

PATHOGENICITY OF THE ROOT-KNOT NEMATODE, *Meloidogyne incognita* RACE 2, IN SOYBEAN-MAIZE INTERCROP

Olaifa G.O. and Adekunle O.K.

Department of Crop Production and Protection, Obafemi Awolowo University Ile Ife

Corresponding author e mail address: gabriel4olaifa@gmail.com 08068618528

ABSTRACT

The screenhouse study one field trial were conducted to investigate the effects of intercropping soybean TGX 1740-2F and 1440-1E and maize cv. Suwan 1 SR in the management of the root-knot nematode, *Meloidogyne incognita* race 2. In the screenhouse thirty six 5-litre plastic pots were filled with sterilized soil. In each pot four seeds of soybean cvs. TGX 1740-2F and TGX 1440-2E and or Suwan-1-SR or combination of both were sown. There were nine treatments in four replicates laid out in Randomized Complete Block Design (RCBD). The field experiment followed the same crop pattern. Under screenhouse conditions, the soybean lowest gall index was recorded in the combination of 25% soybean TGX 1440-1E + 75% maize. An increasing trend was observed in the gall index as the proportions of soybean in the intercrop increased. The population of *M. incognita* in the soil at the termination of the study followed the same trend for all the treatments. In the field trial, the gall index at the end of the experiment was highest in monocultures of the soybean cvs. while the combination of 25% soybean TGX 1440-1E + 75% maize had the lowest gall index. Similarly the highest percentage increase in nematode population was recorded in plots sown to soybean TGX 1740-2F alone, while that sown to maize alone had the lowest percentage increase. The monoculture of soybean cv TGX 1740-2F had the highest soybean yield while 25% soybean TGX 1740 – 2F + 25% maize had lowest. Also maize alone had the highest maize yields while 50% soybean TGX 1440 – 1E + 50% maize had lowest. Seventy-five percent (75%) soybean TGX 1740 – 2F + 25% maize had the highest Land Equivalent Ratio (LER) value of 2.24 while 50% soybean TGX 1440 – 1E + 50% Maize recorded the lowest value (1.14). These results indicate that there were weak yield advantages as a result of intercropping soybean and maize and different mixtures of the intercrop were effective for management of *M. incognita*.

INTRODUCTION

Root-knot nematodes (*Meloidogyne spp.*) are serious pest of many food crops in the tropics. They are being considered as a threat to agricultural crops production and cause economic losses by feeding, primarily on plant root tissues. They exert a direct effect on crop production/systems by reducing the yield as well as the quality of susceptible crops (Jonathan and Hedwig 1991, Luc et al., 2005). They cause disease

known as root-knot disease (Rahman, 2003). *Meloidogyne spp* are among the major groups of pathogens affecting food supply globally because of their wide host range (Sasser 1977). Based on extensive surveys (Koenning et al. 1999), it has been estimated that overall yield losses average over 10%, with this figure approaching 20% for some crops. Estimated annual yield losses of major crops due to plant parasitic nematodes in the world is about 14% in developing countries and about 12.3% in the developed

countries (Agrios, 2005). In monetary terms, worldwide losses have been reported to exceed US \$ 100 billion annually (Brand et al., 2010). Most of the damage is caused by a relatively small number of dozens of nematodes general that attack crops, principally the sedentary root knot (*Meloidogyne* spp) and cyst (*Globodera* and *Heterodera* spp.) nematodes, as well as several migratory nematodes (including *Pratylenchus* and *Radopholus* spp.) Another way to consider the impact of plant-parasitic nematodes is through the management strategies employed in their control. It has been reported that about 109 million pounds of nematicide active ingredient were applied to crops in the U.S.A. at a cost exceeding US \$ 1 billion (Landel, 1989).

Soybean is highly susceptible to *Meloidogyne* spp. and over 100 species (Schmitt and Noel 1984) have been associated with soybean in the U.S.A. Although the economic importance in financial terms has not been determined in Nigeria Soybean grain losses due to nematode infection are enormous. Akeem (1996) reported that *M. incognita*, which is ubiquitous in soybean fields, is the most destructive *Meloidogyne* species in the country.

Although agricultural research originally focused on monoculture or sole cropping and ignored the potential of intercropping (Willey and Osiru 1972), there has been a gradual recognition of the value of this type of mixed cropping system (Blade 1992). Research on the effects of mixed cropping systems on soil nematode communities has been largely limited to

work done in Asia and Africa (Abd-Elgawad and Saad 1989; Idowu 1989). Intercropping legumes with non-legumes has been reported to be an important feature of many cropping systems in the tropics (CIAT 1986). In Nigeria, root crops, cereals, legumes and vegetables are grown in close associations on most traditional agricultural fields. Intercropping has been shown to reduce damage cause by pests diseases, with great yield stability in a wide range of component combinations (Ofori and Stern 1987). Influences of host however become more difficult to explain and quantify under mixed cropping systems (Idowu 1991). The incidence and pathogenicity of plant-parasitic nematodes under mixed cropping systems may be influenced by cultivars of the crops in such systems as well as by their degree of susceptibility. At present, most farmers rely on the use of synthetic chemical products applied to the soil for the control of plant-parasitic nematodes. Chemical control stands supreme in its quick action and effectiveness. However, this practice encompasses many problems such as contamination of ground water, health risks to agricultural workers, and the suppression of free living nematodes, many of which may be natural antagonists to plant-parasitic nematodes. The reduction in availability, increasing cost and environmental problems associated with synthetic nematicides have resulted in the development of alternative methods of nematode control. This study was conducted to investigate the effects of intercropping soybean and maize for the control of *M.incognita* and to identify the optimum ratios of each crop appropriate for effective management of the nematode.

Materials and methods

Source of seeds

Seeds of maize cv. Suwan-1-SR, resistant to *M. incognita* and soybean cvs. TGX 1440-1E and TGX 1740-2F, susceptible to the nematode were obtained from the Institute of Agricultural Research and Training (I.A.R and T), Moor plantation, Ibadan, Nigeria.

Production of cultures of M. Incognita and Extraction of Eggs

Pure cultures of *M. incognita* race 2 were maintained on *Celosia argentea* in the screenhouse of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife. At the age of 8 weeks, *Celosia* plants were harvested and the roots were washed with tap water to remove adhering soil particles and then cut into 2cm pieces. Eggs on the galled roots were extracted as described by Hussey and Barker (1973).

Screenhouse Studies

In the screenhouse, 36 10-liter plastic pots were filled with steam sterilized top soil. Seeds of two *M. incognita*- susceptible soybean cultivars cvs. TGX 1440-1E and TGX 1740-2F and nematode resistant maize cv. Suwan-1 SR were sown in the pots. In each pot, four seeds of soybean or maize or a combination of both were sown. There were nine treatments in four replicates laid out in Randomized Complete Block Design (RCBD). The treatments were: sole soybeans TGX 1440-1E (Four seeds of soybean); sole soybean TGX 1740-2F (Four seeds of soybean), 75% soybean TGX 1440-1E +25% maize; 75% soybean TGX 1740-

2F+25% maize; 50% soybean TGX 1440-1E+50% maize; 50% soybean TGX 1740-2F+50% maize; 25% soybean TGX 1440-1E+75% maize; 25% soybean TGX 1740-2F+75% maize; sole maize. The screenhouse study was conducted twice.

Inoculation of Test Plants

Nine days after planting, fresh *M. incognita* eggs were inoculated close to the roots of the seedlings in each pot at the rate of 5,000 eggs per pot. The plant were watered regularly during the period of experiments.

Data Collection

The studies were terminated 60 days after nematode inoculation. Plants were carefully remove from each pot and washed. Eggs were extracted from root of soybean plants and their population was estimated under a stereomicroscope.

Nematode Analysis

At harvest, soil samples (200ml) were collected from each of the pots following thorough mixing of soil in each pot and nematodes were extracted from the soil samples using the method of Whitehead and Hemming (1965). Nematodes were killed by heat and fixed in 4% formaldehyde. The nematodes were counted in a Doncaster dish under a stereomicroscope (250 X magnification).

Field study

Experimental Site and Layout

A piece of land was selected at the Teaching and Researching Farm of Obafemi Awolowo Universty, Ile Ife, located at

latitude 07⁰28¹N and longitude 04⁰33¹E at 244m a.s.l., in the tropical rain forest zone of Nigeria. The plot was naturally infested with *M. incognita* race 2. It was cleared, ploughed and harrowed in April at the onset of rains and planted to Okra cv.47-4 to increase the population of *M. incognita* in the plot. At the onset of late rainy season, the Okra plants were uprooted from the experimental plot. The plot was ploughed and harrowed. An area 30m x 25m was selected and divided into four blocks of 30m x 5m with each divided into nine plots of 3m x 5m. There was a space of 1m between blocks and 0.5m between plots. The experiment was laid out in a RCBD with nine treatments in four replicates. The treatments were same as those of the screenhouse trials. Sole soybean treatments was made up of eight rows of soybean while sole maize consisted of eight rows of maize. Fifty percent soybean + 50% maize treatment consisted of four rows of soybean planted alternately with four rows of maize. Soybean and maize seeds were planted at a spacing of 60cm x 5cm and 60cm x 50cm respectively. Hand weeding was carried out as necessary and the study was terminated 12 weeks after planting and or at maturity.

Nematode and Soil Analyses

At planting soil samples were collected from each of the 36 plots in the experimental field for nematode and soil analyses. A bulk sample consisting of 20 cores (diameter 18mm; dept 0-200mm) was taken from the plot. Nematodes were extracted from a well-mixed 200ml soil sample drawn from the bulk sample from each plot. Nematodes were extracted using the methods of

Whitehead and Hemming (1965) killed in heat and fixed in 4% formaldehyde then counted in a Doncaster counting dish under a stereomicroscope (x250 Magnification). Individual nematodes from each sample were further identified under a light microscope (x 100-400 magnification) to genus and in a few cases to species level.

Gall Rating

At harvest, 20 randomly selected maize and/or soybean plants per plot were carefully uprooted and assessed for galling using the method describe by Taylor and Sasser (1978), where 0=no galls or egg masses, 1=1-2 galls or egg masses, 2=3-10 galls or egg masses, 3=11-30 galls or eggs masses, 4=31-100 galls or eggs masses and 5=more than 100 galls or egg masses/root.

Crop Yield

Soybean pods and/or maize cobs were harvested from each plot. Harvesting of soybean commenced from a few days after flowering, and every 5 day thereafter until fruiting ceased while dry maize cobs were harvested. They were shelled and weighed per treatment. Yield due to intercropping was estimated by the Land. Equivalent Ratio (LER).

Statistical Analysis

All data obtained were subjected to analysis of variance (ANOVA) while treatment means were separated by Duncan's Multiple Range Test (DMRT) at 5% level of probability.

Results

Screenhouse Studies

In the first trial of the screenhouse study. *M. incognita* populations in the sole soybean cultivars, treatment were not significantly different from that intercropped (Table 1). The lowest gall index was recorded in intercrop 25% soybean TGX 1440-1E +

75% maize. A decreasing trend was observed in the galling of soybean roots as the proportions of soybean in the intercrops decreased. The results of nematode population and root gall index in the second trial of the screenhouse study followed the same trend as in the first trial (Table 2).

Table 1. Effect of Intercropping soybean and maize in *Melodigoyne incognita* infested soil on nematode population and gall index under greenhouse conditions (Trial 1)

Treatments	Population <i>M. incognita</i> (Number/200ml soil X 100)	Transformed value Gall rating
Sole soybean TGX 1440-1E	192a	3.5b (2.2)
Sole soybean TGX 1740-2F	185a	3.7a (2.1)
75% soybean TGX 1740-1E: 25% suwan-1SR maize	86ab	2.2d (1.8)
75% soybean TGX 1440-1E: 25% suwan-1SR maize	76ab	2.8c (1.9)
50% soybean TGX 1440-1E: 50% suwan-1SR maize	62ab	1.5e (1.6)
50% soybean TGX 1740-2F: 50% suwan-1SR maize	9ab	1.7e (1.6)
25% soybean TGX 1440-1E: 75% suwan-1SR maize	58ab	0.1g (1.1)
25% soybean TGX 1740-2F: 75% suwan-1SR maize	26b	0.6f (1.3)
Sole suwan-1SR maize	17b	1.00h (0.0)

* Rating after Taylor and Sasser (1978) were 0 = No gall or egg mass, 1 = 1-2 galls or egg masses, 5= over 100 galls or egg masses. Each value in parenthesis are means of original values. Means with the same letter in a column are not significantly different (P=0.05). by Duncan’s New Multiple Range Test (DMRT)

Table 2. Effect of Intercropping soybean and maize in *Melodigoyne incognita* infested soil on nematode Population and gall index under greenhouse conditions (Trial 2)

Treatments	Population <i>M. incognita</i> (Number/200ml soil X 100)	Transformed value Gall rating
Sole soybean TGX 1440-1E	357a	2.21a (3.2)
Sole soybean TGX 1740-2F	240ab	2.21a (3.9)
75% soybean TGX 1740-1E: 25% suwan-1SR maize	214ab	1.76d (2.1)
75% soybean TGX 1440-1E: 25% suwan-1SR maize	166ab	1.90c (2.6)
50% soybean TGX 1440-1E: 25% suwan-1SR maize	160ab	1.55f (1.4)
50% soybean TGX 1740-2F: 50% suwan-1SR maize	156ab	1.67e (1.8)
25% soybean TGX 1440-1E: 75% suwan-1SR maize	103b	1.06h (0.13)
25% soybean TGX 1740-2F 75% suwan -1SR maize	58b	1.22g (0.5)
Sole Suwan – 1-SR maize	24ab	1.00h (0.0)

* Rating after Taylor and Sasser (1978) were 0=No gall or egg mass, 1=1-2 galls or egg masses, 10 galls or eggs 3= 11-30 galls or egg masses, 4=31-100 galls or egg masses, 5= over 100 galls or egg masses. Each value is a mean of four replicates, Galls were transformed using $\sqrt{n + 1}$ value in parenthesis are means of untransformed values with the same letter in a column are not significantly different (P=0.05). by Duncan’s New Multiple Range Test (DMRT).

Field Study

Under field conditions the root gall index of soybean was significantly different among treatment (Table 3). Gall index was highest in sole soybean TGX 1440-1E and TGX 1740-2F. Among intercrops, 75% soybean TGX 1440-1E + 25% maize had highest gall index which was significantly higher than those of other intercrop treatments. Twenty five percent soybean TGX 1740-2F + 75% maize had the lowest gall index while sole maize was not galled. Sole soybean cv. TGX 1740-2F has the highest percentage increase in population of *M.incognita* (45%) while population of *Xiphinema* spp. increased by 12% *Longidorus* spp. 22% and *Pratylenchus* spp. by 10%. Among intercrop treatment, the highest percentage increase in *M.incognita* population was recorded in 75% soybean TGX 1740-2F + 25% maize (42%) and the population of *Xiphinema* spp. increased by 10% *Longidorus* spp. by 21% and *Pratylenchus* spp. by 10%. This was followed by 75% soybean TGX 1440-1E + 25% maize where *M.incognita* population

increased by 40% while population of *Xiphinema* spp. Increased by 10%, *Longidorus* spp. by 20% and *Pratylenchus* spp. by 10%. Sole maize had the lowest percentage in *M.incognita* population (5%) while population of *Xiphinema* spp. Increased by 10%, *Longidorous* spp. by 15% and *Pratylenchus* spp. by 5%. Among intercrops, 75% soybean TGX 1440-1E + 25% maize had the highest soybean yield of 1.06 t/ha, but this was not significantly higher than the soybean yield in other intercrops. Sole maize recorded the highest maize yield of 1.93t/ha, but this was significantly higher than yield of 25% soybean TGX 1740-2F + 75% maize and 25% soybean TGX 1440-1E + 75% maize. There was significant difference in LER in some treatment and in all intercrop treatments it was more than 1 (Table 4). The lowest LER value of 1.14 was recorded in 50% soybean TGX 1440-1E + 50% maize, significantly different to the two 75% soybean + 25% maize treatment but not different from other treatments.

Table 3. Effect of Intercropping soybean and maize in *Meloidigyne incognita* infested soil on root gall index under field conditions.

Treatments	Population Gall rating
Sole soybean TGX 1440-1E	3.10* (2.02) a
Sole soybean TGX 1740-2F	3.20 (2.04) a
75% soybean TGX 1740-1E: 25% suwan-1SR-maize	2.70 (1.92) b
75% soybean TGX 1440-1E: 25% suwan-1SR-maize	2.60 (1.90) b
50% soybean TGX 1440-1E: 50% suwan-1SR-maize	1.50 (1.64) c
50% soybean TGX 1740-2F:50% suwan-1SR-maize	0.88 (1.58) d
25% soybean TGX 1440-1E 75% suwan-1SR-maize	0.40 (1.26) e
25% soybean TGX 1740-2F: 75% suwan-1SR-maize	0.20 (1.18) e
Sole suwan-1SR-maize	0.00 (1.0) f

* Rating after Taylor and Sasser (1978) were 0=No gall or egg mass, 1=1-2 galls or egg masses, 5=over 100 galls or egg masses. Each value is a means of four replicates. Galls were transformed using $n + 1$. value in parethesis are means of original values. Means with the same letter in a column are not significantly different ($p=0.05$). by Duncan's New Multiple Range Test (DMRT).

Table 4. Effect of intercropping soybean and maize in nematode-infested soil on crop yield and Land Equivalent Ratio (LER)

Treatments	Mean Yield (tones/per ha)		
	Soybean	Maize	LER
Sole soybean TGX 1440-1E	1.26ab	-	-
Sole soybean TGX 1740-2F	1.58abc	-	-
75% soybean TGX 1740-1E: 25% suwan-1SR-maize	1.06abc	1.23bc	1.84ab
75% soybean TGX 1440-1E: 25% suwan-1SR-maize	1.04bc	1.32b	2.24a
50% soybean TGX 1440-1E: 50% suwan-1SR-maize	0.71bc	0.88c	1.14c
50% soybean TGX 1740-2F: 50% suwan-1SR-maize	0.92c	1.34b	1.66abc
25% soybean TGX 1440-1E: 75% suwan-1SR-maize	0.68c	1.63ab	1.55abc
25% soybean TGX 1740-2F: 75% suwan-1SR-maize	0.61c	1.65ab	1.59abc
Sole suwan-1-SR -maize	-	1.93a	-

LER = Land Equivalent Ratio. Each value is a mean of four replicates. Means with the same letter in a column are not significantly different (P=0.05). by Duncan’s New Multiple Range Test (DMRT).

Discussion

Under screenhouse conditions, intercropping soybean and maize resulted in a reduction in soil populations of *M. incognita* but increased populations of the nematode and correspondingly high root gall index were recorded in the sole soybean treatments. The soybean cultivars, being good hosts of *M. incognita* enhanced rapid nematode reproduction and multiplication. It is assumed that most of the hatched second stage larva (J₂) from the egg inoculum penetrated soybean roots. Since there was no competition between the nematodes and any other pathogen under the screenhouse conditions, the nematode had a favourable environment to multiply as well as to induce infection. There was a decreasing trend in the gall index and nematode population as the proportion of the soybean in the intercrop decreased. The lowest nematode population and lowest gall index were recorded in sole maize. This was because

maize being a poor host of the nematode did not support *M. incognita* reproduction.

Intercropping soybean and maize in nematode infested field showed a significant difference in soil nematode population and root gall index across treatments. The results further showed that gall index was highest in the sole soybean cultivars. This may also indicate that continuous planting of susceptible crops in continuous rows increased build up of nematode populations. The nematode apparently had a favourable environment to multiply because the source of nutrition was more concentrated than what would obtain in a crop mixture. Like in the screenhouse trials, there was a decrease in level of galling in the intercrops as the proportion of soybean decreased in the mixture. Intercropping a nematode-susceptible crop with a poor host tended to slow down the build up of nematode population and consequently reduced the damage cause by the pest in the field. These results corroborate the findings of Idowu

(1981) who reported that in a field experiment, root-knot nematode damage was found as small knots or was completely absent in roots of maize planted in nematode-infested soil in association with a nematode susceptible crop. Also Idowu and Fawole (1992) reported that in field experiments where maize and cowpea were intercropped in nematode infested soil, there were no galls or egg masses on maize roots. Furthermore soybean is important in biological nitrogen fixation with appropriate *Rhizobium* strains. It is presumed that when nitrogen is readily available in the soil, plant growth will be enhanced and consequently a higher nematode population may result since the nematodes will have more food substances to feed on. The trend observed in percentage change in *M.incognita* population was similar to that noted for *Xiphinema* spp. *Longidorus* spp. and *Pratylenchus* spp. suggesting that the factors responsible for the increase in *M.incognita* may be at play with respect to populations of these three nematode genera. The present work are in contrast to the work of Abd-Elgawad and Saad (1989). They reported that, in the field studies higher populations of *Tylenchrohynchus claris* and *Pratylenchus* spp. occurred when *Phaseolus Vulgaris* was intercropped with maize than when grown in pure stands. Intercropping soybean and maize in the nematode infested field showed that yield advantages were obtained since all LER values for intercrops were higher than 1. The values increased as the proportion of soybean increased in the intercrop treatments. These results corroborate the findings of Clement *et al.*, (1992) who reported that in field

experiments, two rows of soybean and a row of maize resulted in more gain yield than planting both crops in single alternate rows. Also, Idowu and Falowe (1992) reported that in field trials, where maize and cowpea were intercropped in nematode-infested soil LER values were often greater than 1 suggesting advantages of intercropping over sole cropping. In a related study in Mexico on intercropping corn-bean squash, Amador (1980) reported that corn yield were considerably higher in the mixture than in pure stands. Similarly, Martins *et al.*, (1987) reported that 67% of the full bean planted with the same rows gave an LER higher than pure stand yield. Furthermore Allen and Obura (1983) also investigated yield of corn, cowpea and soybean under different intercropping systems and reported that the total LER value obtained indicated that the intercropping outyielded monocrops by 13%. However Idowu and Fawole (1992) investigated effects of intercropping maize and cowpea on the pathogenicity of *Meloidogyne* spp. and yield of cowpea in two locations and found that effects of sole cropping and intercropping and root-knot nematode reproduction were inconsistent between locations, although LER values were greater than unity they therefore suggested that beneficial yield responses as a result of intercropping could be due to a combination of factors other than root-knot nematode disease reduction alone in the present study, it was noted that though the two cultivars of soybean suffered higher root damage than the soybean in the intercrop treatments they produced slightly higher yields than the intercrops indicating that under natural field conditions, there are

many factors responsible for yield other than effects of plant-parasitic nematodes.

This study established the yield advantages of intercropping in comparison of sole cropping with corresponding reduction in nematode population. The optimum crop mixture of soybean and maize of management of *M.cognita* identified in this study was 25% soybean + 75% suwan – 1 maize.

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