-1} of soil. One seedling was maintained per pot. The pots were inoculated as described in section 3.1.4. The experiment was terminated 99 days after inoculation and disease assessment and plant growth parameters determined as described in section 3.1.5.3 and 3.1.6, respectively.

3.2.3 Greenhouse test 2: The damage potential of varying nematode densities on maize

A greenhouse test was carried out between August and November, 2002 to determine the effect of different inoculum densities on maize. The damage potential of three nematode inoculum densities (0(D0), 1000(D1), 2000(D2) and 4000(D4)-nematodes/2000cm^3 of soil) on eight maize varieties (H513, HB614, H625, H627, Emap11, Pan5195, Pan5355 and Pioneer) was evaluated. Treatments (H513 +D0, H513 + D1, H513 + D2, H513 +D4, HB614 + D0, HB614 + D1, HB614 + D2, HB614 + D4 H625 + D0, H625 + D1, H625 + D2, H625 + D4, H627 + D0, H627 + D1, H627 + D2, H627 + D4, Emap11 + D0, Emap11 + D1, Emap11 + D2, Emap11 + D4, Pan5195 + D0, Pan5195 + D1, Pan5195 + D2, Pan5195 + D4, Pan5355 + D0, Pan5355 + D1, Pan5355 + D2, Pan5355 + D4, Pioneer + D0, Pioneer + D1, Pioneer + D2 and Pioneer +D4) were replicated six times in a RCBD. Growth media preparation and seed sowing t were as described in 3.1.3 and 3.2.2, respectively. At inoculation, each inoculum density was adjusted to 10 ml aqueous nematode suspension and dispensed into a 6 cm deep depression made around the rhizosphere of the seedling and covered with soil. The experiment was terminated 99 days after inoculation and disease assessment and plant growth parameters determined as described in section 3.1.5.3 and 3.1.6, respectively.

3.2.4 Greenhouse test 3: Response of leguminous crops to lesion nematodes

A greenhouse test was carried out concurrently with greenhouse test 1 to investigate response of legumes to lesion nematodes. Four leguminous crops (*C. ensiformis*, *C. ochroleuca, L. purpureus* and *M. pruriens*) were evaluated for response to 0(D0) and and 1 (D1)-nematode/cm^3 of soil. Treatments (*C. ensiformis* + D0, *C. ensiformis* + D1, *C. ochroleuca* + D0, *C. ochroleuca* + D1, *L. purpureus* + D0, *L. purpureus* D1, *M. pruriens* + D0 and *M. pruriens* + D1) six times in a RCBD. Pre-germination of cover crop seeds and planting was as described in section 3.2.2. Treatments were inoculated as described in section 3.1.4. The experiment was terminated 60 days after inoculation and disease assessment and plant growth parameters determined as described in section 3.1.5.3 and 3.1.6, respectively.

3.2.5 Greenhouse test 4: Effect of intercropping maize with non-host leguminous crops on lesion nematodes

A greenhouse test was carried out between August and November, 2001 to investigate the effect of intercropping tolerant or moderately susceptible maize varieties with non-host leguminous crops on lesion nematodes population and disease severity on maize. Three maize varieties (H627, Pan5195 and Emap11) and two legumes (*C. ensiformis* and *M. pruriens*) were used in this test. Nine treatments (Pan5195 alone, Pan5195+*C. ensiformis*, Pan5195+*M. pruriens*, H627 alone, H627+ *C. ensiformis*, H627+ *M. pruriens*, Emap11 alone, Emap11+ *C. ensiformis* and Emap11+ *M. pruriens*) with sole maize serving as controls for respective intercrops were evaluated for their efficacy in suppressing nematodes and improving maize yield. Pre-germination of cover crop and

maize seeds, planting, inoculum density, inoculation and experimental design was as described in section 3.2.2. The experiment was terminated 99 days after inoculation and disease assessment and plant growth parameters determined as described in section 3.1.5.3 and 3.1.6, respectively.

3.2.6 Greenhouse test 5: Efficacy of soil amendment against lesion nematodes

A greenhouse test was carried out between July and September, 2002 to assess the efficacy of soil amendment against lesion nematodes. Efficacy of cattle-manure and green manure of *M. pruriens* and *C. ensiformis* against lesion nematode population build-up was evaluated using Pan5195 and Emap11 maize varieties. Treatments were as follows: Pan5195 alone, Pan5195 + *M. pruriens*, Pan5195 + *C. ensiformis*, Pan5195 + cattle-manure, Emap11 alone, Emap11 + *M. pruriens*, Emap11 + *C. ensiformis* and Emap11 + cattle-manure. Pre-germination of maize seeds, planting, inoculum density, inoculation and experimental design was as described in section 3.2.2. The experiment was terminated 99 days after inoculation and disease assessment and plant growth parameters determined as described in section 3.1.5.3 and 3.1.6, respectively.

3.2.7 On-farm test 1 (February - June, 2002)

Effect of intercropping maize with non-host leguminous crops on lesion nematodes

An on-farm test was conducted to determine the effect of intercropping tolerant or moderately susceptible maize varieties with non-host leguminous crops on nematode population and disease severity on maize. Each of the nine treatments (Pan5195 alone, Pan5195+*C. ensiformis*, Pan5195+*M. pruriens*, H627 alone, H627+ *C. ensiformis*, H627+

M. pruriens, Emap11 alone, Emap11+ *C. ensiformis* and Emap11+ *M. pruriens*) in greenhouse test 4 was established on 3m × 4m micro-plot and replicated thrice in a RCBD. Both maize and legumes were planted on the same day. Maize was planted at 75cm × 25cm inter and intra row spacing, respectively. *Canavalia ensiformis* and *M. pruriens* were then planted between maize rows in appropriate plots at intra row spacing of 25 cm. For both maize and cover crops, one seed was planted per hill and fertilizer applied at a rate of 60 kg $P_2 0_5$ ha^{-1}. Maize was top-dressed with 60 kg N ha^{-1} four weeks after planting.

Nematode population per 200 cm^3 of sub-soil sample from micro-plots was determined before planting. In addition, nematode population per 200 cm^3 of soil sub-sample obtained from the rhizosphere of maize at 45 (mid-season) and 90 (end-season) days after planting was also determined. In addition, root necrosis and nematode population in roots of maize were determined 90 days after planting. Maize from each treatment was dried, shelled and yield determined 150 day after planting.

3.2.8 On-farm test 2 (September - December, 2002)

Efficacy of soil amendments against lesion nematodes

An on-farm test was carried out to determine the efficacy of soil amendments in suppressing lesion nematodes. The eight treatments (Pan5195 alone, Pan5195 + *M. pruriens*, Pan5195 + *C. ensiformis*, Pan5195 + cattle-manure, Emap11 alone, Emap11 + *M. pruriens*, Emap11 + *C. ensiformis* and Emap11 + cattle-manure) evaluated in the greenhouse test 5 were established on micro plots measuring 3 m × 4 m and replicated thrice in a RCBD.. Green manure from 150 days old shrubs, *C. ensiformis* (CE) and *M.*

pruriens (MP), was obtained from a farmer's field and well decomposed cattle manure (CM) from a cattle boma. Cattle-manure and chopped green manure were applied on micro plots at a rate of 5.0 tonnes/ha (500 g/m^2) and plots ploughed to incorporate the amendments to a depth of 15 - 20 cm. Plots were then watered regularly for four weeks before sowing maize seeds. One maize seed was planted per hill at 75 cm × 25 cm using 60 kg P_2O_5 ha^{-1} and top-dressed with 60 kg N ha^{-1} four weeks after planting.

Nematode population per 200 cm^3 of soil sub-sample obtained from micro-plots was determined before planting. In addition, nematode population per 200cm^3 of soil obtained from the rhizosphere of maize at 45 (mid - season) and 90 (end - season) days after planting was determined. Root necrosis and nematode population in roots were also determined 90 days after planting. Maize grain yield was determined 150 days after planting.

CHAPTER 4

4.0 RESULTS AND DISCUSSIONS

4.1 Diversity and density of nematodes of maize in Embu and Kirinyaga districts

4.1.1 Frequency of occurrence of plant parasitic nematode genera in soil and maize roots

Twelve nematode genera, *Pratylenchus* Filipjev, *Meloidogyne* Goeld, *Ditylenchus* Filipjev, *Hemicycliophora* de Man, *Tylenchus* Bastian *Tylenchorhynchus* Cobb, *Paratylenchus* Micoletzky, *Scutellonema* Andrassy, *Tetylenchus* Bastian, *Heterodera* Schmidt, *Trichotylenchus* White and *Helicotylenchus* Steiner were found to be associated with maize in the study areas, Embu and Kirinyaga districts. Both, *Pratylenchus* and *Meloidogyne* spp. were recovered from all soil samples (100%). *Pratylenchus* spp. were however, recovered in 95% of root samples while *Meloidogyne* spp. were recovered from 19% of root samples (Table 1). *Pratylenchus* spp. occurred in all soil and most (95%) root samples confirming that they are important maize parasite (Luc *et al.*, 1993). Correlation between number of *Pratylenchus* spp. in soil and in root revealed a coefficient of determination (r^2) of 0.83 further confirming *Pratylenchus* as major maize nematode. *Meloidogyne* spp. (root-knot nematodes) were recovered in all soil samples possibly due to the common practice of intercropping maize and beans. *Meloidogyne* spp are major nematode pests of beans (Luc *et al.,* 1993). Low percent (19%) recovery of *Meloidogyne* spp. from maize roots confirm that maize is a poor host of root knot nematodes (Idowu, 1981). Occurrence of *Heterodera* spp. in soil samples from maize rhizosphere corroborates Swarup *et al.*, (1964a) and Ibrahim *et al.*, (1986) findings that members of this genus are associated with maize in the tropics. Absence of *Heterodera* in root samples is consistent with previous reports (Maqbool, 1988).

Table 1. Frequency of occurrence of plant parasitic nematode genera in soil and maize roots obtained from the study areas.

	% frequency of nematode occurrence in eight locations.								
Nematode genus	Kagumo	Kagio	Mwea	Kibirigwi	Baricho	Nembure	Manyatta	Karurina	Means
SOIL									
Pratylenchus	100	100	100	100	100	100	100	100	100
Meloidogyne	100	100	100	100	100	100	100	100	100
Ditylenchus	89	100	88	100	83	100	80	40	85
Tylenchus	67	75	25	100	50	100	80	100	75
Paratylenchus	78	75	75	60	100	100	60	40	74
Scutellonema	67	25	88	60	83	100	40	60	65
Hemicycliophora	56	25	63	80	33	100	40	20	55
Heterodera	0	25	88	60	33	100	40	20	48
Tetylenchus	33	0	38	40	50	100	20	0	35
Tylenchorhynchus	56	25	0	80	17	29	20	20	31
Helicotylenchus	0	0	0	40	0	0	0	0	5
Trichotylenchus	0	0	0	0	0	14	0	0	2
ROOT									
Pratylenchus	100	75	100	100	83	100	100	100	95
Hemicycliophora	100	50	88	50	50	43	40	80	63
Tylenchus	33	50	13	83	33	29	20	60	40
Tylenchorhynchus	56	0	25	33	17	86	20	40	35
Ditylenchus	0	0	0	100	0	0	20	40	20
Meloidogyne	44	0	0	17	33	57	0	0	19
Paratylenchus	0	0	0	33	0	14	40	40	16
Tetylenchus	11	0	0	33	0	14	20	0	10

4.1.2 Density of parasitic nematodes associated with maize

There were no significant (p>0.05) differences in population density of *Pratylenchus* spp. in the soil between irrigated (Kagio, Mwea and Karurina) and non-irrigated (Nembure, Manyatta, Karurina, Baricho and Kagumo) locations (Table 2). In the study area, locations with irrigation facilities, cultivated maize in rotation with horticultural crops. Conversely, in locations without irrigation facilities, maize was continuously cultivated. In view of the different maize cultivation practices, lack of significant difference in population density of *Pratylenchus* spp. in the soil between the two locations confirms that the crop rotation being practised is ineffective in management of polyphagus nematodes such as *Pratylenchus* spp. (Luc *et al.*, 1993). Although there was no significant difference in population density of *Pratylenchus* spp. in the soil between irrigated and non-irrigated locations, the former had relatively low population of *Pratylenchus* spp. in the soil (Table 2). The relatively low population of *Pratylenchus* spp. in irrigated locations is consistent with Estioko and Reyes, (1984) findings on negative correlation between nematode population and soil moisture levels and could have attributed to low population of *Pratylenchus* spp. in Kagio, Mwea and Kibirigwi. Mean population density of *Pratylenchus* spp. in soil and roots of maize dominated over the other genera (Table 2). These results confirm report by Bridge, (1994) which rated *Pratylenchus* spp. as the most important nematodes associated with maize in Kenya.

Table 2. Mean† population density of plant parasitic nematode genera recovered from soil and maize root samples.

Nematode genus	Mean population density in 200 cm^3 of soil or 20 grams of roots per location.								
	Kagumo	Kagio	Mwea	Kibirigwi	Baricho	Nembure	Manyatta	Karurina	Means
SOIL									
Pratylenchus	705**	304	331	329	428**	636**	540**	615**	486**
Paratylenchus	504b**	250b	122b	32b†	477b**	1601a**	157b	302b	431**
Meloidogyne	516ab**	230b	301b	275ab	333ab	305ab	281ab	740a**	373
Tylenchus	207b	297ab	51b	194b	36b	972a**	416ab**	724ab**	362
Ditylenchus	408**	358	277	151	126	420**	388	280	301
Heterodera	0	135	378	243	189	216	891**	351	300
Scutellonema	621**	27	111	38	239	131	70	205	180
Hemicycliophora	243	27	78	70	32	62	146	16	84
Tylenchorhynchus	216	14	0	49	18	27	16	0	43
Tetylenchus	75	0	41	16	59	77	38	0	38
Helicotylenchus	0b	0b	0b	11a	0b	0b	0b	0b	1
Trichotylenchus	0	0	0	0	0	8	0	0	1
ROOT									
Pratylenchus	168b	41b	169ab	306a	90b	174ab	103b	130b	148
Hemicycliophora	84a	14b	57ab	18b	36ab	12b	11b	49ab	36
Tylenchorhynchus	39	0	7	14	9	66	11	49	24
Tylenchus	18	14	3	50	23	23	11	27	21
Ditylenchus	0b	0b	0b	113a	0b	0b	38b	16b	21
Paratylenchus	0	0	0	9	0	10	32	38	11
Meloidogyne	12	0	0	6	9	15	0	0	5
Tetylenchus	9	0	0	9	0	8	5	0	4

†Means followed by the same letter(s) within a row are not significantly different at $P < 0.05$ by Tukey's test.

**Density above 2 nematodes/cm^3 of soil.

Other than *Pratylenchus* spp., *Scutellonema* spp. have also been reported to damage maize in Kenya (Kandji *et al.*, 2001). In this study, population densities of *Paratylenchus*, *Tylenchus*, *Ditylenchus* and *Heterodera* spp. in the soil samples obtained from Kagumo, Baricho, Nembure, Manyatta and Karurina were as high (> 2 - nematodes per cm^3 of soil) as those of *Pratylenchus* and *Scutellonema* spp. (Table 2). It's therefore, prudent that the economic importance of these genera in maize production in Kenya be evaluated.

4.1.3 Prominence of parasitic nematodes associated with maize

The prominence of parasitic nematodes in soil and roots was not consistent for all genera except that of *Pratylenchus* (Table 3). *Pratylenchus* spp. maintained prominence over the other nematodes both in soil and roots. These results confirm that *Pratylenchus* spp. are the major nematodes associated with maize in Kenya (Bridge, 1994). The decimal prominence value of *Paratylenchus* in root samples compared to soil samples (Table 3) confirms the ectoparasitic nature of this species (Mai & Lyon, 1975). Although *Tylenchorhynchus* was ranked ninth in soil prominence, it was third in root prominence and maintained nearly the same prominence value confirming its ability to exist both ecto and endoparasitically depending on conditions (Luc *et al.*, 1993).

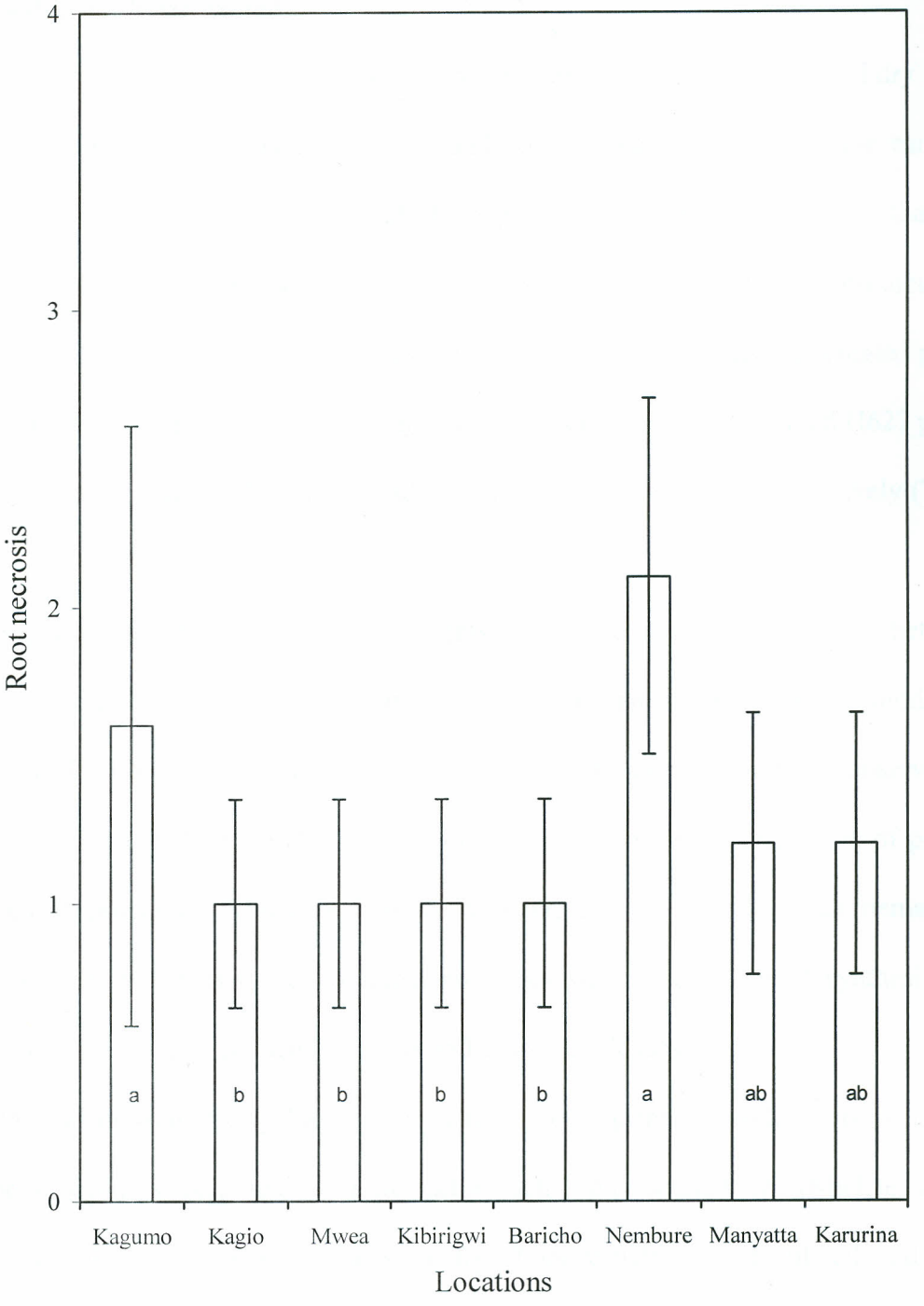
Table 3. Prominence of plant parasitic nematodes from soil and maize roots.

Nematode genus	Prominence[1] value of nematode genus		
	Absolute frequency	Absolute density	Prominence value
		SOIL	
Pratylenchus	100	486	4860
Meloidogyne	100	373	3730
Paratylenchus	74	431	3707
Tylenchus	75	362	3135
Ditylenchus	85	301	2775
Heterodera	48	300	2078
Scutellonema	65	180	1451
Hemicycliophora	55	84	622
Tylenchorhynchus	31	43	239
Tetylenchus	35	38	224
Helicotylenchus	5	1	2.24
Trichotylenchus	2	1	1.41
		ROOT	
Pratylenchus	95	148	1443
Hemicycliophora	63	36	286
Tylenchorhynchus	35	24	142
Tylenchus	40	21	133
Ditylenchus	20	21	94
Paratylenchus	16	11	44
Meloidogyne	19	5	22
Tetylenchus	10	4	13

[1]Prominence value = Absolute density √ Absolute frequency (Beals, 1960).

4.1.4 Root necrosis (disease severity) in the study area

Root necrosis (disease severity), characteristic of *Pratylenchus* spp. root infection significantly ($p<0.05$) differed among the locations under study. Maize varieties in Nembure recorded the highest disease severity on roots (2.1) followed by Kagumo (1.6), Manyatta and Karurina (1.2) while Kagio, Mwea, Kibirigwi and Baricho locations recorded the lowest disease severity of 1.0 as shown in figure 2. The difference in root necrosis among the locations was unexpected because the locations didn't vary significantly in population of *Pratylenchus* spp. soil infestation and root infection (Table 2). Root necrosis is known to be influenced negatively by water availability to the crop (Malcolm, 1986). Relatively high soil moisture levels in irrigated location could therefore, have attributed to low root necrosis in kagio, Mwea and Kibirigwi locations. Inherent genetic difference is also known to influence root necrosis. This could possibly have accounted for low root necrosis in Baricho location where maize is not cultivation under irrigation.



Bars bearing the same letter(s) are not significant ($p>0.05$) different by Tukey's test.

Figure 2. Mean root necrosis of maize roots obtained from different locations in Kirinyaga and Embu ditricts.

4.2 Greenhouse test 1: Response of maize to lesion nematodes

4.2.1 Plant growth assessment

Highly significant ($p<0.001$) differences in plant heights, fresh root and dry shoot weights were observed between inoculated and non-inoculated plants of some varieties (Table 4). Non-inoculated Pan5355 and H625 plants were significantly taller than the respective inoculated plants by 8% and 5%, respectively. Fresh roots of non-inoculated H625 and Pan5355 plants were significantly heavier than the respective inoculated plants by 11% and 10 %, respectively. The fresh shoots and roots of non-inoculated H627 plants were significantly heavier than the inoculated plants by 3% and 6%, respectively (Table 4).

Significant differences in plant heights, fresh shoot and root weights between inoculated and non-inoculated plants attest to the significant influence of nematodes on growth and development of maize. The reduced heights and low weights observed in nematode inoculated Pan5355, H625 and H627 could been due to disruption of plants' physiological processes. Melakeberhan & Webster, (1993) observed that nematodes disrupt many of the plants' physiological processes such as chlorophyll synthesis and photosynthesis leading to reduced biomass and crop yield losses.

Dry shoot weight (dry biomass) as a measure of primary productivity is a good indicator of a plants' response to biotic factor such a phytonematodes (Michael *et al.*, 1986). Lack of significant differences in dry shoot weights of inoculated and non-inoculated Pan5195, H627 and HB614 plants (Table 4) would therefore, be an indication that these varieties were tolerant to lesion nematodes. This was supported by the fact that, although these varieties supported an increase in nematode population (Table 5) their dry biomass was not significantly affected (Dropkin, 1955). Seinhort (1967) observed that

suppression of plants' growth or yield occurred only when their nematode density tolerant limit is exceeded. These results therefore, suggest that the tolerant limit of HB614, H627 and Pan5195 is above 1-lesion nematode per cm^3 of soil.

Dry shoot weights of inoculated H625, Pan5355 and Emap11 plants on the other hand were significantly ($p<0.05$) reduced by 25%, 23% and 11%, respectively, compared to the respective non-inoculated plants (Table 4). In addition, H625, Pan5355 and Emap11 plants supported a relatively high nematode population (Table 5). The reduction in dry biomass of H625, Pan5355 and Emap11 and increase of nematode populations (Rf > 1) supported by these varieties was an indication that these plants were susceptible and good hosts of lesion nematodes (Seinhort, 1967).

Table 4. Mean* plant heights, fresh shoot, dry shoot and fresh root weights of maize 99 days, after inoculation, greenhouse test 1.

Maize variety	Plant parameters	Inoculated	Non-inoculated	% change	*t*-test (means)
Pan5195	Plant height	115.20	115.20	0.0	NS
	Fresh shoot weight	116.40	119.34	-2.0	NS
	Dry shoot weight	16.02	17.18	-7.0	NS
	Fresh root weight	32.05	33.33	-4.0	NS
H627	Plant height	139.50	139.67	-0.1	NS
	Fresh shoot weight	190.42	196.59	-3.0	S
	Dry shoot weight	23.57	24.74	-5.0	NS
	Fresh root weight	37.76	40.11	-6.0	S
HB614	Plant height	109.42	111.30	-2.0	NS
	Fresh shoot weight	128.51	129.95	-1.0	NS
	Dry shoot weight	13.57	15.43	-12	NS
	Fresh root weight	27.99	29.00	-4.0	NS
EMAP11	Plant height	143.30	145.48	-2.0	NS
	Fresh shoot weight	141.72	142.05	-0.2	NS
	Dry shoot weight	19.61	22.14	-11	S
	Fresh root weight	31.25	32.65	-4	NS
H625	Plant height	132.57	138.97	-5.0	HS
	Fresh shoot weight	180.97	186.96	-3.0	S
	Dry shoot weight	19.62	26.06	-25	HS
	Fresh root weight	39.48	44.50	-11	HS
Pan5355	Plant height	132.67	144.50	-8.0	HS
	Fresh shoot weight	142.88	152.81	-7.0	HS
	Dry shoot weight	18.79	24.42	-23	HS
	Fresh root weight	44.74	49.75	-10	HS

* Mean of six replications.

NS-Not significant ($p>0.05$), S-significant ($p<0.05$), HS-Highly significant ($p<0.001$).

4.2.2 Plant disease assessment

Dry shoot weight (dry biomass) of varieties (H625, Pan5355 and Emap11) that had more than 500-nematodes per gram of dry root (Table 5) was significantly ($p<0.05$) reduced (Table 4). Dry shoot weight of varieties that had less than 500-nematodes per gram of dry root was not significantly ($p>0.05$) affected. These results corroborate Malcolm's, (1986) findings which reported 500-nematodes per gram of dry root as a critical threshold for maize growth.

The numbers of nematodes in roots were positively and significantly correlated ($r^2=0.85$; $p=0.05$) to root necrosis index, which significantly ($p<0.05$) differed among maize varieties (Table 5). Pan 5195, H627 and HB614 supported some of the fewest nematodes in roots while Pan5355, H625 and Emap11 had some of the highest nematodes population in roots (Table 5). For instance, the number of nematodes in roots of H627 was significantly ($p < 0.05$) lower by 89%, 87% and 56%, compared to Pan5355, H625 and Emap11, respectively. Low nematode population in the roots of H627, HB614 and Pan5195 can be attributed to nematodes exiting the roots due to lack of readily available food or inadequate food or inability to establish normal feeding sites (Huang, 1985). This speculation, however, requires further experimentation.

Lesion nematodes kill cells as they feed and migrate within the root tissue. This causes extensive root destruction (root necrosis) especially when nematode population in the root is high (Luc *et al.*, 1993). Higher root necrosis in Emap11 (plate 5) and lower root necrosis in Pan5195 (Plate 3) and H627 (Plate 4) therefore, could be attributed to difference in number of nematode population in the roots (Table 5). This is further

supported by strong positive and significant correlation (r^2=0.85; p=0.05) between the number of nematodes in roots and the root necrosis index.

There was a negative correlation (r = -0.68; p=0.05) between number of nematodes in roots and that in the soil for all the varieties. Low lesion nematodes population in the soil is often associated with high root population (Luc *et al.*, 1993). The low nematode population in the soil and high nematode population in roots of H625 and Pan5355 (Table 5) supports this finding.

Table 5. Mean* root necrosis, nematodes population in root and nematode reproductive factor (Rf) in maize 99 days, after inoculation, greenhouse test 1.

Maize varieties	Root necrosis†	Nematodes/gram of dry root	Nematodes/200 cm^3 of soil	(Rf) **	Host status
Pan5195	0.9	405	122	1.3	M
H627	0.9	284	297	1.8	G
HB614	1.0	325	356	2.1	G
Emap11	1.2	646	499	2.9	G
H625	2.0	2122	32	6.4	G
Pan5355	2.0	2679	59	6.9	G
$LSD_{(0.05)}$	0.21	98	38	0.2	
SED	0.1	47.6	18	0.09	
CV(%)	13.3	7.7	0.02	4.3	

*Mean of six replications.

†Necrosis index: 0-no root damage, 1-slight root damage, 2-moderate root damage, 3-severe root damage, 4-very severe root damage (Bridge and Gowen, 1993).

** Rf > 10-excellent host, 10 ≥ Rf ≥1.5-good host, 1≤ Rf<1.5-maintenance host and Rf <1- a poor host or non-host (Ferris *et al.*, 1993).

M-Maintenance host, G-Good host.

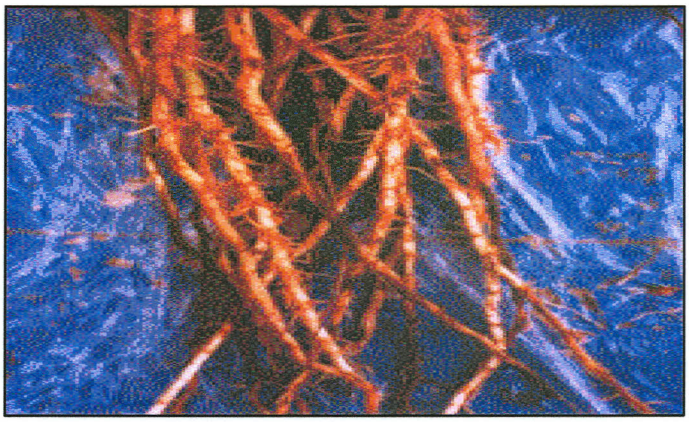


Plate 3. Disease severity of <1 on Pan5195, a tolerant variety to lesion nematodes (Photo Arim O. J)

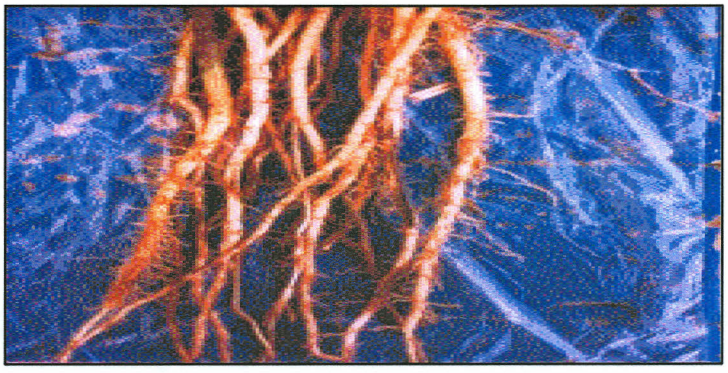


Plate 4. Disease severity of 1<2 on H627, a moderately tolerant variety to lesion nematodes (Photo Arim O. J)

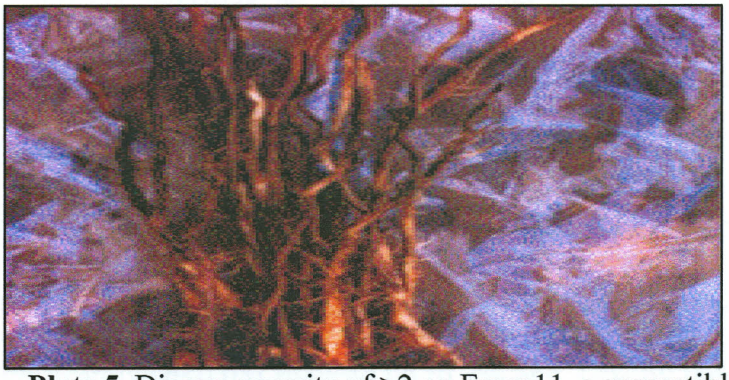


Plate 5. Disease severity of >2 on Emap11, a susceptible variety to lesion nematodes (Photo Arim O. J)

4.3 Greenhouse test 2: The damage potential of varying nematode densities on maize

4.3.1 Plant growth assessment

Increasing nematode inoculum density had significant ($p<0.05$) effect on plant heights, fresh root weights and fresh and dry shoot weights of some varieties (Table 6). The highest (4000), intermediate (2000) and lowest (1000) nematode inoculum densities reduced Phb plant height by 11%, 3% and 2%, respectively. Plant heights of H513, Pan5195, H625, HB614, Pan5355 and Emap11 were not affected by nematode density of 1000 and 2000 but were significantly ($p<0.05$) reduced by nematode density of 4000 by between 1% and 14% (Table 6). The highest nematode density significantly ($p<0.05$) reduced fresh shoot weights of all varieties except Pan5195 and H627 by between 5% and 10%. Pan5195 and H627 dry shoot weights were not significantly ($p<0.05$) affected by increasing nematode density (Table 6). The highest, intermediate and lowest nematode densities significantly reduced dry shoot weight of HB614 by 29%, 9% and 7%, respectively, and that of Phb by 23%, 9% and 5%, respectively (Table 6). Although H513, H625, Pan5355 and Emap11 dry shoot weights were not significantly ($p>0.05$) affected by the lowest density, they were significantly ($p<0.05$) reduced by the intermediate and highest densities by up to 10% and 40%, respectively. Increasing nematode density had no significant ($p>0.05$) effect on fresh root weight of Pan5195 (Table 6). The highest, intermediate and lowest densities significantly reduced the root weight of Phb by 40%, 19% and 6%, respectively, and that of HB614 by 29%, 12% and 4%, respectively (Table 6). Fresh root weights of H513, H627, H625, Pan5355 and Emap11 were significantly ($p<0.05$) reduced up to 7% and 37% by the intermediate and

highest densities, respectively, while the lowest density had no significant ($p>0.05$) effects.

Increasing nematode density led to significant reduction in plant growth of H513, H625, HB614, Pan5355, Phb and Emap11 but had no effect on H627 and Pan5195 possibly due to inherent genetic differences between the varieties. These results validate Ko *et al.*, (1984) findings which reported increased growth suppression with increasing inoculum density of *Heterodera glycines*. The reduction in plant growth can be attributed to increased nematode population in roots with increasing nematode density. High nematode population in roots exerts significant energy demand on the crop for nematodes' growth and reproduction (Melakeberhan & Webster, 1993). In addition, low root biomass under high nematode density, as observed in the current study, results in reduced and imbalanced production of growth hormones (Carmi & Heuer, 1981), impedes uptake of water, nutrient and their translocation. These subsequently reduce the rate of physiological processes such chlorophyll synthesis and photosynthesis resulting in diminishing crop productivity.

Table 6. Mean† plant heights, fresh shoot, dry shoot and fresh root weights under varying nematode densities (Pi) ††, 99 days after inoculation, greenhouse test 2.

Maize varieties	Nematode density	Plant height (cm)	Fresh shoot weight (g)	Dry shoot Weight (g)	Fresh root weight (g)
H513					
	0	138.2	172.6	26.2	25.5
	1000	137.8 (0.3)[b]	172.1 (0.3)	25.6 (2)	24.9 (2)
	2000	137.7 (0.4)	171.7 (0.5)	24.6 (6)	23.9 (6)
	4000	122.0 (12)	161.5 (6)	19.0 (27)	16.0 (37)
Pan5195					
	0	119.4	182.3	25.4	23.3
	1000	118.8 (0.5)	182.1 (0.1)	24.9 (2)	23.2 (0.4)
	2000	118.1 (1)	181.8 (0.3)	24.2 (5)	23.2 (0.4)
	4000	117.9 (1)	181.4 (0.5)	24.0 (6)	23.1 (0.9)
H627					
	0	131.4	183.4	22.2	26.7
	1000	131.0 (0.3)	183.2 (0.1)	22.0 (0.9)	25.8 (3)
	2000	130.8 (0.5)	182.7 (0.4)	21.9 (1)	24.9 (7)
	4000	130.5 (0.7)	182.6 (0.4)	21.5 (3)	23.6 (12)
H625					
	0	117.6	208.0	22.5	32.7
	1000	117.0 (0.5)	207.6 (0.2)	21.9 (3)	32.4 (0.9)
	2000	116.9 (0.6)	207.1 (0.4)	20.3 (10)	30.8 (6)
	4000	104.7 (11)	197.1 (5)	14.3 (36)	24.3 (26)
HB614					
	0	129.0	184.5	25.0	27.3
	1000	128.7 (0.2)	183.8 (0.4)	23.3 (7)	26.2 (4)
	2000	128.5 (0.4)	182.4 (1)	22.9 (8)	23.9 (12)
	4000	113.5 (12)	172.9 (6)	17.8 (29)	19.5 (29)
Phb					
	0	117.5	163.8	21.3	40.0
	1000	115.7 (2)	162.8 (0.6)	20.1 (6)	37.5 (6)
	2000	113.7 (3)	162.8 (0.6)	19.4 (9)	32.3 (19)
	4000	104.1 (11)	149.1 (9)	16.4 (23)	24.0 (40)
Pan5355					
	0	111.7	153.2	20.9	42.4
	1000	111.1 (0.5)	152.7 (0.3)	20.3 (3)	41.8 (1)
	2000	110.9 (0.7)	152.1 (0.7)	19.2 (8)	39.7 (6)
	4000	96.0 (14)	139.9 (9)	16.4 (22)	33.9 (20)
Emap11					
	0	151.6	162.9	24.3	34.5
	1000	151.3 (0.2)	162.8 (0.1)	24.2 (0.4)	34.5 (0)
	2000	151.2 (0.3)	162.8 (0.1)	22.9 (6)	32.8 (5)
	4000	146.4 (3)	147.4 (10)	14.5 (40)	27.7 (20)
$LSD_{(0.05)}$		1.31	1.18	1.08	0.94
SED		0.65	0.59	0.54	0.47
CV %		0.60	0.40	3.10	2.00

†Mean for six replication

††Nematode density at planting

[b] values in parentheses are percent (%) reduction in growth parameters at different nematode densities

4.3.2 Plant disease assessment

Number of nematodes in maize roots of all varieties significantly ($p<0.05$) increased with increasing nematode density (Figure 3). H513, H625, Pan5355 and Emap11 had less than 500-nematodes in their roots under the lowest nematode inoculum density but had more than 500-nematodes under the intermediate and highest nematode densities. HB614 had less than 500-nematodes under the lowest and intermediate nematode densities but had more than 500-nematodes under the highest nematode density. Phb supported more than 500-nematodes in it's roots under the three nematode inoculum densities. Pan5195 and H627 had less than 500-nematodes in their roots under the three nematode densities (Figure 3). Root necrosis significantly ($p<0.05$) increased discriminately among varieties with increasing nematode density (Figure 4). Root necrosis of H625, Phb and Pan5355 increased by up to 323%, 276% and 233%, respectively, with increasing nematode density (Figure 4). In addition, number of nematodes in roots was highly correlated (r=0.91; $p=0.05$) to root necrosis.

Increased number of nematodes in roots resulting from increasing nematode inoculum densities validates findings by Griffin & Jensen (1997) which showed a positive correlation (r=0.92; $p=0.05$) between increasing *Pratylenchus neglectus* inoculum density and invasion of alfalfa root. The increased nematode population in roots at high nematode densities can be attributed to increased root penetration and enzymatic activities (Wallace, 1973). Lesion nematodes kill cells as they feed and migrate within the root tissue. This causes extensive root destruction (root necrosis) especially when nematode population in the root is high (Luc *et al.*, 1993) and could have

accounted for increased root necrosis observed in H625, Phb and Pan5355 varieties under high nematode densities.

In all varieties, there was an increase in nematode reproduction with increasing nematode density. However, the rate (Rf) of increase in nematode reproduction decreased with increasing nematode density (Figure 5). This corroborates the results by Griffin and Gray (1990) which revealed that the reproduction rate of *Pratylenchus neglectus* on alfalfa decreased with increasing nematode inoculum densities. The decreasing rate of nematode reproduction can be attributed to diminishing availability of root mass per nematode with increasing inoculum densities. In addition, severe root damage at 2000 and 4000 nematodes per pot could have affected nematode reproduction.

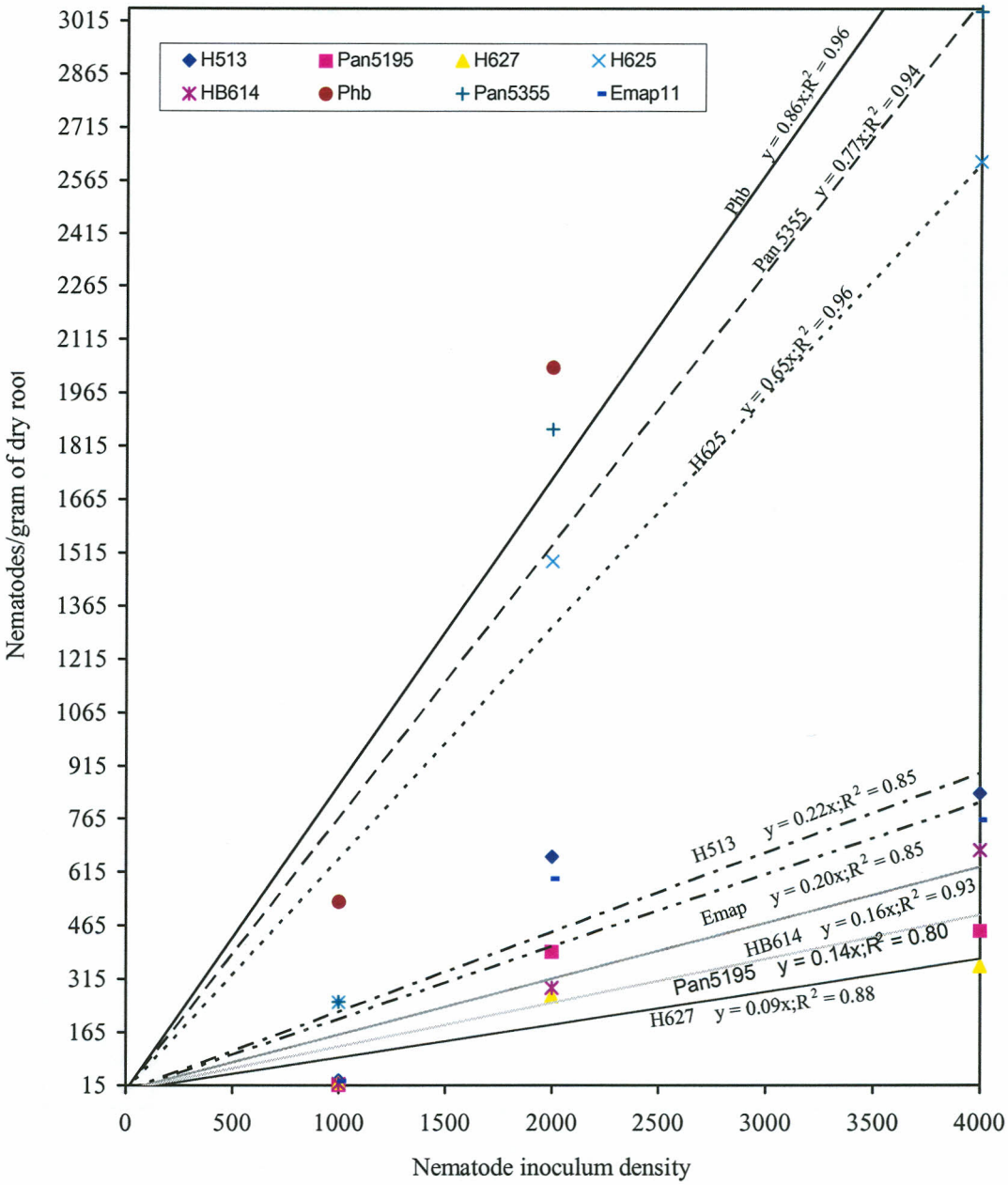


Figure 3. Relationship between nematode inoculum density and nematode population in roots, 99 days after inoculation, greenhouse test 2.

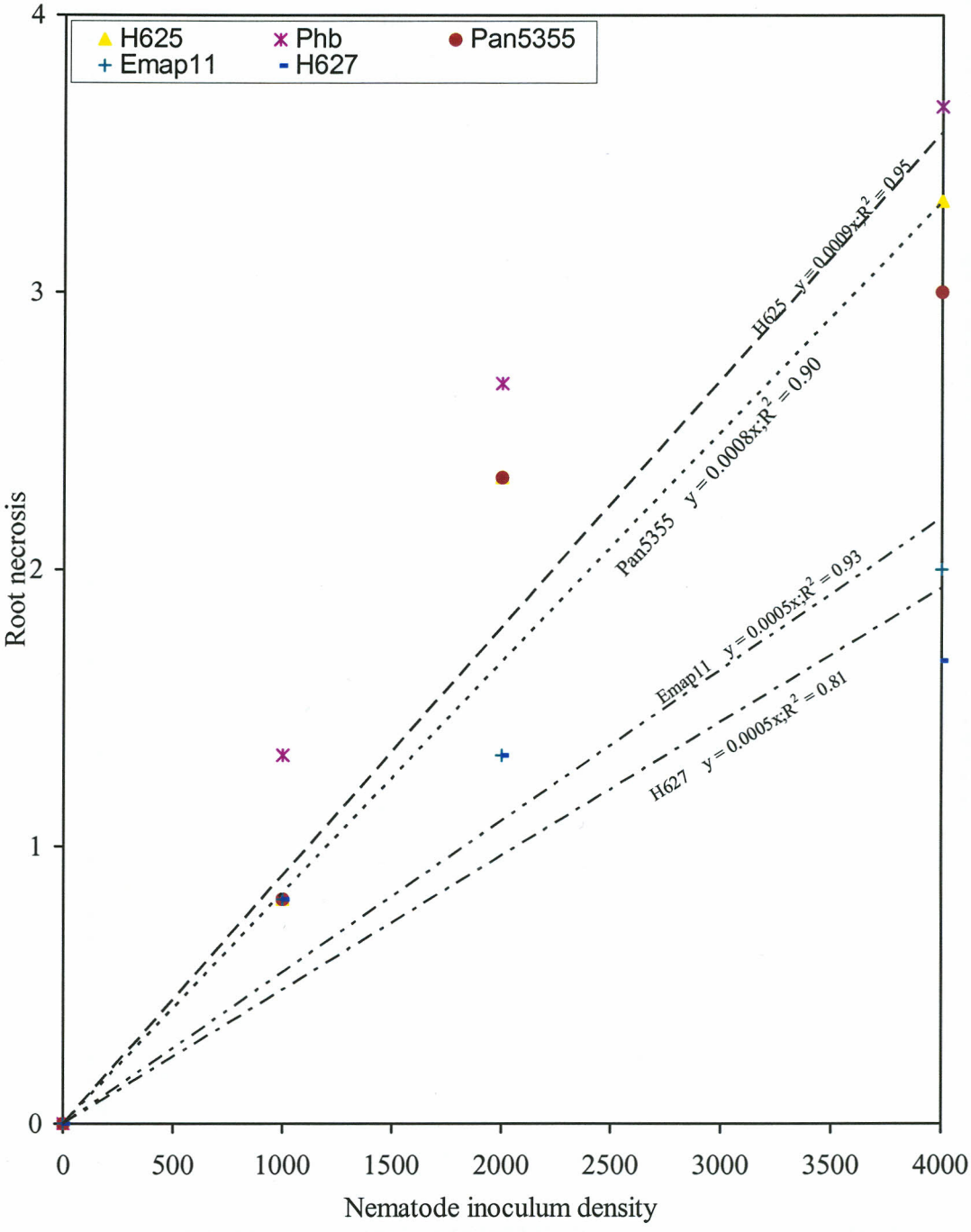


Figure 4. Relationship between nematode inoculum density and root necrosis, 99 days after inoculation, greenhouse test 2.

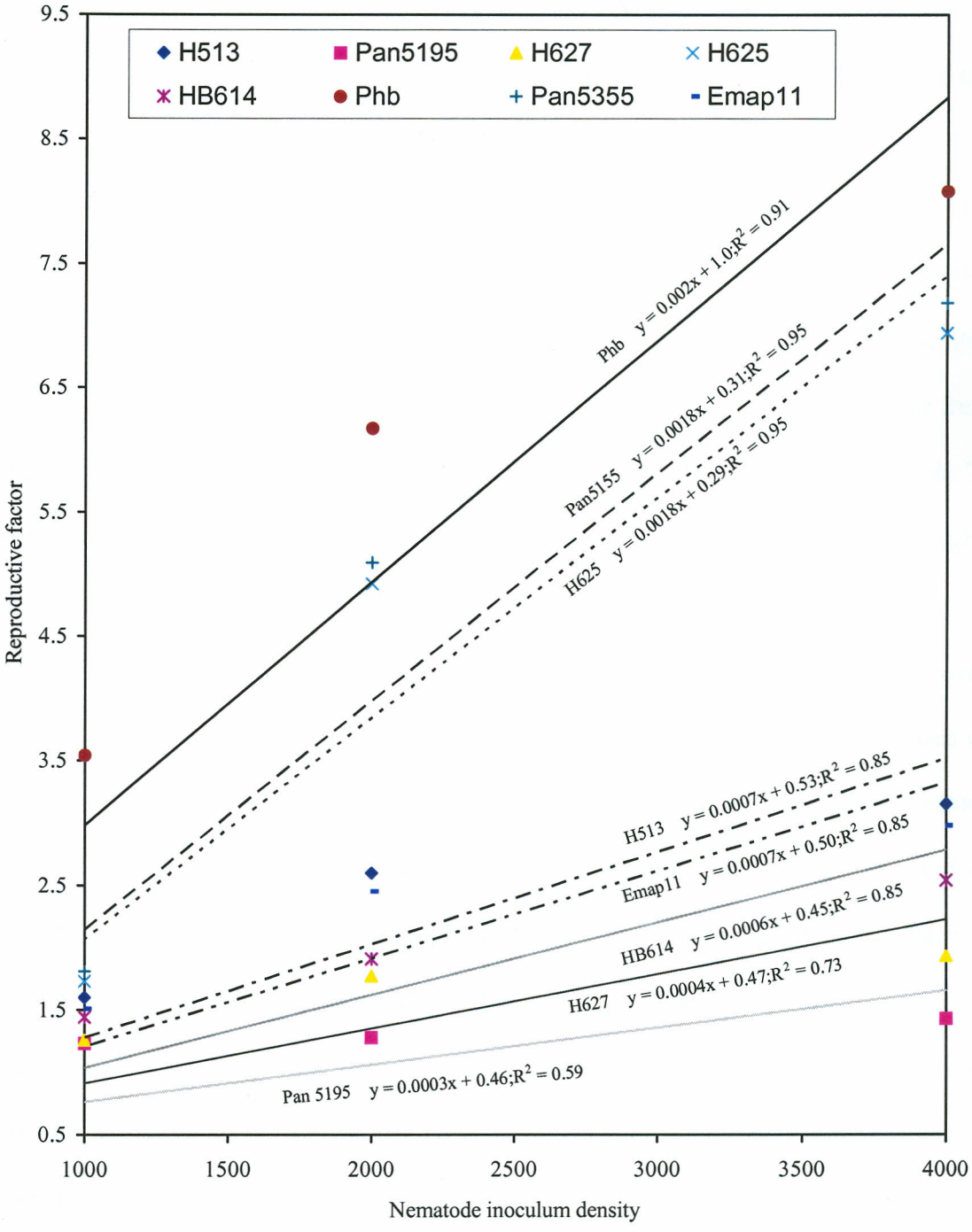


Figure 5. Relationship between nematode inoculum density and reproductive factor, 99 days after inoculation, greenhouse test 2.

4.4 Greenhouse test 3: Response of leguminous crops to lesion nematodes

4.4.1 Plant growth and disease assessment

Highly significant ($p<0.001$) differences in plant heights, fresh root weights and fresh and dry shoot weights were observed between inoculated and non-inoculated *C. ochroleuca* plants. Non-inoculated *C. ochroleuca* plants were taller than inoculated plants by 14% (Table 7). In addition, non-inoculated *C. ochroleuca* plants had higher fresh root weight, dry shoot and fresh shoot weights than inoculated plants by 43%, 36% and 25%, respectively. Non-inoculated *L. purpureus* plants had significantly higher fresh root and shoot weights than inoculated plants by 18% and 5%, respectively (Table 7). There were no significant ($p>0.05$) differences in plant heights and dry shoot weights between inoculated and non-inoculated plants of *L. purpureus*.

Significant differences in plant heights, fresh shoot and root weights between inoculated and non-inoculated plants attest to the significant influence of nematodes on growth and development of leguminous crops. Reduced heights observed in nematode inoculated plants of *C. ochroleuca* and low weights observed in *L. purpureus* and *C. ochroleuca* could be attributed to disruption of plants' physiological processes such as chlorophyll synthesis and photosynthesis leading to reduced heights and low crop biomass. The results on plant disease assessment indicate that nematode reproductive factor (Rf) significantly ($p<0.05$) differed in all the cover crops. *Canavalia ensiformis*, *M. pruriens*, *L. purpureus* and *C. ochroleuca* had nematode reproductive factors of 0.79, 0.95, 1.23 and 2.09, respectively (Table 8). From these results, *L. purpureus* supported an increase in nematode population (R.f >1) with no significant effect on its dry biomass (Table 7) suggesting that this variety has tolerant limit of above 1- nematode per cm^3 of

soil (Seinhort, 1967; Dropkin, 1955). Nematode population declined (Rf<1) under the two leguminous crops (Table 8) indicating that the two legumes are non-hosts to lesion nematodes (Seinhort, 1967). On the contrary, nematode population increased (Rf>1) under *C. ochroleuca* (Table 8) and subsequently decreased the it's biomass (Table 7) suggesting that this variety was susceptible and good host of lesion nematodes.

Table 7. Mean* plant heights, fresh shoot, dry shoot and fresh root weights of cover crops, 60 days after inoculation, greenhouse test 3.

Cover crops	Plant parameters	Inoculated	Non-inoculated	% change	*t*-test (means)
Canavalia ensiformis	Plant height	179.00	179.50	-0.3	NS
	Fresh shoot weight	45.22	45.75	-1.0	NS
	Dry shoot weight	10.19	11.05	-8.0	NS
	Fresh root weight	2.89	2.97	-3.0	NS
Mucuna pruriens	Plant height	277.50	278.83	-1.0	NS
	Fresh shoot weight	63.05	64.00	-1.0	NS
	Dry shoot weight	12.30	13.33	-8.0	NS
	Fresh root weight	13.61	14.42	-6.0	NS
Lablab purpureus	Plant height	199.60	203.25	-2.0	NS
	Fresh shoot weight	37.14	39.10	-5.0	S
	Dry shoot weight	7.97	9.09	-12.0	NS
	Fresh root weight	7.50	9.13	-18.0	S
Crotalaria ochroleuca	Plant height	110.00	127.26	-14.0	HS
	Fresh shoot weight	21.54	28.90	-25.0	HS
	Dry shoot weight	5.32	8.27	-36.0	HS
	Fresh root weight	5.62	9.92	-43.0	HS

* Mean of six replications.

NS-Not significant ($p>0.05$), S-significant ($p<0.05$), HS-Highly significant ($p<0.001$)

Nematode population in the roots of the crops significantly ($p<0.05$) differed. Number of nematodes in roots of *C. ensiformis* was significantly lower by 66% and 44%

compared to *C. ochroleuca* and *L. purpureus*, respectively (Table 8). Nematode population in roots of *M. pruriens* was significantly lower by 60% and 34% compared to *C. ochroleuca* and *L. purpureus*, respectively (Table 8). No significant (p>0.05) difference in number of nematodes in roots was observed between *C. ensiformis* and *M. pruriens*. Nematode population in roots was positively and highly correlated (r^2=0.85; p=0.05) to root necrosis which was 30% and 28% lower in *C. ensiformis* compared *C. ochroleuca* and *L. purpureus*, respectively (Table 8). This results validate report by Luc *et al.* (1993) which associated severe root necrosis with high population of extensively damaging nematode species such as the lesion nematodes. Nematode population in under cover crops significantly (p<0.05) differed. Nematode population in the soil under *C. ochroleuca* was higher by 146% and 121% compared to *C. ensiformis* and *M. pruriens*, respectively (Table 8). *Lablab purpureus* supported (up to 48%) significantly higher number of nematodes in the soil compared to *C. ensiformis* and *M. pruriens*. However, nematode population in the soil under *C. ensiformis* and *M. pruriens* was not significantly (p>0.05) different (Table 8).

Low nematode population in the roots has been attributed to nematodes exiting the roots due to lack of readily available food or inadequate food or inability to establish normal feeding sites (Huang, 1985). While such speculation requires further experimentation, decline in nematode population under *C. ensiformis* and *M. pruriens* could also be attributed to the negative effects of phytochemicals produced by legumes. The germinating seedlings of the two legumes release alkaloids (Bisby *et al.*, 1994) which have nematicidal effects on *P. penetrans* and *P. zeae*. These effects could have attributed to low nematode population in the root and soil under *C. ensiformis* and *M.*

pruriens. Although the alkaloids mode of action on nematodes is scantly documented, they cause feeding deterency in insect pests (Grewal *et al.*, 1995). Bioactive natural products such as triacontanol and tricontyl tetracosanate, which are present in some *Mucuna* spp., have nematicidal effect causing reduced mobility, paralysis and death of juveniles (Marisa *et al.*, 1996).

Table 8. Mean* root necrosis, nematode population in root and nematode reproductive factor (Rf) for legumes, 60 days after inoculation, greenhouse test 3.

Legumes	Root necrosis†	Nem./gram of dry roots	Nem./200cm^3 of soil	(Rf) **	Host status
C. ensiformis	0.7	125	194	0.8	N
M. pruriens	0.8 (-12) [b]	147 (-15)	216 (-11)	0.9 (-11)	N
L. purpureus	0.9 (-22)	222 (-44)	288 (-33)	1.2 (-33)	M
C. ochroleuca	1.0 (-30)	371 (-66)	477 (-59)	2.1 (-62)	G
$LSD_{(0.05)}$	0.13	40	28	0.11	
SED	0.07	20	13	0.06	
CV(%)	12	15	0.02	7	

*Mean of six replications.

†Necrosis index: 0-no root damage, 1-slight root damage, 2-moderate root damage, 3-severe root damage, 4-very severe root damage (Bridge and Gowen, 1993).

**Rf > 10-excellent host (E), 10 ≥ Rf ≥1.5-good host (G), 1≤ Rf<1.5-maintenance host (M) and Rf <1- non-host (N) (Ferris *et al.*, 1993).

[b] values in parentheses are percent (%) of disease assessment parameters in comparison *C. ensiformis*.

4.5 Effect of intercropping maize with non-host legumes in the greenhouse and field

4.5.1. Greenhouse test 4: Maize plant growth assessment

Inter-cropping maize with leguminous crops significantly ($p<0.05$) increased discriminately plant heights, fresh root weights and fresh and dry shoot weights of varieties in the greenhouse test (Table 9). Inter-cropping *C. ensiformis* with Emap11 significantly ($p<0.05$) increased plant height by 5% but had no significant effect on Pan5195 and H627 plant heights (Table 9). Significant ($p<0.05$) differences in fresh shoot weights were observed among the cropping systems of H627 and Emap11 but not Pan5195. Fresh shoot weight of H627 inter-cropped with *C. ensiformis* was significantly ($p<0.05$) higher than the control by 4% (Table 9). Inter-cropping Emap11 with either *C. ensiformis* or *M. pruriens* significantly ($p<0.05$) increased fresh shoot weights by 8% and 5%, respectively, (Table 9). Dry shoot weights significantly ($p<0.05$) differed among the cropping systems of Pan5195 and Emap11 but not in H627. Inter-cropping Pan5195 with *C. ensiformis* significantly ($p<0.05$) increased dry shoot weight by 11% (Table 9). *Canavalia ensiformis* and *M. pruriens* significantly ($p<0.05$) increased dry shoot weight of Emap11 by 34% and 21%, respectively (Table 9). There were no significant ($p>0.05$) difference in fresh root weights among the cropping systems Emap11, H627 and Pan5195 (Table 9).

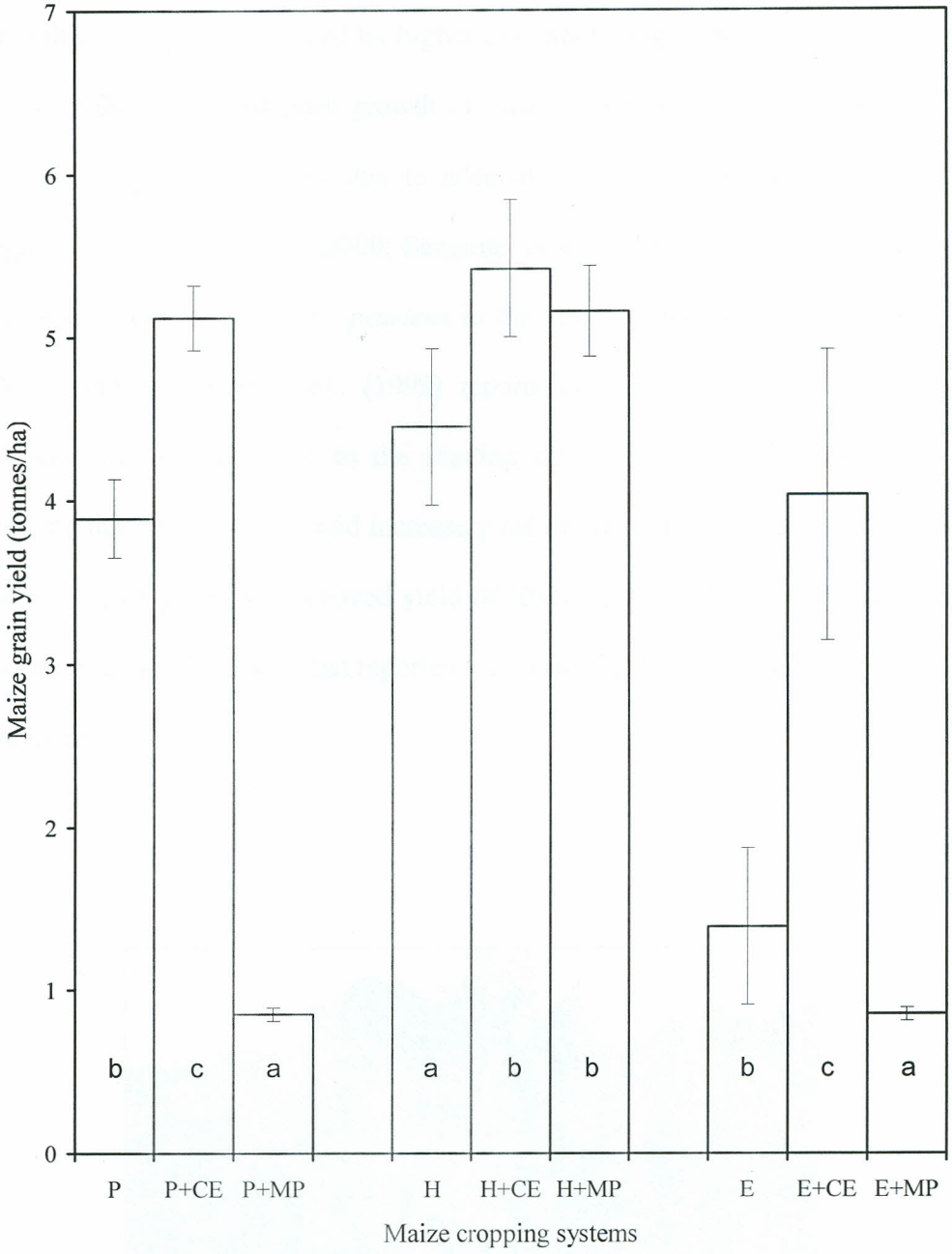
Table 9. Mean† plant heights, fresh shoot, dry shoot and fresh root weights of Pan5195, H627 and Emap11 in sole stand and intercropped with either *C. ensiformis* or *M. pruriens* (MP), 90 days after inoculation, greenhouse test 4.

Treatment	Plant height(cm)	Fresh shoot weight(g)	Dry shoot weight(g)	Fresh root weight(g)
Pan5195	99.8	97.7	14.9	28.1
Pan5195+MP	100.8 (1) [b]	99.0 (1)	15.7 (5)	28.4 (1)
Pan5195+CE	101.3 (2)	99.9 (2)	16.6 (11)	28.8 (2)
H627	117.7	95.0	14.5	33.8
H627+MP	119.3 (1)	97.5 (3)	15.5 (7)	34.1 (1)
H627+CE	120.0 (2)	99.2 (4)	15.9 (10)	34.4 (2)
Emap11	91.2	86.2	10.8	30.1
Emap11+MP	94.0 (3)	90.2 (5)	13.1 (21)	30.6 (2)
Emap11+CE	96.2 (5)	92.7 (8)	14.5 (34)	30.8 (2)
$LSD_{(0.05)}$	2.96	3.70	1.49	1.27
SED	1.47	1.83	0.74	0.63
CV(%)	2.4	3.3	8.7	3.5

† Mean for six replications. [b] values in parentheses are percent (%) increase in growth assessment parameters in comparison to the respective unintercropped maize

4.5.2. On-farm test 1: Maize grain yield assessment

Maize grain yield significantly ($p<0.05$) differed among the cropping systems in the on-farm test (Figure 6). Inter-cropping Emap11 and Pan5195 with *C. ensiformis* significantly ($p<0.05$) increased maize grain yield by 190% and 29%, respectively, compared to the respective monocrop. Maize grain yield of Pan5195 and Emap11 inter-cropped with *M. pruriens* was significantly ($p < 0.05$) lower than that of the respective monocrop by 79% and 40%, respectively. Inter-cropping H627 with either *C. ensiformis* or *M. pruriens* significantly ($p<0.05$) increased maize yield by 22% and 12%, respectively. There was no significant ($p>0.05$) difference between maize grain yield of H627 inter-cropped with either *C. ensiformis* or *M. pruriens* (Figure 6).



Bar means for a maize cropping systems followed with the same letter aren't significantly different.

Figure 6. Mean grain yield of Pan5195 (P), H627 (H) and Emap11 (E) in sole stand and intercropped with either *C. ensiformis* (CE) or *M. pruriens* (MP), 150 days after planting, on-farm test 1.

The study revealed that inter-cropping maize with legumes improved plant growth of maize as evidenced by higher dry shoot weights and grain yields (Michael *et al.*, 1986). Increased plant growth of inter-cropped maize both in the greenhouse and on-farm tests could be due to adequate supply of nitrogen by the N_2-fixing legumes (Wortmann *et al.*, 2000; Sanginga *et al.*, 1996). However, reduced yield of maize inter-cropped with *M. pruriens* in the current study validates Gachene *et al.*, (2000) and Manyong *et al.*, (1999) reports on the potential of *Mucuna* spp. to suppress maize yields due to the shading effect (Plate 6). The results, however, indicate that *M. pruriens* could increase yield of tall maize varieties such as H627. In this study, *M. pruriens* improved yield of H627 by 12%. This corroborates findings by Onyango *et al.*, (2000) that reported increased yield of maize intercropped with *M. pruriens*.



Plate 6. Shading effect of *M. pruriens* in a maize inter-crop, on-farm test 1.

4.5.3. Nematode disease assessment: Greenhouse test 4 and On-farm test 1

There was significant ($p<0.05$) difference in disease severity (root necrosis) among the cropping systems in the greenhouse test (Table 10) and on-farm test (Table 11). Inter-cropping Emap11 with either *C. ensiformis* or *M. pruriens* significantly ($p<0.05$) reduced disease severity by 26% and 23%, respectively (Table 10). These results are consistent with on-farm test in which inter-cropping Emap11 with either *C. ensiformis* significantly ($p<0.05$) reduced disease severity by 23%, respectively (Table 11). There was no significant ($p>0.05$) difference in disease severity among the cropping systems of Pan5195 and H627 in the greenhouse (Table 10). Contrary, Pan5195 and H627 cropping systems significantly ($p<0.05$) differed in disease severity in the on-farm test (Table 11). Pan5195 inter-cropped with either *C. ensiformis* or *M. pruriens* had significantly ($p<0.05$) lower disease severity compared to sole maize by 50% and 35%, respectively (Table 11). Inter-cropping H627 with *C. ensiformis* and *M. pruriens* significantly ($p<0.05$) reduced disease severity by 43% and 26%, respectively (Table 11).

Table 10. Mean* root necrosis, nematode population in roots and soil, nematode reproductive factor (R.f) and nematode suppressive ability ((NSA) of Pan5195, H627 and Emap11 in sole stand and intercropped with either *C. ensiformis* (CE) or *M. pruriens* (MP), 90 days after inoculation, greenhouse test 4.

Treatments	Root necrosis†	Nem./g dry root	Nem./200cm^3 of soil	R.f	NSA**
Pan5195	0.96	419	378	1.34	S
Pan5195+MP	0.81 (16) [b]	410 (2)	194 (49)	0.93 (31)	MS
Pan5195+CE	0.76 (21)	390 (9)	167 (56)	0.84 (37)	MS
H627	0.90	305	626	1.91	LS
H627+MP	0.86 (4)	255 (16)	239 (62)	0.95 (50)	MS
H627+CE	0.81 (10)	208 (32)	234 (63)	0.86 (55)	MS
Emap11	1.17	652	864	2.93	LS
Emap11+MP	0.90 (23)	476 (27)	131 (85)	0.95 (68)	MS
Emap11+CE	0.86 (26)	456 (30)	117 (86)	0.87 (70)	MS
$LSD_{(0.05)}$	0.23	16	17	0.03	
SED	0.11	8	8	0.01	
CV(%)	22.1	3.5	4	1.9	

*Mean for six replications.

†Root necrosis: 0-no root damage, 1-slight root damage, 2-moderate root damage, 3-severe root damage, 4-very severe root damage (Bridge and Gowen, 1993).

**Rf <1-Most suppressive (MS), 1≤ Rf <1.5- suppressive (S) and Rf ≥ 1.5-least suppressive (LS) (Ferris *et al.*, 1993).

[b] values in parentheses are percent (%) decrease in disease assessment parameters in comparison to the respective unintercropped maize.

Table 11. Mean* root necrosis and nematode population in roots of Pan5195, H627 and Emap11 in sole stand and intercropped with either *C. ensiformis* (CE) or *M. pruriens* (MP), on-farm test 1.

Treatments	Root necrosis†	Nematodes in roots
Pan5195	2.0	470
Pan5195+MP	1.3 (35)[b]	439 (7)
Pan5195+CE	1.0 (50)	417 (11)
H627	2.3	350
H627+MP	1.7 (26)	271 (23)
H627+CE	1.3 (43)	245 (30)
Emap11	3.0	656
Emap11+MP	2.7 (10)	491 (25)
Emap11+CE	2.3 (23)	488 (26)
$LSD_{(0.05)}$	0.6	78
SED	0.3	37
CV(%)	17.7	11

*Mean for six replications.

†Root necrosis: 0-no root damage, 1-slight root damage, 2-moderate root damage, 3-severe root damage, 4-very severe root damage (Bridge and Gowen, 1993).

[b]values in parentheses are percent (%) decrease in root necrosis and nematode population in roots in comparison to the respective unintercropped maize.

Nematode population in roots of maize significant ($p<0.05$) differed among the cropping systems in the greenhouse test (Table 10) and on-farm test (Table 11). Inter-cropping H627 with either *C. ensiformis* or *M. pruriens* in the greenhouse significantly ($p<0.05$) lowered nematode population in the root compared to sole H627 by 32% and 16%, respectively (Table 10). These results are consistent with on-farm test which revealed that inter-cropping H627 with either *C. ensiformis* or *M. pruriens* significantly ($p<0.05$) reduced nematode population in roots of maize by 30% and 23%, respectively

(Table 11). *Canavalia ensiformis* and *M. pruriens* significantly ($p<0.05$) reduced nematode population in roots of Emap11 by 30% and 27%, respectively (Table 10). Similar results were observed on-farm where Emap11 inter-cropped with either *C. ensiformis* or *M. pruriens* had significantly ($p<0.05$) lower nematode population in roots compared to sole maize by 26% and 25%, respectively (Table 11). In addition, inter-cropping Emap11 with either *C. ensiformis* or *M. pruriens* reduced nematode population in roots to below 500-nematodes/g of dry root (Table 10). Similar reduction in nematode population in roots of Emap11 intercropped with either *C. ensiformis* or *M. pruriens* was observed in the on-farm test (Table 11). The results of this study indicate that *C. ensiformis* was more effective than *M. pruriens* in reducing nematode population in roots of maize (Table 10). Inter-cropping H627, Pan5195 and Emap11 with *C. ensiformis* significantly ($p<0.05$) reduced nematode population in roots of maize by 18%, 5% and 4%, respectively, compared to the respective maize- *M. pruriens* inter-crops (Table 10).

Inter-cropping maize with leguminous crops significantly ($p<0.05$) reduced nematode population in soil in the greenhouse test (Table 10) and on-farm test (Figure 7). Inter-cropping Emap11, H627 and Pan5195 with *C. ensiformis* significantly ($p<0.05$) reduced nematode population in the soil by 86%, 63% and 56%, respectively, compared to the respective sole maize (Table 10). These results were consistent with on-farm test which revealed that inter-cropping Pan5195, H627 and Emap11 with *C. ensiformis* significantly ($p<0.05$) reduced nematode population in the soil by 13%, 10% and 6%, respectively, 90 days after planting (Figure 7). Inter-cropping maize with *C. ensiformis* was more effective in reducing nematode population in the soil both in the greenhouse (Table 10) and on-farm (Figure 7).

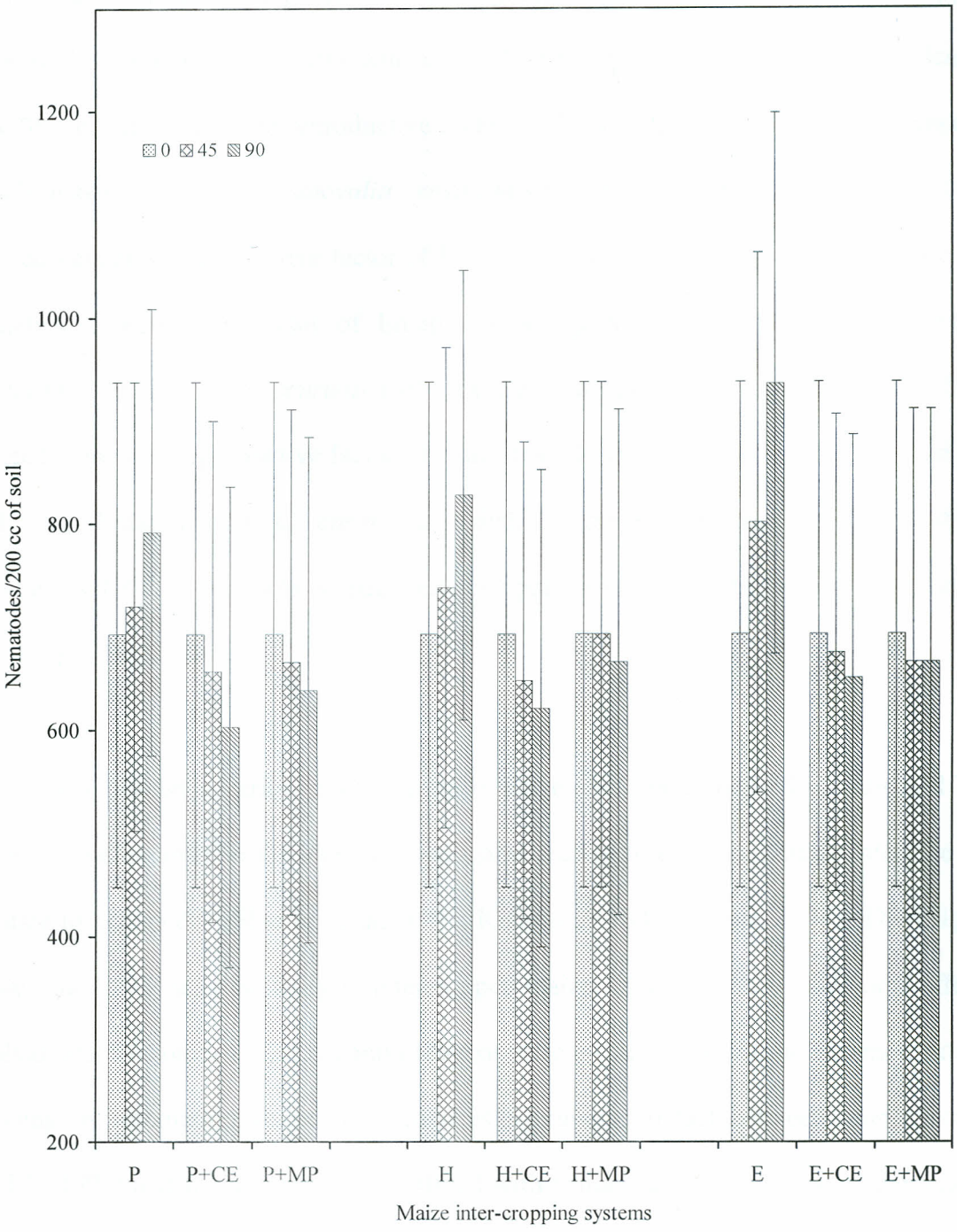


Figure 7. Mean nematode population in soil at planting (0) and at 45 and 90 days after interplanting Pan5195 (P), H627 (H) and Emap11 (E) with *C. ensiformis* (CE) and *M. pruriens* (MP), on-farm test 1.

The rate of nematode reproduction (Rf) significantly ($p<0.05$) varied among cropping systems. Inter-cropping Pan5195 with either *C. ensiformis* or *M. pruriens* significantly ($p<0.05$) reduced nematode reproductive factor by 37% and 31%, respectively, compared to sole maize (Table 10). *Canavalia ensiformis* and *M. pruriens* significantly ($p<0.05$) reduced nematode reproductive factor of H627 by 55% and 50%, respectively (Table 10). Nematode reproductive factor of Emap11 was significantly ($p<0.05$) reduced by *C. ensiformis* (70%) and *M. pruriens* (68%). *Canavalia ensiformis* significantly ($p<0.05$) reduced nematode reproductive factor of maize than *M. pruriens*. Inter-cropping Pan5195, H627 and Emap11 with *C. ensiformis* reduced nematode reproductive factor of the varieties by 10%, 9% and 8%, respectively, compared to the respective maize- *M. pruriens* inter-crops (Table 10).

Low disease severity in inter-cropped maize could be attributed to increased N_2 supply by the legumes to the inter-cropped maize leading to enhanced maize tolerance or resistant to nematodes (Sharma *et al.*, 1997; Ko *et al.*, 1984; Wallace, 1973). This might explain the low disease severity in intercropped maize. In addition to nematicidal effect of alkaloids, triacontanol and tricontyl tetracosanate emanating from the legumes, inter-cropping by alternating single row produces maximum contact between crop species (RAFR, 1991) and this might have interfered with nematodes ability to locate the maize roots (Risch *et al.*, 1983). This also could have accounted for low root infection (hence low nematode population in roots) and disease severity in the inter-cropping systems. Although *C. ensiformis* has a tap root (Fischler, 1997), the root systems for maize and the two legumes is concentrated within 30 cm of the plow layer (Jose *et al.*, 2000).

Inter-cropping maize with other leguminous crops such as soybeans increases bacterial population in the rhizosphere of maize (Keswani *et al.*, 1977). Naturally occurring rhizobacteria might have had significant antagonistic effects on plant parasitic nematodes (Sikora, 1992) and could have led to low nematode population in the rhizosphere of inter-cropped maize. Whilst it's generally accepted that nematodes' movement in the soil is limited, reduced nematode immigration and increased emigration from a maize row between rows of non-host legumes is likely to occur (Risch *et al.*, 1983; Altiera & Letournean, 1982). Such a phenomenon would result in low nematode population similar to that observed in the inter-cropped maize compared to that of maize monocrop. Low nematode population in the inter-cropping systems can also be attributed to the low nematode reproductive factor. The rate of nematode multiplication is dependent on the initial nematode density (Seinhorst, 1967). When the initial nematode density is high, there is a considerably high rate of multiplication than at lower initial densities. The low nematode population in roots of inter-cropped maize might therefore, have been unfavorable for rapid nematode multiplication in the inter-cropping systems. This speculation however, needs to be confirmed by further experimentation.

The results of this study indicated that maize-*C. ensiformis* inter-crops were more effective than those of *M. pruriens* in reducing nematode population in the soil, roots and alleviating disease severity in maize. The apparent superiority of *C. ensiformis* over *M. pruriens* could be attributed to two factors. *Canavalia ensiformis* takes up more P from the soil than *M. pruriens* (Wortmann *et al.*, 2000), this is likely to impede increase in lesion nematode population since P favours increase in lesion nematodes population (Yeates, 1976). In addition, when inter-cropped with maize, *C. ensiformis* fixes more

nitrogen than *M. pruriens* (Wortmann *et al.*, 2000), under such circumstances maize inter-cropped with *C. ensiformis* would be more tolerant to nematodes and therefore, suffer less disease severity (Sharma *et al.*, 1997).

4.6. Efficacy of soil amendments against lesion nematodes

4.6.1. Greenhouse test 5: Maize plant growth assessment

Soil amendments significantly ($p<0.05$) increased discriminately plant heights, fresh root weights and fresh and dry shoot weights of different varieties in the greenhouse test (Table 12). Cattle manure, *M. pruriens* and *C. ensiformis* significantly ($p<0.05$) increased plant heights of Pan5195 by 8%, 7% and 3%, respectively (Table 12). In addition, cattle manure and *M. pruriens* significantly ($p<0.05$) increased plant heights of Emap11 by 11% and 6%, respectively, but *C. ensiformis* did not. Cattle manure was more effective in increasing plant heights of Emap11 than *C. ensiformis* and *M. pruriens* by 8% and 5%, respectively (Table 12).

In soils amended with cattle manure, *M. pruriens* and *C. ensiformis*, fresh shoot weights of Pan5195 were significantly ($p<0.05$) higher than the controls by 25%, 19% and 18%, respectively. Cattle manure, *M. pruriens* and *C. ensiformis* amended soils increased fresh shoot weights of Pan5195 equally. Cattle manure, *M. pruriens* and *C. ensiformis* amended soils significantly ($p<0.05$) increased fresh shoot weights of Emap11 by 32%, 28% and 23%, respectively. Cattle manure was more effective in increasing fresh shoot weights of Emap11 by 8% than *C. ensiformis*. Cattle manure was as effective as *M. pruriens* while *C. ensiformis* was as effective as *M. pruriens* in increasing fresh shoot weights of Emap11 (Table 12).

Table 12. Mean[†] plant heights, fresh root weights, fresh and dry shoot weights of Pan5195 and Emap11 cultivated in unamended soils, soil amended with *M. pruriens* (MP), *C. ensiformis* (CE) and cattle manure (CM), 90 days after inoculation greenhouse test 5.

Treatment	Plant height (cm)	Fresh root weight (g)	Fresh shoot weight (g)	Dry shoot weight (g)
Pan5195	86.0	20.7	79.3	16.7
Pan5195+MP	92.3 (7)[b]	26.4 (27)	94.1 (19)	24.9 (49)
Pan5195+CE	88.3 (3)	26.1 (26)	93.3 (18)	23.0 (38)
Pan5195+CM	93.0 (8)	27.8 (34)	99.0 (25)	27.2 (63)
Emap11	105.2	26.2	73.3	10.8
Emap11+MP	111.0 (6)	31.9 (22)	93.8 (28)	20.5 (90)
Emap11+CE	108.0 (3)	30.4 (16)	90.0 (23)	19.4 (81)
Emap11+CM	116.8 (11)	32.2 (23)	97.1 (32)	22.2 (106)
$LSD_{(0.05)}$	4.7	1.9	5.6	2.6
SED	2.5	1.0	2.8	1.3
CV(%)	4.0	6.0	5.3	10.6

[†] Mean for six replications.

[b]values in parentheses are percent (%) increase in growth assessment parameters in comparison to the respective maize in non-amended soil.

Dry shoot weights differed significantly ($p<0.05$) among treatments. Cattle manure, *M. pruriens* and *C. ensiformis* significantly ($p<0.05$) increased dry shoot weights of Pan5195 by 63%, 49% and 38%, respectively. Cattle manure was more effective in increasing dry shoot weights of Pan5195 compared to *C. ensiformis* but was equally as effective as *M. pruriens* amended soils. Soil amended with cattle manure, *M. pruriens* and *C. ensiformis* significantly ($p<0.05$) increased dry shoot weights of Emap11 by 106%, 90% and 81%, respectively. Cattle manure significantly ($p<0.05$) increased dry shoot weights of Emap11 by 14% compared *C. ensiformis*. Amending soil with cattle

manure was as effective as *M. pruriens* in increasing dry shoot weight of Emap11. Similarly, amending the soil with *C. ensiformis* and *M. pruriens* was equally effective in increasing dry shoot weight of Emap11 (Table 12).

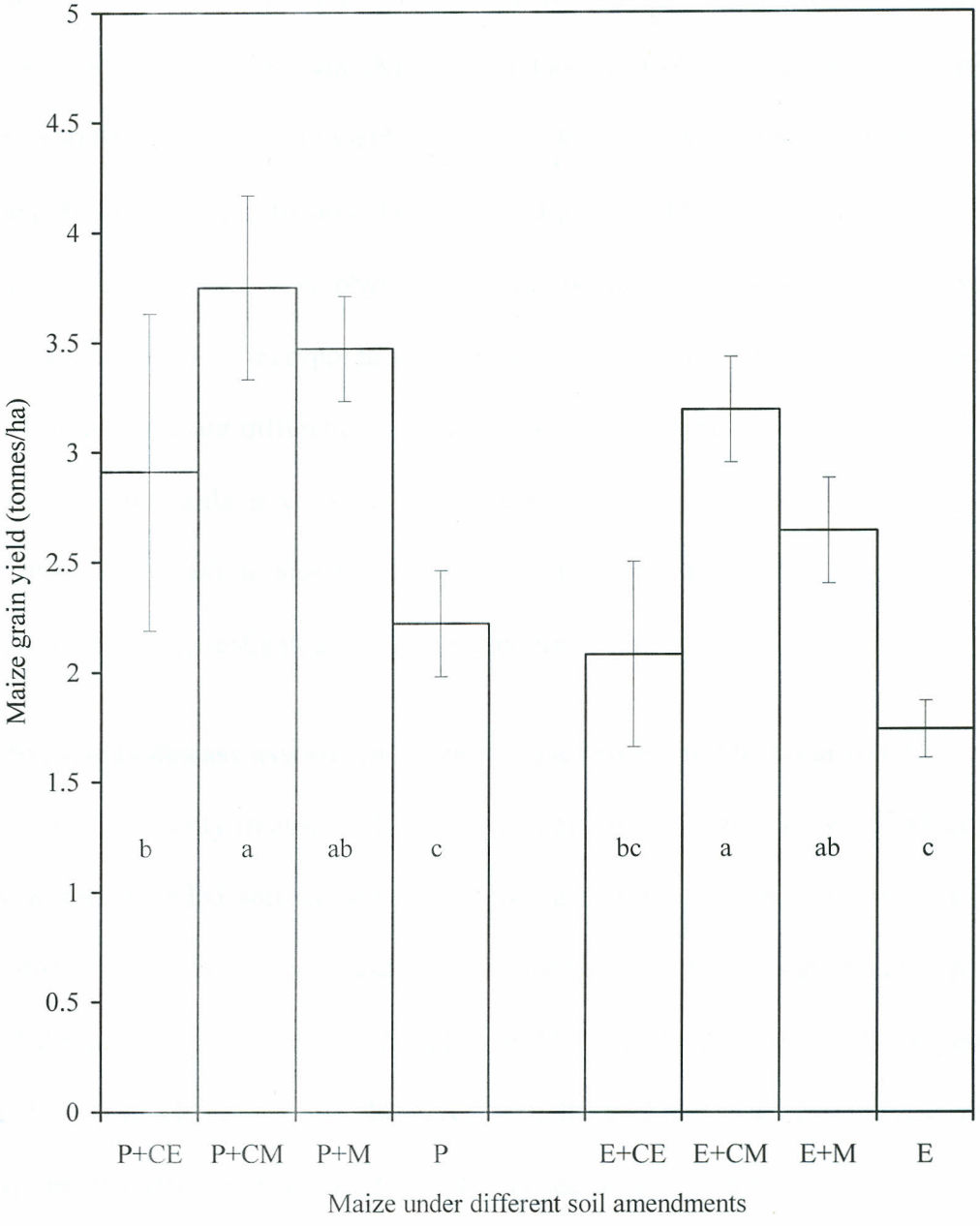
Soil amendments significantly ($p<0.05$) increased fresh root weights. Cattle manure, *M. pruriens* and *C. ensiformis* amended soils significantly ($p<0.05$) increased fresh root weights of Pan5195 by 34%, 27% and 26%, respectively, and that of Emap11 by 23%, 22% and 16%, respectively. The three amendments, however, didn't differ significantly ($p>0.05$) in their efficacy to increase fresh root weights of Pan5195 and Emap11 (Table 12).

The ability of cattle-manure, *M. pruriens* and *C. ensiformis* amended soils to improved plant growth is consistent with findings by Patel *et al.*, (1999b), Ritzinger & McSorley (1998) and Waceke *et al.*, (1993). The significant increase in plant growth in amended soils could be attributed to increased soil nutrient status and enhanced root growth. Improved nutrient status has been reported to increase crop tolerance or resistance to nematodes (Sharma *et al.*, 1997) and might have therefore, contributed to improved plant growth in amended soil. In addition, improved root growth due to amendments might have enhanced production of phytohormones such as gibberellins and cytokinins (Carmi & Heuer, 1981) thus minimizing the effect of nematode root damage on plant growth (Scott & Horgan, 1984) and increasing root growth.

4.6.2. On-farm test 2: Maize grain yield assessment

The three amendments, cattle manure, *M. pruriens* and *C. ensiformis*, significantly ($p<0.05$) increased maize grain yield (Figure 8). Cattle manure, *M. pruriens*

and *C. ensiformis* significantly ($p<0.05$) increased grain yield of Pan5195 by 73%, 59% and 32%, respectively. In addition, cattle manure significantly ($p<0.05$) increased grain yield of Pan5195 by 31% compared to *C. ensiformis*. However, *C. ensiformis* amended soil was as effective as *M. pruriens* in increasing grain yield of Pan5195. Similarly, cattle manure and *M. pruriens* didn't differ significantly in their effectiveness to increase grain yield of Pan5195. Amending soil with cattle manure and *M. pruriens* significantly ($p<0.05$) increased grain yield of Emap11 by 88% and 53%, respectively. Cattle manure significantly ($p<0.05$) increased grain yield of Emap11 by 52% compared to *C. ensiformis*. *Canavalia ensiformis* was as effective as *M. pruriens* in increasing grain yield of Emap11. Cattle manure and *M. pruriens* didn't differ significantly in their effectiveness to increase grain yield of Emap11 (Figure 8).



Bar means followed by the same letter (s) are not significantly ($p<0.05$) different.

Figure 8. Mean grain yield of Pan5195 (P) and Emap11 (E) cultivated in *C. ensiformis* (CE), cattle-manure (CM), and *M. pruriens* (M) amended soils, 150 days after planting, on-farm test 2.

Significant increase in grain yield of Pan5195 and Emap11 in cattle-manure and *M. pruriens* amended soils can partly be attributed to the reduction in nematode population in the roots and soil (Muller and Gooch, 1982). The negative correlation (r^2=0.5, p=0.05) between grain yield and nematodes in roots underscores the importance of nematodes in maize production. The increased grain yield could also have been due to improved nutrient status, soil physical conditions, soil biological activities and crop viability associated with incorporation of organic matter into farm land (Hungalle *et al.*, 1986). The insignificant difference in grain yield of maize between cattle-manure and *M. pruriens* amended soils is consistent with earlier report by Gitari *et al.*, (2000). The ability of *C. ensiformis* to significantly increase grain yield of Pan5195 but not that of Emap11 need to be investigated by further experimentation.

4.6.3. Nematode disease assessment; Greenhouse test 5 and On-farm test 2

Disease severity in amended soils was significantly (p<0.05) lower than in plants grown in non-amended soils in the greenhouse test (Table 13) and on-farm test (Table 14). Cattle manure, *M. pruriens* and *C. ensiformis* amended soils significantly (p<0.05) reduced disease severity in Pan5195 and Emap11 by up to 21% and 46%, respectively (Table 13). Amendments didn't, however, equally and consistently suppress disease severity in Pan5195 and Emap11 in the on-farm test (Table 14). Cattle manure significantly (p<0.05) reduced disease severity on Pan5195 by 60%, 20% and 20% compared to control, *M. pruriens* and *C. ensiformis*, respectively, while *M. pruriens* and *C. ensiformis* significantly (p<0.05) reduced disease severity on Pan5195 by 50% (Table 14). However, the three amendments were equally effective in suppressing disease severity on Emap11 (Table 14). Cattle manure and *M. pruriens* or *C. ensiformis*

significantly ($p<0.05$) reduced disease severity on Emap11 by 70% and 67%, respectively (Table 14).

Table 13. Mean* root necrosis (RN) †, nematode population in roots of Pan5195 and Emap11, nematode reproductive factor (Rf), and nematode suppression ability (NSA) of *M. pruriens* (MP), *C. ensiformis* (CE) and cattle manure (CM) amended soils, 90 days after inoculation, a greenhouse test 5.

Treatment	RN	Nematodes/g of dry root	Rf	NSA
Pan5195	0.9	433	1.41	S
Pan5195+MP	0.7 (22) [b]	51 (88)	0.70 (50)	MS
Pan5195+CE	0.7 (22)	59 (86)	0.82 (42)	MS
Pan5195+CM	0.7 (22)	46 (89)	0.70 (50)	MS
Emap11	1.3	650	2.91	LS
Emap11+MP	0.7 (46)	69 (89)	0.79 (73)	MS
Emap11+CE	0.7 (46)	80 (88)	0.89 (69)	MS
Emap11+CM	0.7 (46)	63 (90)	0.73 (75)	MS
$LSD_{(0.05)}$	0.2	8.0	0.04	
SED	0.1	4	0.02	
CV(%)	23.0	3.6	2.60	

*Mean for six replications.

†Root necrosis: 0-no root damage, 1-slight root damage, 2-moderate root damage, 3-severe root damage and 4-very severe root damage (Bridge and Gowen, 1993).

S-Suppressive, MS-Most suppressive, LS-Least suppressive.

[b]values in parentheses are percent (%) decrease in disease assessment parameters in comparison to the respective maize in non-amended soil.

Amended soil significantly ($p<0.05$) reduced nematode population in roots of maize in the greenhouse test (Table 13) and on-farm test (Table 14). In the greenhouse test, amending soil with cattle manure, *M. pruriens* and *C. ensiformis* significantly ($p<0.05$) reduced nematode population in roots of Pan5195 by 89%, 88% and 86%, respectively (Table 13). In addition, cattle manure, *M. pruriens* and *C. ensiformis* significantly ($p<0.05$) reduced nematode population in roots of Emap11 by 90%, 89% and 88%, respectively (Table 13). These results are consistent with the on-farm test, which revealed that amending soil with cattle manure, *M. pruriens* and *C. ensiformis* significantly ($p<0.05$) reduced nematode population in roots of Emap11 by 87%, 85% and 83%, respectively (Table 14). On-farm results also indicated that amending soils with cattle manure significantly ($p<0.05$) reduced nematode population in roots of Pan5195 by 83% while *M. pruriens* and *C. ensiformis* equally reduced nematode population in roots of Pan5195 by 81% (Table 14). Cattle manure and *M. pruriens* were equally more effective and suppressed nematode population in roots than *C. ensiformis* (Tables 13 & 14).

Table 14. Mean* root necrosis (RN) [†] and nematode population in roots of Pan5195 and Emap11 cultivated in unamended soils and *M. pruriens* (MP), *C. ensiformis* (CE) and Cattle manure (CM) amended soils, 90 days after planting on-farm test 2.

Treatment	RN[†]	Nematodes/g of dry root
Pan5195	2.0	460
Pan5195+MP	1.0 (50) [b]	87 (81)
Pan5195+CE	1.0 (50)	89 (81)
Pan5195+CM	0.8 (60)	77 (83)
Emap11	3.0	664
Emap11+MP	1.0 (67)	98 (85)
Emap11+CE	1.0 (67)	112 (83)
Emap11+CM	0.9 (70)	84 (87)
$LSD_{(0.05)}$	0.14	13
SED	0.07	6.1
CV(%)	6.0	3.6

*Mean for six replications.

[†]Root necrosis: 0 - no root damage, 1- slight root damage, 2 -moderate root damage, 3 - severe root damage and 4 - very severe root damage (Bridge and Gowen, 1993).

[b]values in parentheses are percent (%) decrease in root necrosis and nematode population in roots in comparison to maize in non-amended soil.

The three amendments significantly ($p<0.05$) suppressed nematode reproduction (Table 13). Cattle manure and *M. pruriens* significantly ($p<0.05$) reduced nematode reproduction on Pan5195 by 50% and 42%, respectively. Amending the soil with cattle manure was more effective in suppressing nematode reproduction on Pan5195 than with *C. ensiformis*. Cattle manure and *M. pruriens* were equally effective in suppressing nematode reproduction. Cattle manure amended soils significantly ($p<0.05$) suppressed nematode reproduction on Emap11 by 75%, 18% and 8% compared to non-amended, *C.*

ensiformis and *M. pruriens*-amended soils, respectively. Amending soil with *M. pruriens* significantly ($p<0.05$) suppressed nematode reproduction on Emap11 by 73% and 11% compared to non-amended and *C. ensiformis* amended soil respectively. *Canavalia ensiformis* amended soil significantly ($p<0.05$) suppressed nematode reproduction on Emap11 by 69% (Table 13).

Cattle manure, *M. pruriens* and *C. ensiformis*, significantly ($p<0.05$) reduced nematode population in soil (Figure 9). Cattle manure significantly ($p<0.05$) reduced nematode population in the rhizosphere of Pan5195 by 68%. *Mucuna pruriens* and *C. ensiformis* amended soils significantly ($p<0.05$) reduced nematode population in the rhizosphere of Pan5195 by 68%. However, nematode population in the rhizosphere of Pan5195 in non-amended soil significantly ($p<0.05$) increased by 12%, ninety days after planting (Figure 9). Amending soil with cattle manure, *M.* pruriens and *C. ensiformis* significantly ($p<0.05$) reduced nematode population in the rhizosphere of Emap11 by 68%, 63% and 62%, respectively, ninety days after planting. However, nematode population in the rhizosphere of Emap11 in non-amended soil significantly ($p<0.05$) increased by 19% ninety days after planting (Figure 9).

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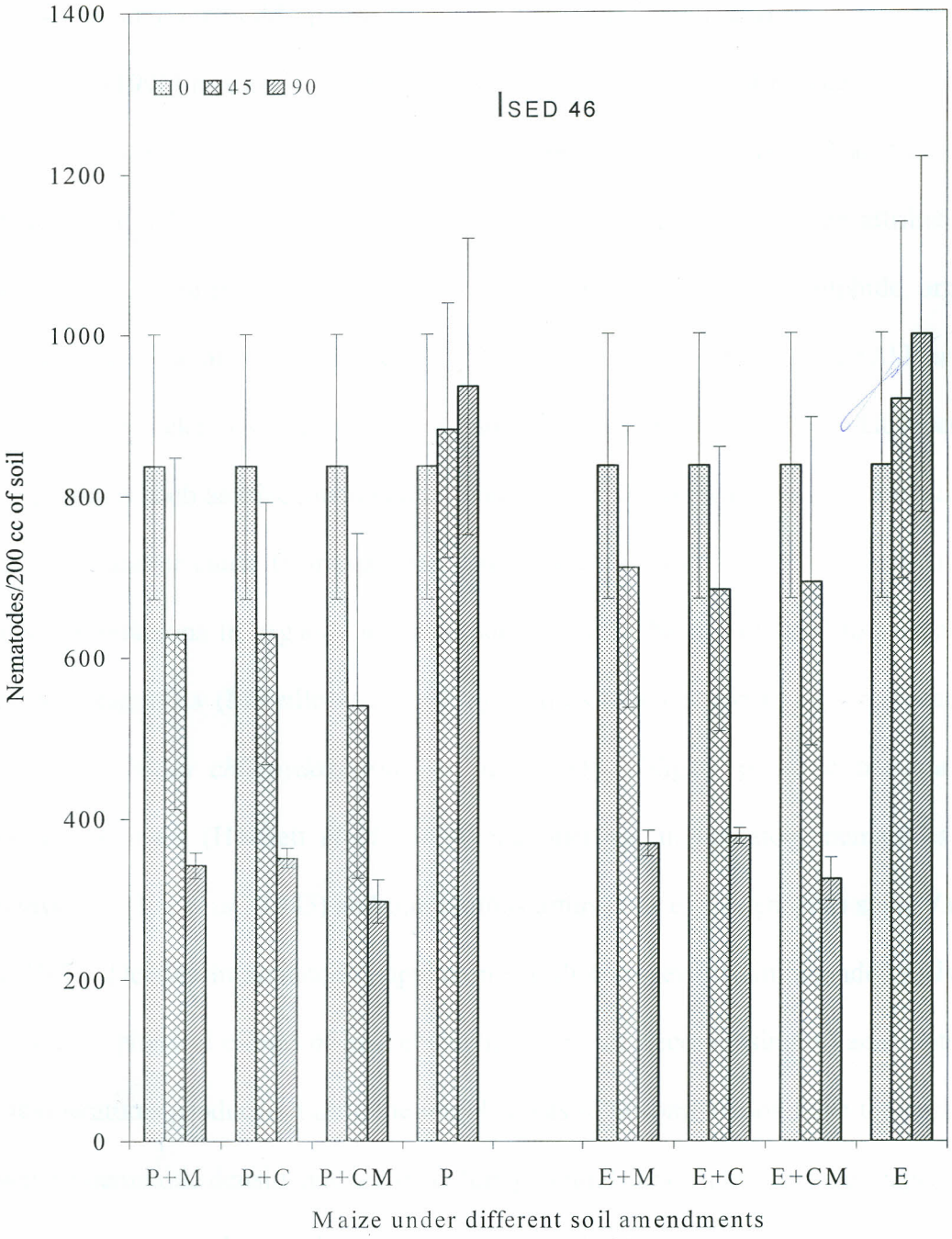


Figure 9. Mean nematode population in soil obtained from the rhizosphere of Pan5195 (P) and Emap11 (E) at 0, 45 and 90 days after planting maize in *M. pruriens* (M), *C. ensiformis* (C) and cattle-manure (CM) amended soils.

Decline in nematode population in the soil and in roots and reduction in disease severity in amended soil indicate that the amendments had suppressive effects on lesion nematodes population build-up and disease development. This corroborates McSorley and Gallaher (1996) results, which revealed that yard waste compost reduced densities of *Pratylenchus* spp. and increased yield of maize by between 10-212%. Nematode suppression through incorporation of organic matter in crop land has been attributed to nematicidal effects of metabolites such as ammonia, nitrites, hydrogen sulphide, organic acids and other chemicals produced by microbes during decomposition (Akhtar and Malik, 2000; Waceke *et al.*, 1993). In addition, *Mucuna* spp. has nematicidal bioactive natural products such as triacontanol and tricontyl tetracosanate which reduce mobility of the juveniles and/or cause their paralysis and death (Marisa *et al.*, 1996). Reduction in nematode populations in organic amended soils has also been attributed to increase in nematode antagonists (Mateille *et al.*, 1995) such as nematode trapping or predacious fungi e.g. *Pochonia chlamydosporia* (stirling, 1991), obligate parasitic bacteria e.g. *Pasteuria penetrans* (Hewlett *et al.*, 1994) and increase in predatory nematodes e.g. *Diplogasterids* (Lal *et al.*, 1983) and omnivorous arthropods e.g. *Adenoplea* spp. (Van de Bund, 1972). Decline in nematode population and disease severity in amended soils can also be due to physical effects of heat emanating from the decomposing organic material. High temperatures produced during the initial stages of decomposition have the potential of lowering nematode density and the inoculum potential. This in turn reduces capacity of the nematodes to reproduce and produce enzymes and consequently make the nematodes highly vulnerable to antagonism (Katan, 1987; Stapleton *et el.*, 1985). The actual

mechanism(s) by which cattle-manure, *C. ensiformis* and *M. pruriens* reduces lesion nematodes population, however needs to be elucidated by further experimentation.

CHAPTER 5

5.1. CONCLUSIONS AND RECOMMENDATIONS

5.1.1 CONCLUSIONS

Results of this study revealed that twelve plant parasitic nematodes were associated with maize in the study areas. Lesion nematodes were the most prominent nematodes both in the soil and roots. Disease severity caused by these nematodes scored an index range of 1 -2.1 out of 4. Lesion nematodes had high damage potential and reproductive capacity on a number of maize varieties screened in this study. Phb, H625 and Pan5355 were the most susceptible, Emap11, H513 and HB614 were moderately susceptible while H627 and Pan5195 were tolerant varieties. Increasing nematode density significantly increased nematode root invasion, disease severity and suppressed growth of all varieties except H627 and Pan5195. The study revealed that *C. ensiformis* and *M. pruriens* are non-host to lesion nematodes while *Lablab purpureus* and *C. ochroleuca* had high nematode reproductive factors and were highly susceptible.

Inter-cropping maize with non-host leguminous crops (*C. ensiformis* and *M. pruriens*) significantly reduced lesion nematode population in the soil, roots and disease severity in maize. In addition, intercropping maize with *C. ensiformis* significantly improved maize grain yield in both short and tall maize varieties. However, *M. pruriens* suppressed maize grain yield of short maize varieties due to the shading effect. Intercropping maize with *C. ensiformis* was more effective than *M. pruriens* in enhancing plant growth and reducing disease severity.

Soil amended with *C. ensiformis* and *M. pruriens* green manures reduced lesion nematode population in the soil, roots and alleviated nematode damage on maize. In addition, the amendments increased maize grain yields.

5.1.2 RECOMMENDATIONS

The results of this study have been informative and have added value to the nematological literature that has been gathered for two and a half years in central Kenya. However, this work should be viewed as a stepping stone for further research that is required to solidify the findings before they are recommended to the farming community. Below are among the areas some I suggest that future research should focus to compliment this work:

(1) This study revealed that a number of plant parasitic nematodes are associated with maize in central Kenya. Since nematodes populations dynamics are influenced by cropping practices, there is a need to develop a medium and long term program to monitor the impact of the intercropping and the soil amendments investigated in this study on the other nematodes that are of significance important to crops that are intercropped or cultivated in rotation with maize.

(2) A number of factors (age of green manure, preparation, quantity applied, method of application etc) directly affect the efficacy of organic manure against soil born phytopathogens. Due to time constrain, these factors were not investigated in the present study yet they are critical for optimum

utilization of organic amendments in managing nematode problems. It is therefore, important that research is conducted to elucidate how the above factors affect the efficacy of the green manures utilized in this study against nematodes.

(3) Contradictory findings on the impact of *Mucuna* spp. on the yields of intercropped maize have been reported. The results of this study indicate that *M. pruriens* suppressed yields of short maize varieties due to shading effect but improved that of tall varieties. Whether to intercrop maize with *Mucuna* or not should therefore, be based on the maize variety at the disposal of the farmer and /or the economic sense of the cost of detrailing *Mucuna* from the intercropped maize so as to avoid the shading effect.

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