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Suitability of eight cotton and nine soybean cultivars for rotation with tobacco based on their reaction to Zimbabwean nematode populations of the genus *Meloidogyne*

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Summary. Eight cotton and nine soybean cultivars were evaluated for their response to *Meloidogyne javanica* and *M. incognita* races 1 and 3 in pot tests to assess appropriate rotation schemes with tobacco. Nine weeks post nematode infestation, root galling, the number of nematode egg masses, the number of eggs per root, and the number of second-stage juveniles (J2) per pot were recorded. A reproduction factor was computed from the data. All the cotton cultivars were susceptible to *M. incognita* race 3 but resistant to *M. javanica*. The cultivars TE-94-4, FQ 92-19, CY889, AG4869, and DF885 were resistant to *M. incognita* race 1. The three cultivars which were susceptible to *M. incognita* race 1 did not show any root damage symptoms, suggesting that assessing for resistance using this criterion alone may be inadequate. The soybean cultivars were all susceptible to the three populations except SNK60, which was resistant to *M. incognita* race 1. This cultivar however, produced galls further indicating the inadequacy of using root damage functions of *Meloidogyne* species for host status evaluation.

Key words: cotton, crop rotation, host status, *Meloidogyne* spp., reproduction factor, soybeans, susceptible, resistant.

Root-knot nematodes, *Meloidogyne* spp. are among the most economically destructive plant parasitic nematodes, with a wide host range and geographical distribution (Sasser & Freckman, 1987). 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-dibromo3-chloropropane) has made plant resistance an important component in root-knot nematode management (Boerma & Hussey, 1992; Kutywayo, 2003).

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 target crop. Crop rotation with non-hosts is an effective way of reducing nematode soil population densities (Baker & Koenning, 1998). However, there are few economically feasible crops that can be used in a rotation for management of *Meloidogyne* spp. (Thomason & Caswell, 1987). This is partly due to the frequent occurrence of these nematodes as a mixture of different species that all have a wide host range. Most known non-host crops reduce the 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 classic 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 (Tobacco Handbook, 2000). In recent years, farmers have been looking for a more profitable rotation crop. Therefore, resistance must be identified in potential rotation crops that are financially lucrative or in their wild relatives to be incorporated into elite germplasm. A reasonable starting point would be to check for resistance in the current cultivars of potential rotation crops. Cotton (*Gossypium hirsutum* L.) and soybeans (*Glycine max* (L.) Merr. are commercially viable in Zimbabwe and have been earmarked for incorporation in a tobacco rotation but their host status to locally occurring *Meloidogyne* spp. is unknown. This study aimed to evaluate the host status of common cotton and soybean cultivars in Zimbabwe to the root-knot nematode populations in the country and develop recommendations for their usefulness in a tobacco rotation and the impact this has for plant breeders.

Table 1. Number of root knot egg masses, eggs and J2 of *Meloidogyne incognita* races 1 and 2 and *M. javanica* from eight cotton cultivars nine weeks post inoculation with an initial density of 5 J2s g-1 soil (5000J2s $1kg^{-1}$ 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	1 2.2	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	1 0.2	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	1 0.2	9.9	0	0	0
DF885	0.8	800	7.8	211.0	1 0.7	1 0.4	48.0	380.0	470.4
BC853	0.8	14 8.4	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)

MATERIAL AND METHODS

Plant establishment. This study was done at Kutsaga Research Station in Zimbabwe. Plants were grown by placing three seeds of the cultivar under test in the centre of 15 cm diameter pots filled with 1kg steam-sterilised, aerated sandy (>90 % sand) soil. Two weeks later, seedlings were thinned to one per pot. To each pot, 3g Compound D (8-14-7), a basal dressing fertiliser; was added and later supplemented with 25 ml of Nutrifol (Zimbabwe Fertiliser Company) (20-20-20) liquid fertiliser fortnightly. Soil water content was maintained at around field capacity and temperature at 20-25°C.

Inoculation procedure and experimental set up: To prepare inoculum, *Meloidogyne* eggs were extracted from roots of ten-week-old tomato (*Lycopersicon esculentum* Mill.) plants eight weeks after inoculation, using NaOCl (Hussey & Barker, 1973). The eggs were counted, their concentration was adjusted to 333 eggs ml^{-1} and they were used immediately. Pots were then inoculated with 4,000 and 5,000 eggs/pot for 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, ensuring a uniform vertical distribution of the eggs (Been & Schomaker, 1986). The holes were immediately filled with soil.

The experiment was designed as a completely randomised block design, with 5 blocks. Each combination of *Meloidogyne* population (3) and crop cultivar (8 for cotton, 9 for soybean) occurred once in each of the five blocks. The cotton cultivars were TE-94-4, FQ902, FQ92-19, CY889, AG4869, SZ-9314, DF885 and BC853. Soybean cultivars were Gazelle, Viking, Soma, Soprano, Solitaire SNK60, A7119, Storm and Prima. Tomato cv. Moneymaker was included as a positive control to ascertain inoculum viability, although no data were recorded from them.

Data collection and analysis: Nine weeks after inoculation, the root knot gall index, number of

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 post inoculation with an initial density of 5 J2 g-1 soil (5000 J2 $1kg^{-1}$ pot) of *Meloidogyne incognita* race 3.

Cultivar	Gall Index ± SE	Reproduction 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]

Table 3. Number of root knot egg masses, eggs and J2 of *Meloidogyne incognita* races 1 and 2 and *M. javanica* from nine soybean cultivars nine weeks post inoculation with an initial density of 4 J2 g-1 soil (4000 J2 $1kg^{-1}$ 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.

egg masses, eggs and J2 per pot were recorded. Where possible, the reproduction factor (RF) was calculated. Root galling was rated on a scale 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, galls very numerous, mostly coalesced, root growth slightly retarded 7 = very heavy, mass invasion, slight root growth; 8 = extremely heavy, mass invasion, no root development (Daulton & Nusbaum, 1961). Root egg masses were counted, after staining with Phloxine B (0.15g/l water) (Daykin & Hussey, 1985), and eggs were counted after extraction with NaOCl (Hussey & Barker, 1973). J2 were extracted from soil samples using the modified Baermann technique (Daykin & Hussey, 1985). Initially a 100 g sample of well mixed soil of each pot was extracted, and all *Meloidogyne* J2 were counted. Additional samples were processed if the number of nematodes recovered per pot was considered too low (<200) to obtain reliable density estimation. If fewer than 200 J2 were counted, additional soil would be extracted from that pot until 200 nematodes had been counted, otherwise, the whole pot would be counted. A reproduction factor [(RF) = (final number of eggs and J2 in soil) ÷ initial number of eggs inoculated] was calculated for each cultivar.

Prior to statistical analysis, nematode reproduction counts (numbers of egg masses, eggs and J2) 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 greater for *M. incognita* (Kofoid & White) Chitwood race 3 than for *M. incognita* race 1 or *M. javanica* (Treub) Chitwood for all cultivars. Based on counts of egg masses, eggs, and J2 and the consequent RFs, all cultivars were equally good hosts for *M. incognita* race 3 ($P<0.05$). This population also caused substantial root galling on all cultivars (Table 2). Cultivars FQ902 and SZ 9314 were better hosts for *M. incognita* race 1 than the other cultivars. TE-94-4 and AG4869 had no egg masses, and the rest very few (Table 1). *Meloidogyne javanica* and *M. incognita* race 1 did not cause root galling even on cultivars that allowed nematode reproduction. DF885 was a better host for *M. javanica* than the other cultivars ($P<0.05$). *Meloidogyne javanica* produced egg masses only on the cultivars FQ 92-19, CY889 and DF885, but without causing any root galling.

Soybeans. All three populations produced high numbers of egg masses, eggs and J2 on all soybean cultivars except on cv. A7119 which completely prevented reproduction of *M. incognita* race 1 (Table 3). However, this population caused some root galling on cultivar A7119 (Table 4). Data on root galling and RFs generally corresponded with egg mass, egg and J2 counts (Table 4). Prima generally was a better host for the three populations than the other cultivars ($P<0.05$) (Tables 3 and 4).

DISCUSSION

Host parasite relationship. All the cotton cultivars were good hosts for *M. incognita* race 3 leading to nematode reproduction and root galling. Three cultivars were hosts to *M. incognita* race 1, and one cultivar to *M. javanica*. Consequently, they are unsuitable for areas where these *Meloidogyne* populations 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 cotton-growing regions in Zimbabwe and most African countries is not well documented (Martin, 1969). This makes it hard to make clear geographic recommendations although it is clear that where *M. incognita* race 3 occurs, the cotton is unsuitable as a crop for reducing this 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 also make them unsuitable as a rotation crop to manage these nematodes. The high reproduction of the three *Meloidogyne* populations on Prima and reduced reproduction on A7119 of *M. javanica* and *M. incognita* race 3 is consistent with survey findings (Fourie *et al.*, 2001), which classified these cultivars as having a high and low *Meloidogyne* prominence respectively.

Reproduction with galling is a common result after infection of a host by *Meloidogyne* (Hussey & Grundler, 1998). However, three cotton cultivars (FQ92, DF885 and BC853) with RF of greater than 1 for *M. incognita* race 1, and one cultivar (DF 885) with RF greater than 1 for *M. javanica* did not exhibit galling. There is no explanation for this as it is generally accepted that *Meloidogyne* spp. are endoparasites that generally cause galling on susceptible hosts. 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 (Callahan *et al.*, 1997). 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. These findings demonstrate that the degree of root galling is not an accurate measure to determine host status for *Meloidogyne*.

Absence of galling and no evidence of reproduction were found on TE 94-4, FQ92-19, CY889, 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 roots. The high numbers of eggs in the roots has implications for soil sampling. Counts of J2 from 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 roots are ignored, yet they

would be off-loaded into the soil within one to two weeks. There is, therefore, a general under-estimation 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 roots are also submitted for investigation.

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 (Martin, 1969; Keetch & Buckley, 1984; Luc *et al.*, 1990). 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 from other species but it would not be too surprising to find some areas where *M. incognita* race 3 is not found.

CONCLUSIONS

This study provides 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 is recommended that resistant cultivars be tested under field conditions.

Table 4. Mean gall indices (0-8; where 0 = no galls and 8 = ca 100% galling) and reproductive factors for *Meloidogyne incognita* races 1 and 2 and *M. javanica* on nine soybean cultivars nine weeks post inoculation with an initial density of 5 J2 g^{-1} soil (5000 J2s $1kg^{-1}$ 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 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 = Gall to the Duncan's multiple range test.

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V. Kutywayo. Оценка пригодности восьми сортов хлопка и девяти сортов сои для ротации при выращивании табака по их реакции на зимбабвийские популяции нематод рода *Meloidogyne.*

Резюме. В контейнерах с почвой проведена оценка возможности использования восьми сортов хлопка и девяти сортов сои для ротации с табаком и контроля *Meloidogyne javanica* и *M. incognita* (расы 1 и 3). Через девять недель после заражения нематодами оценивали число галлов, количество скоплений яиц, число яиц, приходящееся на один корень, и число личинок второй стадии. По этим данным вычисляли фактор размножения. Все сорта хлопка были восприимчивы к *M. incognita* расы 3, но устойчивы к *M. javanica.* Сорта хлопка TE-94-4, FQ 92-19, CY889, AG4869 и DF885 были устойчивы к расе 1 *M. incognita.* Три сорта, чувствительные к расе 1 *M. incognita*, не показывали симптомов повреждения корней, что указывает на возможную неадекватность оценки устойчивости SNK60, который был устойчив к расе 1 *M. incognita.* На этом сорте, однако, образовывались галлы, что так же указывало на неадекватность использования такого показателя, как повреждение корней для оценки пригодности растений как хозяев нематод рода *Meloidogyne.*