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Plant parasitic nematodes associated with common bean in Kenya and the effect of *Meloidogyne* infection on bean nodulation

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ABSTRACT

A study to determine the distribution and population densities of plant parasitic nematodes associated with beans was undertaken in Kakamega, Kiambu, Machakos and Siaya districts of Kenya. Soil and root samples were taken from 25 randomly selected farms in each district. Nematodes in the genera *Meloidogyne, Pratylenchus, Scutellonema* and *Helicotylenchus* were frequently recovered in the rhizophere of bean plants with varying densities in the different locations of the study. *Meloidogyne* spp. and *Pratylenchus* spp. were the most predominant endoparasites occurring in 86 and 61% of the root samples, respectively. *Scutellonema* and *Helicotylenchus* species were present in 80 and 59% of the soil samples, respectively. Other nematodes found in association with bean plants were in the genera *Tylenchorhynchus, Tylenchus, Criconemella, Aphelenchus, Hemicyliophora*, and *Trichodorus*. Greenhouse tests were conducted to determine the effect of *M. incognita* infection on nodulation of bean genotypes. With the exception of bean genotype M28, *Meloidogyne* infection caused significant ($P \leq 0.05$) reductions in nodulation. In a second pot experiment, bean cv. GLP-24 was inoculated with *Rhizobium leguminosarum* bv. *phaseoli* alone and in various combinations with *M. incognita*. Both nodulation and the dinitrogen fixation processes were adversely affected especially in plants where nematode inoculation preceded *rhizobial* inoculation.

Key Words: Bean genotypes, East African highlands, *Helicotylenchus* spp., *Meloidogyne incognita, Pratylenchus* spp., *Scutellonema* spp., *Tylenchorhynchus* spp.

RÉSUMÉ

Une étude pour déterminer la distribution et la densité de la population de nématodes parasitaires de plantes associés aux haricots a été initié dans les districts de Kakamega, Kiambu, Machakos et Siaya au Kenya. Les échantillons des racines et de sols ont été pris dans 25 fermes sélectionnées aléatoirement dans chaque districts. Les nématodes des genres *Meloidogyne, Pratylenchus, Scutellonema* et *Helicotylenchus* ont été fréquemment trouvés dans la rhizosphère des plantes de haricot avec des densité variées dans differentes parties du pay. *Meloidogyne* spp. et *Pratylenchus* spp. étaient des endoparasites les plus dominants apparaissant dans 86 et 67% d'échantillons de racines, respectivement. Les espèces de *Scutellonema* et *Helicotylenchus* étaient présents dans 80 et 59% d'échantillons de sol respectivement. Autres nématode trouvés en assoication avec la plante de haricot étaient des genres de *Tylenchorhynchus, Tylenchus, Criconemella, Aphelenchus, Hemicyliophora* et *Trichodorus.* Avec exception du génotype M28, l'infection du *Meloidogyne* a causé de réduction significative ($P \leq 0.05$) dans la nodulation. Dans le deuxième essai en pots des combinaisons de haricot avec *M. incognita* ont été considérées. Ensemble les processus de dinitrification et de nodulation ont été défavorablement affectés spéciallement dans les plantes où les nématodes ont précédé l'inoculation du *Rhizobium*.

Mots Clés: Genotype de harricot, haute terre de l'Afrique de l'Est, *Helicotylenchus* spp., *Meloidogyne incognita*, *Pratylenchus* spp., *Scutellonema* spp., *Tylenchorhynchus* spp.

Introduction

Common beans (*Phaseolus vulgaris* L.) are grown on an estimated 500,000 ha, in Kenya mainly in association with other crops, especially maize in Kenya (Wortmann and Allen, 1994; Gethi *et al*., 1997). The average yields of 750 kg ha^{-1} are often low compared to a potential 1500 - 2000 kg ha^{-1} (Rheenen *et al*., 1981). The main constraints to bean production in descending order of importance are diseases, low soil fertility and insect pests (Otsyula and Ajang, 1995).

Root-knot (*Meloidogyne* spp.) nematodes are recognised as a major constraint to bean farming, causing up to 60% yield losses in heavily infested fields (Ngundo and Taylor, 1974). Infection by root-knot nematodes is also known to suppress nodulation and hence nitrogen fixation in leguminous plants (Karanja, 1988; Siddiqui and Mahmood, 1994). Although *Meloidogyne* species are known to cause enormous losses on common bean, information on nematode populations in the bean growing areas of Kenya is lacking. This study was undertaken to determine the population density and frequency of occurrence of plant parasitic nematodes associated with common bean in Kenya and to determine the effect of root-knot nematodes on bean nodulation.

Materials and Methods

Sample collection. A survey of nematodes associated with dry bean was carried out in Kakamega, Kiambu, Machakos and Siaya districts between May and July 1998. Twenty soil and bean root samples were collected from each of the 25 randomly selected farms in each district. Plants were gently uprooted and a trowel was used to dig out soil from the bean rhizosphere to a depth of 25 cm. All the roots obtained from each farm were placed in a polythene bag but only about 3 kg of the composite soil sample from each farm was transported to the laboratory.

Nematode damage assessment and extraction. Ten plants from each sample were examined for damage by root-knot nematodes. Damage due to root-knot nematodes was rated using a root-gall index scale of 0-10 (Bridge and Page, 1980). Nematodes were extracted from two 200 cm^3 soil volumes using the sieving and filtration method described by Hooper (1990). The root-knot nematodes were extracted from two batches of 5 g roots each using the maceration/filtration technique (Hooper, 1990). Extracted nematodes were fixed in hot 4% formalin. Identification of nematodes to genus level was done using an identification key and descriptions by Mai and Lyon (1975). Nematode population levels were determined from a counting slide under a compound microscope and expressed either as number per 200 cm^3 soil or 5 g roots.

Effect of *Meloidogyne* infection on bean nodulation in different bean genotypes. Twenty bean genotypes were used in this test. Bean lines E1, E3, E4, NOB, M14, M24, M26, M28, M29, M30, L31, L32, L40 and L45, improved for multiple disease resistance, were obtained by the University of Nairobi Bean Improvement Project (Kimani *et al*., 1993). Bean lines

KK8, KK14, KK15 and KK22 were selected because of their resistance to root rot pathogens (Otsyula and Ajanga, 1995) while two of the commonly grown cultivars, GLP-24 and GLP-1004, were included as checks.

Plastic pots with a 15 cm diameter were filled with 2:1 (v/v) heat-sterilised loam soil: sand mixture and placed in a greenhouse. Bean seeds were surface sterilised using procedures described by Somasegaran and Hoben (1994). Four bean seeds were planted in each pot but thinning was done at emergence to leave two seedlings per pot. All plants were inoculated at emergence with *Bradyrhizobium leguminosarum* biovar *phaseoli* strain 446 supplied by the Microbial Resource Centre (MIRCEN) of the University of Nairobi. Two ml of a rhizobial culture containing ca. $1x10^9$ cfu ml^{-1} was pipetted into indentations made in the root zone of each plant (Somasegaran and Hoben, 1994).

For each bean line, five pots were infested with 2000 juveniles (J2) of *M. incognita* at emergence. The juveniles were obtained by immersing galled tomato roots in sterile distilled water for 7 days (Omwega *et al*., 1988). The juveniles were suspended in 10 ml sterile distilled water. Surface soil was removed to expose some roots to which the inoculum was added and the soil was replaced. Treatments were arranged in a completely randomised design with 5 replications. Plants were maintained in the greenhouse with regular watering. The experiment was terminated six weeks after soil infestation with nematodes. Plants were gently uprooted from the pots and shaken to remove adhering soil. The total number of nodules on each plant was recorded before taking a random sample of 10 nodules for use in determining the percentage of those that were effective (pinkish in colour).

Effect of time of inoculation with *Meloidogyne* on bean nodulation. Pots were filled with heat-sterilised loam soil: sand mixture as described above. Three bean cv. GLP-24 seeds were planted in each pot but thinning was done at emergence to leave one seedling per pot. Treatments consisted of plants inoculated with *R. leguminosarum* bv. *phaseoli* strain 446 alone at emergence, plants inoculated with strain 446 and *M.incognita* at emergence, plants inoculated with *Rhizobium* at emergence and *M. incognita* 10 days after emergence (DAE), and plants inoculated with *M. incognita* at emergence and strain 446 at 10 DAE. Treatments were arranged in a completely randomized design with 10 replications. Analysis of variance of the data was done using MSTAT-C (1990), and means compared using Least Significant Difference (LSD) tests.

Results

Nematodes associated with beans. Nematodes belonging to ten genera, *Meloidogyne* Goeldi, *Pratylenchus* Filipjev, *Scutellonema* Andrassy, *Helicotylenchus* Steiner, *Tylenchorhynchus* Cobb, *Tylenchus* Bastian, *Hemicycliophora* de Man, *Criconemella* Taylor, *Aphelenchus* Bastian and *Trichodorus* Cobb, were found associated with beans in Kakamega, Kiambu, Machakos and Siaya districts (Table 1). The predominant ectoparasites were in the genera *Scutellonema* and *Helicotylenchus*, with overall occurrences of 80 and 59%, respectively. The endoparasitic nematodes, *Meloidogyne* and *Pratylenchus* species, were present in 86 and 61% of the root samples, respectively.

Table 1. Occurrence of plant parasitic nematodes in soil and bean roots collected from Kakamega, Kiambu, Machakos and Siaya districts in Kenya

Nematode genus	% frequency of nematode occurrence per district				
	Kakamega	Kiambu	Machakos	Siaya	Overall
Soil					
Meloidogyne	96	42	84	72	74
Pratylenchus	96	48	80	48	68
Scutellonema	100	80	64	76	80
Helicotylenchus	76	80	24	56	59
Tylenchorhynchus	12	36	52	32	33
Tylenchus	4	8	12	0	6
Criconemella	0	0	8	0	2
Aphelenchus	0	8	4	0	3
Hemicycliophora	0	44	0	0	11
Trichodorus	0	8	0	0	2
Roots					
Meloidogyne	96	80	88	80	86
Pratylenchus	76	32	76	60	61

Numbers of the predominant nematodes belonging to the genera *Meloidogyne, Pratylenchus, Scutellonema* and *Helicotylenchus* varied significantly ($P \leq 0.05$) between districts (Table 2). Numbers of all plant parasitic nematodes in soil and root samples were highest in Kakamega district (Table 2). Nematode densities were lowest in soil and root samples from Siaya and Kiambu districts, respectively.

Table 2. Mean population density of the predominant plant parasitic nematodes recovered from soil and bean roots collected from Kakamega, Kiambu, Machakos and Siaya districts in Kenya[1]

Nematode	Mean population density in 200 cm^3 soil or 5 g roots per district			
	Kakamega	Kiambu	Machakos	Siaya
Soil				
Meloidogyne	127 a*	43 c	102 ab	54 bc
Pratylenchus	251 a	21 b	42 b	56 b
Scutellonema	244 a	102 b	33 b	62 b
Helicotylenchus	71 a	41ab	11 b	17 b
Other nematodes	99	36	33	7
Roots				
Meloidogyne	564 a	250	393 b	320 bc
Pratylenchus	79 a	10 b	18 b	40 b

[1]Data are means of 25 samples
*Means followed by the same letter (s) along rows are not significantly ($P \leq 0.05$) different by Least significant difference test

Nematode damage. The most obvious symptoms of nematode damage were galls on roots caused by *Meloidogyne* species. Levels of damage varied ($P \leq 0.05$) among the districts, with highest and lowest root galling being observed in Kakamega and Kiambu districts, respectively (Fig. 1). Symptoms of damage by nematodes from the other groups were non-characteristic, appearing mainly as reduced growth and yellowing of foliage.

Effect of *Meloidogyne* infection on nodulation of different bean genotypes. Numbers of nodules on bean plants infected with *M. incognita* were significantly ($P \leq 0.05$) lower than in control, with the exception of lines E1 and M28 (Table 3). Reduction in nodulation ranged from 35.4% in line NOB to 100% in line E4. Numbers of nodules in plants inoculated with *M. incognita* were significantly ($P \leq 0.05$) different among the bean genotypes. For instance, no nodules were observed on the roots of bean line E4 inoculated with nematodes as compared to more than 111 nodules on roots of bean line NOB. *Meloidogyne* infection caused significant ($P \leq 0.05$) reduction in proportions of effective nodules in the bean genotypes tested, with the exception of bean cv. GLP-24 (Table 3).

Table 3. Numbers of nodules and percentage of effective nodules in *Meloidogyne*-infected and non-infected bean plants

Bean genotype	Nodule numbers in:		Effective nodules (%) in	
	Inoculated[1]	Control[2]	Inoculated	Control
GLP-24	90.0	166.8	70.0	98.0*
GLP-1004	20.0	58.6	14.0	50.0
NOB	111.2	172.2	72.0	90.0
KK8	70.2	132.2	36.0	78.0
KK14	88.6	161.8	38.0	88.0
KK15	77.4	157.6	40.0	80.0
KK22	38.0	124.6	32.0	62.0
E1	42.6	92.8	20.0	74.0
E3	19.4	121.4	8.0	40.0
E4	0	54.0	0	76.0
M14	1.4	30.2	0	68.0
M24	45.4	99.8	22.0	62.0
M26	41.6	92.4	10.0	58.0
M28	25.4	39.2*	20.0	70.0
M29	27.6	59.4	46.0	74.0
M30	9.2	28.0	8.0	38.0
L31	32.2	116.6	36.0	70.0
L32	32.4	107.8	34.0	66.0
L40	4.6	40.0	12.0	44.0
L45	19.0	46.2	18.0	50.0
LSD ($P \leq 0.05$)	19.9	24.7	15.2	12.6

[1]Inoculated = Nematode-infected, [2]Control = Nematode-free; Data are means of five replications

Effect of time of inoculation with *Meloidogyne incognita* on bean nodulation. The number of nodules was significantly ($P \leq 0.05$) higher in bean cv. GLP-24 plants inoculated with *rhizobia* alone than in plants inoculated with combinations of *rhizobia* and *M. incognita* (Table 4). Plants inoculated with nematodes at emergence and with *rhizobia* 10 days after emergence (DAE) had the lowest number of nodules on their roots. The percentage of effective nodules was significantly ($P \leq 0.05$) higher in bean plants inoculated with *rhizobia* alone than those inoculated with *rhizobia* and *M. incognita* (Table 4). Plants inoculated with nematodes at emergence had lower ($P \leq 0.05$) proportions of functional nodules than those inoculated with nematodes 10 DAE.

Table 4. Effect of Meloidogyne infection on bean nodulation and functioning of nodules in bean cv. GLP-24 plants[1]

Treatments	Nodule number/plant	Effective nodules (%)
Rhizobium alone at emergence	96.5 a[2]	83.0 a
Nematode + Rhizobium (both at emergence)	74.4 b	48.8 c
Nematode (at emergence) + Rhizobium (10 DAE)	54.2 c	47.4 c
Rhizobium (at emergence) + Nematodes (10 DAE)	78.2 b	66.0 b

DAE = Days after emergence
[1]Data are means of 10 replications. [2]Means followed by the same letter along the columns are not significantly ($P \leq 0.05$) different by Least significant difference test

Discussion

The present study revealed that nematodes in the genera *Meloidogyne, Pratylenchus, Scutellonema* and *Helicotylenchus* are widely distributed in bean fields in Kenya. Density and frequency of occurrence of nematodes in the four genera were highest in Kakamega district. Warm and wet conditions prevailing in the district (Jaetzold and Schmidt, 1983), coupled with a high cropping intensity of *P. vulgaris* are ideal for most plant parasitic nematode population build-up. Incidence and population densities of the predominant nematodes were, however, low in Kiambu district in spite of high cropping intensities in the district. This could be attributed to the high amount of cow manure, obtained from zero-grazing units, incorporated into the soil in most farms (Woomer *et al*., 1998).

The high frequency of occurrence of *Meloidogyne* species in bean fields in Kakamega, Kiambu, Machakos and Siaya districts supports previous reports that root-knot nematodes are commonly associated with beans in Kenya (Hollis, 1962). Damage caused by the nematodes varied significantly among the districts. Variation in damage can be attributed to many factors such as differences in bean cultivars and types, environmental factors and their interactive effect on nematode densities and parasitism (Egunjobi, 1974; Griffin *et al*., 1996).

Our study showed that *Pratylenchus* spp. are common inhabitants of the rhizosphere of bean plants. Lesion nematodes, especially *P. zeae*, is a serious pest on maize in Kenya (Kimenju *et al*., 1998). Maize and beans are usually grown together in the small-scale holdings in Kenya (Wortmann and Allen, 1994; Gethi *et al*., 1997). Therefore, pathogenicity of *Pratylenchus* spp. associated with beans and their influence on bean growth needs to be determined.

The finding that *Meloidogyne* infection suppresses bean nodulation in bean plants is consistent with previous reports (Karanja, 1988; Siddiqui and Mahmood, 1994). Biotic factors that affect nodule formation or dysfunction of existing nodules include nematode, viral and fungal infections (Tu *et al.*, 1970; Bowen, 1978; Orellana *et al.*, 1978; Khan, 1993). Several plant parasitic nematodes with different modes of parasitism have adverse effects on nodulation through competition for ecological niches and nutrients, and suppression of lateral root formation, thus reducing sites for nodule formation and early degradation of nodules because of nematode infection (Taha, 1993).

Numbers of nodules on plants inoculated with *M. incognita* were significantly different among the bean genotypes tested in this study. This indicates that nematodes had variable effects on nodulation in different bean genotypes. Conflicting observations have been made on the effect of nematode infection on bean nodulation (Hussey and Barker, 1976; Verdejo *et al.*, 1988; Siddiqui and Mahmood, 1994). Our results show that the bean genotype used may influence the impact of nematode infection on nodulation.

In the present study, bean nodulation was more adversely affected when inoculation with *M. incognita* preceded *Rhizobium* inoculation. In a related study, Sharma and Khurana (1991) reported that growth and nodulation of bean plants were normal in treatments where *Rhizobium* inoculation preceded nematode inoculation. However, inoculation with *M. incognita* prior to *Rhizobium* interfered with growth and nodulation in beans.

Variation in nodulation ability among the bean lines used in this study was clearly demonstrated. These findings indicate that nitrogen fixation can be improved through selection of bean genotypes with high nodulation potential. However, presence of nodules is only a prerequisite for symbiotic nitrogen fixation but not a reliable parameter for measuring nitrogen fixation in legume-rhizobia symbiosis.

Conclusions

Plant parasitic nematodes in the genera *Meloidogyne*, *Pratylenchus*, *Scutellonema* and *Helicotylenchus* are widespread in bean growing areas in Kenya. The wide distribution and the enormous losses associated with root-knot (*Meloidogyne* spp.) nematodes justifies the development of control strategies that are acceptable to small scale bean growers. The influence of especially *Pratylenchus* spp. (which are recognised as important pests on maize) on growth and yield of this crop needs to be determined.

Interference of root-knot nematodes with bean nodulation and effectiveness of the nodules formed was clearly demonstrated. This implies that nematode control should be incorporated in programmes aimed at improving soil fertility through symbiotic nitrogen fixation.

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