WFL Publisher
Science and Technology
Meri-Rastilantie 3 C, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.4 (3&4) : 223-227. 2006 *www.world-food.net*

Reaction of cotton and soybean cultivars to populations of *Meloidogyne javanica* and *M. incognita* in Zimbabwe

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Received 3 May 2006, accepted 26 August 2006.

Abstract

The response of common cotton (*Gossypium hirsutum* L.) and soybean [*Glycine max* L. (Merr.)] cultivars in Zimbabwe to infestation by *Meloidogyne javanica* (Treub) Chitwood and *Meloidogyne incognita* (Kofoid & White) Chitwwod races 1 and 3 was evaluated. This was done under greenhouse conditions in two separate experiments at Kutsaga Research Station, Zimbabwe, in a program aimed at identifying alternative rotation crops for root-knot nematode management in tobacco (*Nicotiana tabacum* L.). Seedlings of each cultivar were raised in sterilised soil in 15 cm diameter pots. The inoculum which was applied three weeks after sowing was a mixture of eggs and second stage juveniles (J2s). The dosage was 4000 mixed eggs and J2s per plant for the soybean trial while it was 5000 for cotton. Nine weeks after infestation, root gall indices, numbers of nematode egg masses and eggs per root system were recorded. The numbers of J2s per pot were also recorded. Reproduction factor (RF) was computed as final population (eggs + J2s) ÷ initial population inoculated. All the cotton cultivars were susceptible (RF>1 and abundant galling) to *M. incognita* Race 3 while they were all resistant (RF<1) to *M. javanica*. The cultivars, TE-94-4, FQ 92-19, CY889, AG4869 and DF885 were resistant to *M. incognita* Race 1. The other three cultivars which were susceptible to *M. incognita* Race 1 did not show any damage symptoms suggesting that assessing for resistance using this criterion alone may be inadequate. The soybean cultivars were all susceptible to the three species except SNK60 which was resistant to *M. incognita* race 1 (RF=0). The cultivar, however, produced galls further indicating the inadequacy of using damage functions of *Meloidogyne* species for host status evaluation.

Key words: Cotton, crop rotation, host status, *Meloidogyne* spp., reproduction factor, soybean, susceptibility, resistant.

Introduction

Root-knot nematodes cause significant economic losses to a lot of crop species. Successful management of root-knot nematodes commonly involves the use of a combination of strategies that include crop rotations with non-host crops, the application of nematicides, the use of resistant cultivars, fallow and organic amendments. The basic principle in these management strategies is to decrease the population densities of the target nematode to below damage threshold before the next susceptible crop is grown. In a sustainable agricultural system, it is imperative that the combination of strategies used do not disrupt the agro-ecosystem. The phase out of fumigant nematicides such as methyl bromide and DBCP (1,2-dibromo-3-chloropropane) has increasingly made plant resistance an important component in root-knot nematode management [3, 12].

Besides being a sustainable and environmentally benign method for limiting damage caused by root-knot nematodes, host plant resistance can be used in two fronts. It can either be incorporated into the target crop or into a suitable rotation crop to be grown before the targeted crop. Crop rotation with non-hosts is an effective way of reducing nematode soil population densities [1]. However, there are a few economically feasible crops that can be used in a rotation for management of *Meloidogyne* spp. [17]. This is partly due to the occurrence of these nematodes as a mixture of different species that all have a wide host range. Most known non-host crops reduce annual average farm revenues because they have little cash value and/or have low regional marketability. The scenario in Zimbabwe tobacco (*Nicotiana tabacum* L.) farming is a classical example. The crop has to be rotated with non-hosts of *Meloidogyne* spp. after every two years of continuous tobacco. Katambora Rhodes grass (*Chloris gayana* Kunth.) has traditionally been used in this rotation as a root-knot nematode management strategy [18]. In recent years, farmers have been looking for a more financially viable rotation crop. Therefore, resistance must be identified in potential rotation crops that are financially lucrative or their wild relatives and incorporated into elite germplasm. A reasonable starting point would be to check for any resistance in the current cultivars of potential rotation crops. Cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L. Merr.) are commercially viable in Zimbabwe and have been earmarked for incorporation in a tobacco rotation, but their host status to the *Meloidogyne* spp. in the country is unknown. This study aimed to evaluate the host status of common cotton and soybean cultivars in Zimbabwe to the root-knot nematode species in the country and come up with recommendations for their usefulness in a tobacco rotation and the implication this has for plant breeders.

Materials and Methods

Plant establishment: This study was done at Kutsaga Research Station in Zimbabwe. Plants were established by placing three seeds of the cultivar under test in the centre of 15 cm diameter pots which were filled with 1000 g sandy (>90% sand) soil. Two weeks after sowing, these were thinned to one plant per pot. The soil had been steam sterilised and then aerated for eight weeks to allow breakdown and evaporation of toxic compounds formed during sterilisation and for re-colonisation by soil microbes. Without this period, the nematodes added to the soil would not have survived [16]. Pots were filled and the soil was gently compressed to the same volume in each pot. Compound D (8-14-7), a basal dressing fertiliser, was applied at a rate of 3 g/kg soil as a source of nutrition for the plants. This was supplemented with 25 ml of Nutrifol (20-20-20) liquid fertiliser fortnightly. Water content was maintained at around field capacity and temperature maintained below 25°C.

Inoculation procedure and experimental set up: Inoculum was obtained from ten week old tomato (*Lycopersicon esculentum* Mill.) plants that had been inoculated with the respective nematode species when they were two weeks old. This was done following the NaOCl solution procedure [9]. The resultant inoculum which comprised of eggs was suspended in water and the eggs were counted under a microscope. The egg concentration was diluted to 333 eggs ml^{-1} and used immediately. Pots were then inoculated at a density of 4 and 5 eggs/g soil in the soybean and cotton trials respectively. This required the inoculation of 12 and 15 ml of the inoculum suspension per pot. To inject an egg suspension into the soil, 3 ml of the suspension was drawn using an ordinary pipette and then inserted into the soil almost to the bottom of the pot. The pipette was then pulled up steadily while air was being blown through. This was repeated until the required volume per pot had been discharged. This ensured that the juveniles were uniformly distributed in a vertical channel from bottom to top [2]. The holes were immediately filled with soil.

Pots were randomised and placed on flat elevated steel beds. For cotton, these were arranged as 5 blocks (sections of green house bench) of three main plots (nematode species) with eight subplots (cultivars) in each block. For the soybean trial, there were three blocks of the same three main plots with nine subplots (cultivars) in each block. The cotton cultivars used were TE-94-4, FQ902, FQ92-19, CY889, AG4869, SZ-9314, DF885 and BC853. Soybean cultivars evaluated were Gazelle, Viking, Soma, Soprano, Solitaire, SNK60, A7119, Storm and Prima. A tomato plant (cv. Moneymaker) was included per main plot as an indicator plant to ascertain inoculum viability although no data were recorded from them.

Data collection and analysis: Nine weeks after inoculation, the root-knot gall index, numbers of egg masses, eggs and J2s per pot (plant) were recorded. Where possible, the reproduction factor (RF) was calculated. The root gall rating system used featured a rating from 0–8, where: 0 = no galls, 1 = trace infection, less than 5 galls; 2 = very slight, trace to 25 galls; 3 = slight, 26 to 100 galls; 4 = moderate, numerous galls, mostly discrete; 5 = moderately heavy, numerous galls, many coalesced; 6 = heavy very numerous galls, mostly coalesced, root growth slightly retarded; 7 = very heavy, mass invasion, slight root growth;8 = extremely heavy, mass invasion, no root development [6]. To facilitate easy counting of egg masses, the roots were stained with Phloxine B (0.15 g/l water) for 15 minutes, removed and rinsed in water to remove excess stain [5]. Eggs were isolated for counting using the NaOCl method [9]. Soil samples for estimation of J2s were processed using the modified Baermann technique [5]. This was done by extracting a 100 g sample of the well mixed soil of each pot. All J2s of the respective species were counted and noted. Additional samples were processed if the number of nematodes recovered per pot was too low to obtain reliable density estimation. For all density estimations of J2s, at least 200 juveniles per pot were counted. If less than 200 juveniles were counted, further extractions would be done from that pot until the 200 nematodes had been counted, otherwise the whole pot would be counted. A reproduction factor [(RF) = (final number of eggs and J2s in soil) ÷ initial number of eggs inoculated] was calculated for each cultivar.

Prior to statistical analysis, nematode reproduction data (numbers of egg masses, eggs and J2s) and the gall indices were transformed using [log 10 (χ + 1)]. The original data are shown in tables. All data were subjected to analysis of variance (ANOVA) and means were separated by Duncan's Multiple Range Test with $P<0.05$.

Results

Cotton: Egg mass, egg and J2 production was higher on *Meloidogyne incognita* (Kofoid & White) Chitwood Race 3 than on *M. incognita* Race 1 and *Meloidogyne javanica* (Treub) Chitwood for all the cultivars. Based on egg mass, egg and J2 counts and the consequent RFs, all the cultivars supported reproduction of *M. incognita* Race 3 to a similar extent ($P>0.05$). However, for *M. incognita* Race 1, FQ902 and SZ 9314 supported more reproduction than the rest of the cultivars. DF885 also supported significantly more reproduction of *M. javanica* than the rest of the cultivars ($P<0.05$). Only three cultivars produced egg masses of *M. javanica* viz; FQ 92-19, CY889 and DF885. For *M. incognita* Race 1, two cultivars, TE-94-4 and AG4869, did not produce any egg masses with the rest having trace to low egg production (Table 1).

Based on the gall index, no cultivars suffered any damage from *M. javanica* and *M. incognita* Race 1. They were, however, all damaged by *M. incognita* Race 3 to a similar extent ($P>0.05$) and all had high RFs (Table 2). Although no damage was recorded on *M. incognita* Race 1, the cultivars FQ 902, SZ 9314 and BC 853 supported nematode reproduction (RF = 56, 75 and 6 respectively). The same phenomenon was observed on DF 885 which had a galling score of zero but a RF of 19.

Soybean: All soybean cultivars had high egg mass, egg and J2 production for the three species except A7119 which did not support reproduction of *M. incognita* Race 1 (Table 3). Subsequent RFs all depict a similar trend (Table 4). Prima supported more egg mass, egg and J2 production of *M. javanica* than all the other cultivars ($P<0.05$). It also had more egg mass formation for both races of *M. incognita* and its RF for *M. incognita* Race 3 was the highest among all the cultivars (Tables 3 and 4). A7119 still had the lowest reproduction of *M. incognita* 3 although this was not statistically significant from the nematode's reproduction on the other cultivars. Despite being

Table 1. Number of egg masses, eggs and J2s from eight cotton cultivars nine weeks after inoculation with an initial density of 5 J2s g^{-1} soil (5000 J2s kg^{-1} pot).

Cultivar	*M. incognita* Race 1			*M. incognita* Race 3			*M. javanica*		
	Egg masses	Eggs ($x10^2$)	J2s ($x10^2$)	Egg masses	Eggs ($x10^5$)	J2s ($x10^5$)	Egg masses	Eggs ($x10^2$)	J2s ($x10^2$)
TE-94-4	0	0	0	315.2	12.5	12.2	0	0	0
FQ902	48.4	141.2	1383.6	310.8	5.3	5.2	0	0	0
FQ92-19	0.6	4	3.9	236.2	10.4	10.2	0.8	8.0	7.84
CY889	0.2	6.5	6.4	161.6	5.0	4.9	1.6	17.5	13.7
AG4869	0	0	0	194.8	4.3	4.2	0	0	0
SZ-9314	32.2	189.2	1854.9	292.0	10.2	9.9	0	0	0
DF885	0.8	800	7.8	211.0	10.7	10.4	48.0	380.0	470.4
BC853	0.8	148.4	145.4	168.3	6.3	6.2	0	0	0
SED	26.51	433.96	1030.50	112.03	4.614	4.18	24.01	244.33	235.22
F-test probability	0.222	0.169	0.391	0.437	0.322	0.390	0.460	0.364	0.463

Values are mean numbers per pot (plant).

Table 2. Mean gall indices (0-8; where 0 = no galls and 8 = ca 100% galling) and reproduction factors for eight cotton cultivars nine weeks after inoculation with an initial density of 5 J2s g^{-1} soil (5000 J2s kg^{-1} pot) of *M. incognita* Race 3.

Cultivar	Gall index ± SE	Reproductive factor ± SE
TE-94-4	5.4 ± 0.40	493.1 ± 119.74
FQ902	5.0 ± 0.55	212.4 ± 64.12
FQ92-19	3.8 ± 0.58	413.6 ± 140.69
CY889	3.4 ± 0.40	197.0 ± 64.50
AG4869	3.6 ± 0.98	171.3 ± 58.86
SZ-9314	4.6 ± 0.40	402.8 ± 64.99
DF885	3.8 ± 1.02	423.3 ± 212.72
BC853	3.0 ± 0.84	251.0 ± 147.52
SED	1.00	168.73
F-test probability	0.253	0.390

Values are mean numbers per pot (plant) ± SE.
Reproduction factor = [final population (J2s + eggs) ÷ initial egg density]

unable to support reproduction of *M. incognita* Race 1, A7119 still formed galls (Table 4). The galling was, however, not as severe as it was on Gazelle, Soprano and Prima ($P<0.05$).

The amount of galling on the roots was similar for all the three species of *Meloidogyne.* The gall index for the two races of *M. incognita* of Prima was higher than that of the other cultivars. On *M. javanica* it was damaged to a similar extent with Storm, A7119, SNK60 and Soma (Table 4).

Discussion

Host-parasite relationship: Three main types of host-parasite relationship feasible between a plant parasitic nematode and a plant are: 1) the nematode can multiply on the plant and the plant is considered to be a host; 2) the nematode cannot multiply on the plant; however, the plant sustains the nematode by supplying a source of food and 3) there is no interaction between the plant and the nematode - the plant is considered a non-host.

The host-parasite relationship between the three *Meloidogyne* species and the cotton cultivars investigated had two different forms. Some cultivars allowed successful invasion with galling

Table 3. Number of egg masses, eggs and J2s from nine soybean cultivars nine weeks after inoculation with an initial density of 4 J2s g^{-1} soil (4000 J2s kg^{-1} pot).

Cultivar	*M. incognita* Race 1			*M. incognita* Race 3			*M. javanica*		
	Egg masses	Eggs ($x10^3$)	J2s ($x10^3$)	Egg masses	Eggs ($x10^3$)	J2s ($x10^3$)	Egg masses	Eggs ($x10^4$)	J2s ($x10^4$)
Gazelle	56.7^{c}	51.1^{b}	50.0^{b}	114.3^{b}	339.5^{ab}	332.8^{a}	33.7^{a}	5.5^{a}	5.3^{a}
Viking	77.0^{c}	63.6^{b}	62.3^{b}	72.3^{ab}	337.0^{ab}	330.2^{a}	26.0^{a}	4.6^{a}	4.5^{a}
Soma	21.7^{bc}	81.8^{b}	80.1^{b}	25.0^{ab}	115.7^{ab}	113.4^{a}	40.0^{a}	3.6^{a}	3.6^{a}
Soprano	97.7^{c}	280.2^{b}	274.6^{b}	44.0^{ab}	168.1^{ab}	164.8^{a}	40.7^{a}	5.9^{a}	5.8^{a}
Solitaire	93.0^{c}	208.9^{b}	204.8^{b}	139.0^{b}	563.9^{bc}	552.6^{b}	40.3^{a}	3.3^{a}	3.3^{a}
SNK 60	6.7^{ab}	4.4^{a}	4.3^{a}	9.3^{a}	316.6^{a}	31.0^{a}	42.3^{a}	3.3^{a}	3.5^{a}
A 7119	0^{a}	0^{a}	0^{a}	1.0^{a}	4.7^{a}	4.6^{a}	45.7^{a}	3.5^{a}	3.4^{a}
Storm	98.7^{c}	224.3^{b}	219.8^{b}	70.3^{ab}	410.6^{a}	402.4^{ab}	35.0^{a}	9.4^{a}	9.3^{a}
Prima	316.7^{d}	340.4^{b}	333.6^{b}	441.0^{c}	983.0^{c}	963.4^{c}	107.3^{b}	23.9^{b}	23.5^{b}
SED	148.51	182.44	178.73	45.26	215.27	210.98	24.46	7.55	7.30
F-test probability	0.010	<0.001	<0.001	<0.001	0.008	<0.001	0.030	0.014	0.019

Values are mean numbers per pot (plant). Means within a column followed by the same letter(s) are not significantly different at the 5% probability level according to the Duncan's multiple range test.

Table 4. Mean gall indices (0-8; where 0 = no galls and 8 = ca 100% galling) and reproductive factors for nine soybean cultivars nine weeks after inoculation with an initial density of 5 J2s g^{-1} soil (5000 J2s kg^{-1} pot).

Cultivar	*M. incognita* Race 1		*M. incognita* Race 3		*M. javanica*	
	GI ± SE	RF± SE	GI± SE	RF± SE	GI± SE	RF± SE
Gazelle	4.3 ± 0.88^{b}	25.3 ± 22.03^{ab}	4.3 ± 0.33^{c}	168.3 ± 50.30^{ab}	3.3 ± 0.33^{a}	27.0 ± 18.315^{a}
Viking	3.0 ± 0.33^{ab}	31.5 ± 20.97^{b}	3.7 ± 0.33^{c}	167.4 ± 134.64^{ab}	4.0 ± 0.58^{ab}	22.9 ± 10.308^{a}
Soma	3.3 ± 0.88^{ab}	40.5 ± 14.62^{b}	3.7 ± 0.33^{c}	57.0 ± 55.31^{ab}	4.7 ± 0.88^{bc}	18.2 ± 7.655^{a}
Soprano	4.0 ± 0.58^{b}	138.7 ± 71.59^{b}	2.7 ± 1.45^{abc}	83.0 ± 63.52^{a}	3.7 ± 1.20^{ab}	29.3 ± 23.254^{a}
Solitaire	3.0 ± 0.58^{ab}	103.5 ± 59.65^{b}	4.0 ± 0.577^{c}	279.0 ± 48.46^{bc}	3.7 ± 0.33^{ab}	16.4 ± 6.426^{a}
SNK 60	2.0 ± 0.58^{a}	2.2 ± 2.20^{ab}	1.0 ± 0.577^{a}	16.2 ± 6.44^{a}	5.0 ± 1.0^{abc}	16.3 ± 8.747^{a}
A 7119	2.0 ± 0^{a}	0 ± 0^{a}	1.3 ± 0.667^{ab}	2.1 ± 2.33^{a}	5.7 ± 0.88^{bc}	17.4 ± 9.186^{a}
Storm	3.3 ± 0.33^{b}	111.0 ± 67.40^{b}	3.0 ± 0^{b}	203.2 ± 146.46^{ab}	5.0 ± 0^{abc}	46.7 ± 42.011^{a}
Prima	6.3 ± 0.33^{c}	168.4 ± 145.2^{b}	7.3 ± 0.333^{d}	487.8 ± 41.269^{c}	7.7 ± 0.88^{c}	118.5 ± 78.462^{ab}
SED	0.75	90.30	0.52	85.20	0.96	36.88
F-test probability	0.042	0.034	0.019	0.008	0.042	0.032

GI= Gall index; RF= Reproduction factor = [final population (J2s + eggs) ÷ initial egg density]; Values are mean numbers per pot (plant) ± SE.
Means within a column followed by the same letter(s) are not significantly different at the 5% probability level according to the Duncan's multiple range test.

and subsequent nematode reproduction. Others showed absence of galling and no evidence of reproduction. Successful invasion with galling and subsequent nematode reproduction was observed on all the cotton cultivars on *M. incognita* Race 3; one cultivar, on *M. javanica* and three cultivars on *M. incognita* Race 1. These cultivars can be classified as hosts of the respective nematode species and renders them unsuitable for growing in areas where the respective *Meloidogyne* species are found. There is no source of resistance in the current cotton cultivars for *M. incognita* Race 3 and breeders have to look at sources of resistance out of this gene pool. The distribution of *M. incognita* Race 3 in Zimbabwe and most African countries where these cultivars are grown is not well documented [14]. This makes it hard to make clear geographic recommendations although it is clear that where *M. incognita* Race 3 occurs, the cultivars are unsuitable as a crop and for reducing soil populations of the nematode. Except for A7119 which is a non-host of *M. incognita* Race 1, all the soybean cultivars evaluated are hosts of *M. javanica* and *M. incognita* Races 1 and 3 which make them unsuitable in a rotation to manage the nematode species. The high reproduction of the three *Meloidogyne* spp. on Prima and reduced reproduction on A7119 on *M. javanaica* and *M. incognita* Race 3 agree with survey findings [8] which classified these cultivars as having a high and low *Meloidogyne* spp. prominence respectively.

Reproduction with galling is a well understood phenomenon of *Meloidogyne* reproduction [10]. However, on three cotton cultivars, FQ92, DF885 and BC853, there was no evidence of galling caused *Meloidogyne* spp. feeding but there was egg and J2 production and a consequent RF of greater than 1 for *M. incognita* Race 1. The *M. javanica* – DF 885 relationship also produced the same phenomenon. There is no explanation for this as it generally accepted that *Meloidogyne* spp. are endoparasites. The soybean cultivar A7119 did not support reproduction of *M. incognita* Race 1 but produced galls suggesting a post-infectional defence mechanism. The cultivar may pose no barrier to initial infection by the nematode but nematode development is arrested after penetration and limited feeding. If this assumption is correct, the plant probably produces a protein that is suspected to be able to disrupt root-knot nematode development [4]. If this trait can be transferred through breeding, this variety may form the basis for breeding for *M. incognita* Race 1 resistance in soybeans. However, the intolerance of the cultivar suggests that it may suffer yield losses as a result of exposure to *M. incognita* Race 1.

Absence of galling and no evidence of reproduction were found on TE 94-4, FQ92-19, CY889 and AG4869 with *M. incognita* Race 1 and *M. javanica*. The reactions of DF 885 on *M. incognita* Race 1 and FQ902, SZ-9314 and BC853 also produced the same result indicating that these cultivars can be considered as non-hosts. This makes them suitable as rotation crops in areas where these nematode species are being targeted either alone or in a mixed population.

Population densities in the organic fraction: The high number of eggs in the organic fraction has implications for soil sampling. Counts of J2s from the mineral fraction of the soil as used in most of the contemporary extraction methods for population density estimation will produce low population density estimates. The high population densities from the organic fraction are ignored yet they would be off-loaded into the mineral fraction within one to two weeks. There is, therefore a general underestimation of densities and errors are made in the advice given to farmers and in scientific research. In order to get a correct estimation of *Meloidogyne* spp. population density, it is absolutely necessary that the organic fraction of the soil (roots) is also submitted for investigation.

Host status evaluation: It may not be a sound idea to research host status reactions on the basis of a gall index alone. In doing so, there is no information on the initial population density and therefore, no assessment of any actual reproduction. As demonstrated in this study, presence of galls does not suffice as enough evidence for being a host. This implies the need for actual reproduction to be confirmed using methodologies that allow a reproduction factor to be determined. Despite this, there are some reports where conclusions on the host status of some plants are based on galling alone [7, 15].

Mapping of nematode distribution: The distribution of *M. incognita* Race 3 in Zimbabwe and other parts of Africa is not well known although its existence is well documented [11, 13, 14]. This information is critical in that it would enable recommendations to use some cotton cultivars as a non-hosts

for root-knot nematode management in the areas *M. incognita* Race 3 is not found. The cultivars tested in this study are grown in most parts of Eastern and Southern Africa. These results therefore are relevant to a large geographical area but the lack of data on the species distribution is critical for the recommendations to be made. It is unlikely that a *Meloidogyne* spp. may exist in isolation of other species but it would not be too surprising to find some areas where *M. incognita* Race 3 is not found.

Conclusions

This study provided valuable information on the resistance to *M. incognita* Race 1 and *M. javanica* by some cotton cultivars and to *M. incognita* Race 1 by a soybean cultivar. It also gave some insight into the flaws of using galling indices alone as a basis for evaluating host status to *Meloidogyne* species. It is recommended that resistant cultivars be tested under field conditions and a tobacco or other susceptible crop be grown thereafter to ascertain accrual of any benefits on the target crop as a result of their non-host status.

Acknowledgements

The Tobacco Research Board, Zimbabwe, funded the research.

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