REACTION OF *AMARANTHUS HYBRIDUS* SUBSP. *INCURVATUS* TO VARYING LEVELS OF *MELOIDOGYNE JAVANICA* INOCULUM

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ABSTRACT

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In greenhouse tests conducted to determine the host suitability of *Amaranthus hybridus* subsp. *incurvatus* L. to *Meloidogyne javanica* (Treub) Chitwood, eleven-day-old seedlings in pots of steam sterilized soil were inoculated with 0, 10, 100, 1000, or 10,000 second-stage larvae. Observations on growth response made 14, 28, 42, and 49 days after inoculation showed stimulated growth in inoculated plants but this effect was short-lived with the highest inoculum. Stimulated growth was reflected in significantly greater shoot heights, number of leaves per plant, length of internodes, and length and weight of inflorescences, especially in plants inoculated with 10,000 larvae per plant, according to the analysis of variance tests. Plants in the 10,000 inoculation were the first to come into flower. The weight of root systems and number of secondary roots tended to increase with an increase in the inoculum levels, but the number of primary roots was reduced. The severity of infestation increased with an increase in inoculum densities. *M. javanica* produced an average of 167 larvae per eggmass. Apparently *Amaranthus hybridus* is tolerant to *M. javanica*.
Additional key words: root-knot nematode, stimulation of plant growth, tolerant plant

RESUMEN

Bafokuazara, N. D. 1983. Reacción del *Amaranthus hybridus* subsp. *incurvatus* a niveles variantes de inoculo de *Meloidogyne javanica* Nematrópica 13:17-26.

En las pruebas de invernadero conducidas para determinar la reacción del *Amarathus hybridus* subsp. *incurvatus* L. al *Meloidogyne javanica* (Treub) Chitwood, plántulas de once dias en potes con suelo esterilizado al vapor fueron inoculadas con 9, 10, 100, 1000 y 10,000 larvas en el segundo estadio. Las observaciones hechas a las 14, 28, 42 y 49 dias después de la inoculación mostraron una estimulación en el crecimiento de las plantas inoculadas, pero este efecto fue de muy corta duración cuando se usó el inoculo más alto. La estimulación en el crecimiento se reflejó significativamente aumentando la altura de los retoños, el número de hojas por planta, la longitud de los entrenudos y la longitud y el peso de las inflorescencias, especialmente en las plantas inoculadas con 10,000 larvas por planta, de acuerdo con las pruebas de análisis de la varianza. Las plantas inoculadas con 10,000 fueron las primeras en florecer. El peso de las raices y el número de raices secundarias tendieron a aumentar con el incremento de los niveles del inoculo pero el número de raices

primarias fue reducido. La severidad de enfectación aumentó con el incremento de la densidad del inoculo. *M. javanica* produjo un promedio de 167 larvas por masa de huevecillos. Aparentemente *A. hybridus* es tolerante al *M. javanica.*
Palabras claves adicionales: nematodo nodulador, estimulatión del crecimiento de las plantas, plantas tolerantes.

INTRODUCTION

Amaranthus spp. are among the most popular local spinaches of many people in East Africa, particularly in Uganda. The various varieties found in this country and their different local names have been published by Goode (4). In the past they have been relatively unimportant in the diet, and have been grown on a rather small scale. However in recent years there has been an increased demand for various vegetables including *Amaranthus* which has stimulated increased production of this crop. The most common species now found on the fresh market is *Amaranthus hybridus* subsp. *incurvatus* L.

Increased production of vegetables could possibly result in increased nematode infestation and crop damage, hence control methods should be planned. In surveys of vegetable gardens in Uganda, *A. hybridus* subsp. *incurvatus* was found virtually free of *Meloidogyne* spp. damage (1). In addition, information gathered in 1975 in field trials (at Kawanda, Uganda), studying the influence of cropping sequences on populations of plant parasitic nematodes in vegetables (Bafokuzara, 1975 unpublished), indicated that this crop had a suppressive effect on populations of *Meloidogyne* spp. A similar effect was observed in field trials carried out at Thika, Kenya in 1977 (2). Apparently, *A. hybridus* is a promising rotational crop in vegetable culture where *Meloidogyne* spp. are present. However, no information is available on the host-parasite relationships of this crop with the common nematodes of vegetables in East Africa.

The objectives of these investigations were: 1) to determine the host status of *Amaranthus hybridus* subsp. *incurvatus* to *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949; 2) to study the effects of various inoculum levels on growth of *A. hybridus;* and 3) to determine the fecundity of this nematode on this crop.

MATERIALS AND METHODS

The inoculum of *M. javanica* for the experiment was initiated from a single eggmass and was increased on *Lycopersicon esculentum* Mill. (cv. Money Maker). For this test, light-brown intact eggmasses were removed from mature females 46 days after inoculation.

A. hybridus seeds were surface sterilized with 0.10% mercuric chloride for 5 min. and rinsed in sterilized water. They were then sown 1 cm deep in steam-sterilized soil in 20 cm plastic pots. Four days before inoculation the plants were thinned to one per pot. All plants were inoculated eleven days after germination with second stage larvae at inoculum levels of 0, 10, 100, 1000, or 10,000 nematodes per pot. For inoculations of 10 and 100 larvae, nematodes were handpicked from a counting dish with a handling needle and placed in vials containing distilled water. The number of vials corresponded to the number of treatments that had to be effected. For inoculations of 1000 and 10,000 larvae per pot, the procedure was as follows: a five ml aliquot was pipetted into a counting dish and the nematodes therein counted. The nematodes were then put into a separate beaker; six such counts were made and the average count found. The nematodes in the beaker were then returned to the original container and transferred to the glasshouse to effect the inoculation. This was done by pipetting the nematode suspensions into shallow depressions made in the soil around the bases of the seedling. The depressions were then filled with soil and water was added. Each treatment was replicated five times in a randomized design. Uninoculated plants served as controls.

Temperatures in the greenhouse where the experiment was conducted fluctuated between 19-37°C during the experimental period. No artificial fertilizers were used since the soil mixture that was used (sand, coffee husks, stone chippings 6mm thick, gravel, and animal manure in the ratio 4:4:2:2:1) was found to support very good plant growth. Plants were well watered throughout the test period. The experiment was conducted at the Faculty of Agriculture Field Station, Kabete, University of Nairobi, Kenya, in 1977.

The following data were collected 14, 28, 42, and 49 days after inoculation: 1) length of stem (length of hypocotyl, not included), 2) number of leaves present on plant, 3) number of leaves that have senesced, 4) length of 3rd leaf from plant apex, 5) length of internode between the 5th and 6th leaves from plant apex, 6) colour of plants, 7) number of days to flowering, 8) length of inflorescences, and 9) plant vigour. However, recording of some data had to be discontinued before the 49th day after inoculation for various reasons. For instance, recording of data on length of 3rd leaf from plant apex, length of internodes between the 5th and 6th leaves and colour of foliage was stopped when the plants flowered, as this was no longer possible.

After 49 days, the plants were up-rooted and the following data were recorded: 1) length of shoot plus that of hypocotyl, 2) weight of stem, 3) girth of stem at cotyledons, 4) length of taproots, 5) weight of root

system, 6) number of primary roots on first 5cm of taproot, 7) number of secondary roots on the first 25cm of primary roots, 8) rootknot index, and 9) number of larvae per eggmass. The 21 eggmasses used in the determination of fecundity were selected from plants in all the inoculations. Eggs were hatched in distilled water in an incubator maintained at 25°C as described previously (2).

RESULTS

Generally, *Meloidogyne javanica* stimulated the growth of *A. hybridus* (Table 1). The length of the main stem was greater for all levels of inoculum above 10 than for the control after 14 days, but by the 49th day after inoculation, the length of stems for only the plants inoculated with 1000 larvae was greater. The foliage of inoculated plants at 42 days after inoculation was noticeably less green, a possible indication of both flowering and progressive senescence (5).

In the 10,000 inoculation, plant and leaf growth were faster than in the control; however leaf senescence did not differ significantly from the control ($P=0.05$). The early leaf senescence and flowering that were observed in this treatment may have been the result of restricted feeder roots. The general level of vigour in these plants was depressed within the first 28 days after inoculation; thereafter plants looked more vigorous or as strong as the controls.

Leaf retention was ultimately highest in the 1000 inoculation. Plants inoculated with low to medium population densities (i.e. 100 and 1000 larvae) had the longest leaves and internodes at 42 days after inoculation, a time when most plants came into flower. This probably reflected persistent stimulated growth in these plants. By the 42nd day after inoculation, plants in the 100 inoculation and the control had small, immature inflorescences; those in the rest of the treatments were much bigger and well developed. Thus for 0, 10, 100, 1000, and 10,000 nematodes per pot, the mean weights of the inflorescences were 0.6, 7.6, 0.7, 7.8, and 11.7 g respectively.

Data recorded at 49 days after inoculation are shown in Table 2. There was a high correlation between the number of leaves and stem weight ($r=0.94^*$) and between number of leaves and stem lengths ($r=0.86$), but lengths of stems in plants inoculated with 1000 larvae were significantly longer than those of the controls. No other treatment differed significantly from the control. Inoculum densities and stem lengths were not significantly correlated (Table 3). Similarly, the relationship between length of stem and that of taproot was not significant ($r=-0.34$).

There was a significant correlation ($r=0.96^*$) between number of leaves and stem girth. Plants that were inoculated with 10 and 10,000

Table 1. Effect of various inoculum levels of *Meloidogyne javanica* on top growth of green *Amaranthus hybridus*.

Data recorded[w]	Time of sampling and inoculum density (2nd stage larvae)									
	14 days after inoculation					28 days after inoculation				
	0	10	100	1000	10,000	0	10	100	1000	10,000
Height of stem (cm)[x]	16.4	11.2*	22.8*	24.6*	24.0*	38.7	21.5*	40.7	48.2	51.4*
Number of leaves per plant	15.8	15.0	14.4	15.2	18.6*	19.6	21.2	19.0	21.4*	27.0*
Number of leaves, senesced	0.0	0.0	0.0	0.0	0.0	0.6	0.4	0.2	0.0	4.2
Length of 3rd leaf from plant apex (cm)	5.7	3.3*	4.1*	5.4	5.5	4.7	3.2*	5.1	4.9	
Length of internode between the 5th and 6th leaves from apex (cm)	0.2	0.2	0.2	0.2	0.3	0.2	0.3	0.2	0.2	
Colour of foliage[y]	4.0	4.0	4.0	4.0	3.9	4.0	3.8	4.0	4.0	2.0
Plant vigour[z]	3.8	3.8	3.5	3.6	2.3*	3.5	1.8*	3.4	3.7	2.2*
Number of days from germination to flowering										26
Length of inflorescence (cm)										3.6

Table 1. Effect of various inoculum levels of *Meloidogyne javanica* on top growth of green *Amaranthus hybridus* (continued).

Data recorded[w]	Time of sampling and inoculum density (2nd stage larvae)									
	42 days after inoculation					49 days after inoculation				
	0	10	100	1000	10,000	0	10	100	1000	10,000
Height of stem (cm)[x]	68.1	42.0*	70.8	83.2*	74.6	73.4	66.7	77.6	90.9*	77.6
Number of leaves per plant	25.0	27.4	25.3	29.2*	23.8	27.0	20.4*	27.0	30.8*	23.5*
Number of leaves, senesced	6.2	4.2*	5.5	6.2	7.4	7.2	5.2	6.6	6.8	7.8
Length of 3rd leaf from plant apex (cm)	2.8		5.5*	3.5*						
Length of internode between the 5th and 6th leaves from apex (cm)	0.20		0.35	0.40*						
Colour of foliage[y]	4.0	2.0	1.5	1.5	0.6					
Plant vigour[z]	1.9	2.6	3.6*	3.7*	2.8*	2.7	2.4	2.6	3.5	2.8
Number of days from germination to flowering	38	38	38	36	26					
Length of inflorescence (cm)	1.1	1.7	1.8	3.4	20.4	3.5	3.4	4.2	5.9	23.1*

[w]Means of five replications; treatments within the same sampling date followed by an asterisk (*) differ significantly (P=0.05) from control according to an analysis of variance followed by LSD test. Some data not recorded (see text).

[x]Length from cotyledons to base of inflorescence; does not include length of hypocotyl.

[y]Colour of foliage: 1=1–25% green (light green); 2=26–50% green; 3=51–75% green; 4=76–100% green (dark green).

[z]Plant vigour: 1=1–25% robustness (low level of vigour); 2=26–50% robustness; 3=51–75% robustness; 4=76–100% robustness (high level of vigour).

Table 2. Effect of *Meloidogyne javanica* on growth of *Amaranthus hybridus* at 49 days after inoculation.[x]

Inoculum level (2nd stage larvae)	Stem length (cm)	Stem weight (g)	Stem girth at cotyledons (cm)	Taproot length[y] (cm)	Weight of root system (g)	Number of lateral roots: Primary roots on first 5cm of taproot (proximal end)	Number of lateral roots: Secondary roots on 25cm of primary roots	Root knot index[z]
0	77.3	73.9	4.5	24.0	49.5	59.4	20.0	0.0
10	69.7	37.2**	3.2*	23.5	34.8**	57.0	15.2	0.0
100	81.5	81.3	4.6	26.5	59.4*	56.8	25.6	1.0
1000	94.5*	104.3	4.9	21.7	60.5*	59.4	31.6*	1.8
10,000	83.4	55.2*	4.0	24.8	67.9**	33.6*	39.4**	3.7

[x]Data are means of five replications. Means in columns followed by asterisks (*,**) differ significantly from the control at P=0.05 and P=0.01 respectively, according to an analysis of variance followed by LSD test.

[y]Stem and hypocotyl lengths, combined.

[z]Root knot index: 1=1–25% roots with visible galls; 2=26–50%; 3=51–75%; 4=76–100%. A sample of 21 representative eggmasses from all treatments averaged 167 larvae per eggmass.

Table 3. Correlation coefficients between *Meloidogyne javanica* inoculum density and various variables for *Amaranthus hybridus* subsp. *incurvatus* measured 49 days after inoculation.

Variable correlated with inoculum density	Correlation coefficient (r)[x]
Plant height[y]	+0.22
Stem weight	–0.26
Stem girth	–0.16
Taproot length	+0.14
Root weight	+0.63
Number of primary roots	–0.98**
Number of secondary roots	+0.80
Number of galls per 50cm of roots	+0.90*
Root knot index	+0.92*

[x]Asterisks (*,**) indicate significance at P = 0.05 and P = 0.01, respectively
[y]Includes length of hypocotyl

larvae had stems that were significantly lighter than those of controls. There was a tendency for large stems to be supported by large root systems (r = 0.53). Roots of plants inoculated with 100, 1000, and 10,000 nematodes were significantly heavier than the ones from controls, but those from plants that received 10 nematodes were lighter. Higher nematode population densities tended to stimulate increased root weight (r = 0.63), though this was not significant.

High population densities reduced the number of primary roots (r = –0.98**) but promoted production of more secondary roots. Root-knot indices increased with increase in nematode population densities (r = 0.92*).

DISCUSSION

Results show that attack of *A. hybridus* by *M. javanica*, particularly at inoculum levels of 100 and 1000 larvae per plant, had a stimulating effect on growth of leaves, and length, girth, and weight of stems. Compared with the control, big increases were recorded in the weight of root systems and in number of secondary roots of these plants, suggesting reparative growth in the affected plants (9). The stimulatory effect in the 10,000 inoculum was generally short-lived; the ultimate measurements of height, weight, and girth of stem, and the rate of plant growth

showed that the nematodes generally had a suppressive effect on plant growth. The increased growth observed at low to medium population densities is in agreement with reports by Wallace (10) that low populations of *M. javanica* increased top growth of several crops. While working with *M. hapla* Chitwood, Olthof and Potter (6) observed a similar phenomenon in cauliflower and cabbage.

The number of leaves which individual plants supported reflected the general development of the stems. This can best be seen in the measurements of stem length, girth, and weight; the last two were significantly correlated with leaf numbers. Apparently stimulated plant growth accelerated the onset of flower and fruit formation, and consequently increased senescence. The data also suggest that high population densities stimulated and accelerated the growth of the inflorescences, probably through changes in growth regulators (7). This hypothesis is supported by the observation that there were increases in the lengths of inflorescences with increases in nematode numbers. The inflorescences in the control were, at the termination of these tests, still relatively young. However the plants apparently did not show high potential for supporting inflorescences that would be much heavier than those observed in higher inoculations. Other data on inflorescences indicate that plants inoculated with 10 and 10,000 larvae had some of the heaviest inflorescences. It is logical to expect that these weights were attained at the expense of stem weights, hence the apparent light weights in these inoculations.

Data from the inoculations of 10 larvae per plant is generally not in accord with that from other inoculations. It is thought that in addition to nematodes, other factors might have influenced the results.

The increase in infestation level with increase in inoculum density indicates that *A. hybridus* is a host of *M. javanica,* though a poor one. Only tiny galls were produced and these supported small eggmasses, each capable of producing 167 larvae. Apparently, *Amaranthus* is tolerant to *M. javanica,* according to the scheme designed by Dropkin and Nelson (3) and adopted by Wallace (8).

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