EFFECTS OF *MELOIDOGYNE INCOGNITA* ON GROWTH AND NUTRIENT CONTENT OF *AMARANTHUS VIRIDIS* AND TWO CULTIVARS OF *HIBISCUS SABDARIFFA*

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ABSTRACT

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Pots with seedlings of callaloo (*Amaranthus viridis*) and the 'Red' and 'White' cultivars of sorrel (*Hibiscus sabdariffa*) were inoculated with 0, 100, 1 000, and 10 000 eggs of *Meloidogyne incognita* race 1 and the plants were grown for 6 weeks in a greenhouse. Nematode reproduction was good on Red sorrel, poor on callaloo, and was inhibited by White sorrel. Dry weights of infested callaloo roots were greater than weights of noninfested roots. Shoot heights, leaf areas, and dry shoot and root weights of infested plants of both sorrel cultivars were less than those of noninfested plants. The concentrations of the nutrients varied with the host, plant organ, and initial nematode density. The shoot/root ratios of the elements were similar at all inoculum levels (Pi) except those of callaloo roots at Pi = 10 000, which had significantly lower and higher ratios of sodium and manganese, respectively, than those of noninfested plants.

Key words: Amaranthus viridis, callaloo, *Hibiscus sabdariffa* cvs. White and Red, host resistance, macronutrients, *Meloidogyne incognita* race 1, micronutrients, pathogenicity, sorrel.

RESUMEN

Heffes, T. P., P. L. Coates-Beckford, y H. Robotham. 1991. Efectos de *Meloidogyne incognita* sobre el crecimiento y contenido de nutrientes en *Amaranthus viridis* y dos cultivares de *Hibiscus sabdariffa*. Nematrópica 21:7–18.

Plántulas de amaranto (*Amaranthus viridis*) y los cultivares de sorrel (*Hibiscus sabdariffa*) 'Red' y 'White' fueron sembradas en maceteros e inoculados con 0, 100, 1 000, y 10 000 huevos de *Meloidogyne incognita* raza 1 y mantenidas por 6 semanas en un invernadero. El nematodo se reprodujo bien en hibisco Red, pobremente en amaranto, y fue inhibido por el hibisco White. Pesos secos de raíces infestadas de amaranto fueron mayores que los no inoculados. La altura de los tallos, superficie foliar, y peso seco de los tallos y pesos radiculares de plantas infestadas de ambos cultivares de hibisco fueron más bajos que los no inoculados. La concentración de nutrientes varió con el hospedador, parte de la planta muestreada, y el inóculo inicial. Las relaciones tallo-raíz de los elementos fueron similares en todos, excepto en aquellas raíces de amaranto con una Pi = 10 000 que presentaron niveles de sodio y magnesio significativamente bajos y altos, respectivamente en relación a aquellas plantas no inoculadas.

Palabras clave: amaranto, *Amarantus viridis*, *Hibiscus sabdariffa*, hibisco, macroelementos, *Meloidogyne incognita* raza 1, microelementos, patogenicidad, resistencia.

INTRODUCTION

The root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is one of the most important pests in Jamaica. Earlier work by Hutton et al. (11) showed that callaloo (*Amaranthus viridis* L.) and sorrel (*Hibiscus sabdariffa* L. cv. White) inhibited the reproduction of *Meloidogyne* spp. in field and greenhouse tests whereas populations increased on 'Red' sorrel. The following studies were designed to investigate, in greater detail, the relationships between these three cultivars and *M. incognita* race 1. They also included investigations of the effects of parasitism on the nutrient status of hosts that show varying degrees of susceptibility to the nematode.

MATERIALS AND METHODS

Populations of *M. incognita*, each initiated from a single egg mass, were identified as race 1 using the North Carolina Differential Host Test (9) and maintained on *Lycopersicon esculentum* Mill. cv. Rutgers in the greenhouse. Eggs for inoculum were obtained by the method of McClure et al. (16).

For White and Red sorrel, two seeds were sown 1.5 cm deep and, for callaloo, two seedlings were transplanted into 12.5-cm-diam plastic pots containing 1 L of steam-sterilized sand. All pots were thinned to leave one seedling per pot just before inoculation of the soil with eggs. Ten pots with 33-day-old callaloo and 10 with 18-day-old White or Red sorrel seedlings were inoculated with 100, 1 000, or 10 000 eggs of *M. incognita* race 1 by pouring 20-ml suspensions in preformed 2-cm-deep cavities adjacent to the seedlings. Ten pots of each cultivar, serving as the controls, were inoculated with supernatant from centrifuged egg suspensions. The cavities were filled with sterile sand. For each test host, the pots were separated randomly into a group with six and another with four of each initial nematode population (Pi). Both groups of each host were arranged in a completely randomized design in a greenhouse with a mean daytime temperature of 28 C. At the time of inoculation, plant heights were recorded and each pot was given 25 ml of Long Ashton nutrient solution (10). Plants were fertilized similarly once each week thereafter.

The plants were harvested at 6 weeks after inoculation. For each plant in those groups with six replicates of each treatment, records were made of the number of leaves produced, total leaf area, shoot height, dry shoot and root weights after drying at 70 C for 5 days, and the number of root galls present. The dried organs were kept for nutrient determinations. The soil in each pot was suspended in 6 L water and the nematodes were extracted from 3 L for 24 hours by the Christie and

Perry technique (3). The nematode extracts were made up to 100 ml with tap water and the populations were estimated from either three or five 1-ml aliquots for high ($\geq$10/ml) and low ($<$10/ml) population densities, respectively, using Peter's counting slide. The number of egg masses produced by the roots of each plant and those groups with four replicates of each treatment were counted after staining with Phloxine B (4). Egg mass and gall production were rated by the indices 0, 1, 2, 3, 4, and 5 representing 0, 1-2, 3-10, 11-30, 31-100, and $>$100 egg masses or galls per root system, respectively.

Nutrient analyses were performed on the organs of each of the six replicates of callaloo. For each sorrel cultivar, the determinations were made on the combined pairs of randomly selected shoots or roots of each treatment. The dried organs of the replicates were ground in a stainless steel mill fitted with a 60-mesh sieve to retain the unwanted fragments. Samples of 200 mg of each organ were acid-digested by Wolf's method (24) modified by Rees and Sidrak (20) and made up to 100 ml with distilled water when cool. Nitrogen concentrations in callaloo were determined as nitrates by Taras' method (23) at a wave length of 470 nm, 30 min after addition of the reagents. Nitrogen in sorrel was determined as ammonium by the method of Fawcett and Stoughton (7) at 450 nm. Phosphorus concentrations were determined from the acid-digested samples by the method of Rees and Sidrak (2). For both elements, the standards ranged from 0 to 50 ppm.

The concentrations of the other essential macroelements (potassium, magnesium and calcium) and seven microelements (manganese, sodium, cobalt, iron, zinc, aluminum, and vanadium) were determined for each plant speices by instrumental neutron activation analysis (1). Each finely ground, homogenized, plant sample was enclosed in a heat-sealed, 1.7-cc polyethylene capsule. Then the samples were irradiated for 1 min at a neutron flux of 10^{12} neutrons cm^{-2} sec^{-1} in a SLOWPOKE-II reactor (1), and allowed to decay for 10 min before counting for 5 min on a Canberra reverse electrode germanium semi-conductor detector. The samples were irradiated again for 2 hours, allowed to decay for 3 weeks, and counted for 3 hours. The resulting spectra were analyzed using the EG & G Ortec Geligam software package and the concentration of each element determined by comparison with similarly treated standards. All elements were quantified from the first irradiation, decay and count scheme except cobalt, iron, and zinc, which were quantified from the second.

All data were subjected to analyses of variance (22) and each treatment mean was compared to that of the control by the least significant difference test (LSD) to determine the significant effects of nematode infestation on host growth and nutrient content.

RESULTS

Population development: Egg masses developed on roots of all inoculated plants except those of White sorrel growing in pots with Pi = 100 (Table 1). At Pi = 10 000, the respective egg mass indices for callaloo and White and Red sorrel were 4, 2, and 4 and were greater than those for plants grown at lower Pi s. Whereas the egg masses on Red sorrel were very large, those on White sorrel and callaloo were quite small with few eggs. At each Pi, the numbers of nematodes recovered were smallest from pots with White sorrel and were largest from those with Red sorrel.

Nematode pathogenicity: Leaf number, leaf area, shoot lengths, and shoot weights of infested callaloo plants did not differ significantly from those of the controls but the roots had significantly larger dry weights at all Pi s (Table 1). Both sorrel cultivars growing in soil with Pi = 10 000 had significantly shorter shoots, smaller leaf areas, and lower shoot and root dry weights than the controls. Some adverse effects on the growth of each sorrel cultivar were observed at Pi = 1 000 also but not at Pi = 100 (Table 1).

Galls, occurring on the feeder roots only of callaloo and White sorrel, never exceeded 3 mm in length but were ubiquitous and larger on Red sorrel roots. Several formed on callaloo and Red sorrel but few occured on White sorrel roots (Table 1).

Nutrient content: At Pi = 1 000 and 10 000, callaloo shoots had higher concentrations of nitrogen and manganese and, at the highest Pi, shoots had higher concentrations of aluminum than the controls (Table 2). In contrast, there was a significantly lower concentration of sodium at the two higher Pi s and of phosphorus and iron at the highest Pi.

In callaloo roots from pots with Pi = 1 000 and 10 000, nitrogen, calcium, magnesium, sodium, and aluminum occurred in higher concentrations than in the controls (Table 2). Nitrogen was also higher at Pi = 100. As in the shoots, phosphorus and iron concentrations were lower at Pi = 10 000 than at Pi = 0.

In White sorrel shoots of infested plants, the concentrations of only four elements differed from those of the controls. At Pi = 1 000 and 10 000, nitrogen, manganese, and vanadium concentrations were higher and, at Pi = 100, those of manganese and sodium were higher than control concentrations (Table 3).

At all Pi s, White sorrel roots had higher concentrations of nitrogen and manganese than the controls; roots from pots with Pi = 10 000 had higher levels of magnesium, zinc, and vanadium; at Pi = 1 000 and 100, concentrations of calcium were higher also (Tables 3). There were lower levels of potassium in the roots at Pi = 1 000 and 10 000 than at Pi = 0 and lower sodium levels at Pi = 10 000. Nematode infestation did not

Table 1. Population development of *Meloidogyne incognita* race 1 and response of *Amaranthus viridis* and two cultivars of *Hibiscus sabdariffa* grown in soil infested for 6 weeks, at four initial nematode densities.

Initial number of eggs/pot	Final population/pot	Egg mass index[y]	Shoot length[z] (cm)	Number of leaves	Leaf area (cm^2)	Dry shoot wt (mg)	Dry root wt (mg)	Root knot index[y]
			A. viridis					
0	0	0	25	11	291	2 544	1 160	0
100	50**	2*	24	11	269	2 495	1 453*	2**
1 000	350**	2*	24	12	276	2 862	1 580**	3**
10 000	3 600**	4**	23	12	252	2 677	1 538**	4**
			H. sabdariffa cv. White					
0	0	0	15	10	134	766	289	0
100	30**	0	15	11	156	773	305	0
1 000	50**	1*	12	10	134	726	277*	2*
10 000	330**	2**	11*	10	98*	503**	194**	2*
			H. sabdariffa cv. Red					
0	0	0	25	11	116	698	154	0
100	150**	2**	20	10	120	775	167	1*
1 000	1 250**	3**	22*	10	105	584*	168	3**
10 000	5 200**	4**	16**	10	90*	566*	190*	4**

Data are means of six replications except for egg mass and root-knot indices, which are means of four replications.
*, ** = significantly different from the control at $P \leq 0.05$ and 0.01, respectively, by the LSD test.
[y]Scale of 0 - 5 where 0=0, 1= 1-2, 2= 3-10, 3= 11-30, 4= 31-100 egg masses or galls/root system.
[z]Final height minus height at inoculation.

Table 2. Nutrient content of shoots and roots of *Amaranthus viridis* from soil infested for 6 weeks with *Meloidogyne incognita* race 1, at four initial nematode densities.

Initial number of eggs/pot	Essential macroelements (%)					Essential microelements (ppm)					Other micro-elements (ppm)	
	N	P	K	Ca	Mg	Na	Mn	Co	Fe	Zn	Al	V
						Shoots						
0	0.30	1.20	4.5	1.30	0.90	355	26	0.5	287	35	100	0.7
100	0.35	1.00	4.5	2.20	1.20	388	26	-	-	-	109	0.6
1 000	0.67**	0.90	4.3	1.80	1.10	214*	37*	-	-	-	114	0.6
10 000	0.80**	0.67*	4.7	1.80	1.20	124**	40**	0.6	242*	39	136**	0.6
						Roots						
0	0.29	0.92	2.9	0.85	0.64	4 300	64	1.0	2 400	30	1 900	11.0
100	0.43**	0.83	3.3	0.89	0.64	4 400	54	-	-	-	2 200	12.0
1 000	0.68**	0.77*	3.0	0.97*	0.84**	5 000	74	-	-	-	3 400	10.0
10 000	0.70**	0.53**	3.0	1.02*	0.88**	7 700	54	1.0	2 000	33	3 600	11.0

Data are means of six replicates.
*, ** = significantly different from the control at $P \leq 0.05$ and 0.01, respectively, by the LSD test.

Table 3. Nutrient content of shoots and roots of *Hibiscus sabdariffa* cv. White from soil infested with *Meloidogyne incognita* race 1 for 6 weeks, at four initial nematode densities.

Initial number of eggs/pot	Essential macroelements (%)					Essential microelements (ppm)					Other micro-elements (ppm)	
	N	P	K	Ca	Mg	Na	Mn	Co	Fe	Zn	Al	V
						Shoots						
0	0.90	0.17	1.42	1.99	0.42	154	255	1	397	42	145	1
100	1.10	0.18	1.40	2.07	0.40	249**	311	-	-	-	150	1
1 000	1.34*	0.17	1.40	2.04	0.45	153	335	-	-	-	183	2**
10 000	1.86**	0.17	1.41	1.96	0.44	143	324*	1	403	47	153	2**
						Roots						
0	0.41	0.10	2.54	1.12	1.46	3 100	119	8	1 600	51	166	20
100	0.61	0.11	2.54	1.26**	1.52	2 700	183*	-	-	-	169	20
1 000	0.58*	0.12	2.30*	1.23**	1.51	2 900	201*	-	-	-	170	22
10 000	1.01**	0.11	2.00*	1.12	1.66*	1 800	205*	10	1 800	67**	165	24*

Data are means of three replicates, each from two, combined, experimental units.
*, ** = significantly different from the control of $P \leq 0.05$ and 0.01, respectively, by the LSD test.

affect the root concentrations of phosphorus, iron, and aluminum, and like callaloo, cobalt.

The concentrations of all but three elements were similar in Red sorrel shoots at all Pi s. At Pi = 10 000, nitrogen, manganese, and iron concentrations were significantly lower, and at Pi = 100 that of nitrogen was higher, than concentrations in control plants (Table 4).

At the highest Pi, Red sorrel roots had significantly lower concentrations of nitrogen, potassium, magnesium, manganese, cobalt, iron, and zinc and higher concentrations of sodium, aluminum, and vanadium than control roots (Table 4). Similar differences occurred at Pi = 100 for magnesium and sodium. Only phosphorus and calcium concentrations remained unaffected by nematode infestation.

There were no macroscopic symptoms of nutrient deficiency or toxicity in any host. Neither were there differences in the shoot/root ratios of any element except sodium and manganese, which were smaller and greater, respectively, in callaloo at Pi = 10 000 than at Pi = 0 (Table 5).

DISCUSSION

The reproduction of *M. incognita* were severely inhibited by White sorrel and the mean egg mass index of 2 at the highest Pi indicated that this host, unlike Red sorrel, was highly resistant to the nematode (9). However, plant productivity was reduced in both cultivars.

The egg mass index of 4 for Red sorrel and callaloo at Pi = 10 000 indicated that both these hosts were more susceptible to the nematode than White sorrel. However, the small size of the egg masses and galls on callaloo roots probably resulted from physiological conditions within the roots being unfavorable to parasitism by the nematode. Also, the lack of growth retardation in callaloo indicated that this host had some degree of tolerance to *M. incognita* as noted for the relative, *Amaranthus hybridus* subsp. *incurvatus* L., which is tolerant to *Meloidogyne javanica* (Treub) Chitwood (2). The poor host status of both plants to *M. incognita* in this study confirms the findings by Hutton et al. (11) for the same hosts and Minton (17) for various lines of *H. sabdariffa*.

The degree of susceptibility of the plants to the nematode was not obviously reflected in the differences in their nutrient status resulting from parasitism. For example, the response of all elements, except phosphorus, potassium, and vanadium in the roots, to nematode infestation in both sorrel cultivars differed markedly without showing a general pattern (Table 3, 4). Also, several elements were less concentrated in the roots of infested Red sorrel than in noninfested ones whereas they have been observed to be more concentrated in the roots of infested cowpeas, another highly susceptible host (unpublished data). Therefore, the concentrations of nutrients in the plants appeared to depend on the host/nematode combination.

Table 4. Nutrient content of shoots and roots of *Hibiscus sabdariffa* cv. Red from soil infested with *Meloidogyne incognita* race 1 for 6 weeks, at four initial nematode densities.

Initial number of eggs/pot	Essential macroelements (%)					Essential microelements (ppm)					Other micro-elements (ppm)	
	N	P	K	Ca	Mg	Na	Mn	Co	Fe	Zn	Al	V
						Shoots						
0	1.26	0.16	1.25	1.63	0.47	185	245	1	551	117	124	2
100	1.44*	0.16	1.24	1.68	0.46	188	248	-	-	-	125	2
1 000	1.26*	0.16	1.28	1.71	0.43	162	255	-	-	-	123	2
10 000	0.78**	0.14	1.22	1.65	0.51	160	186**	1	312**	97	122	2
						Roots						
0	0.90	0.11	2.12	1.04	1.78	2 700	163	12	7 900	86	154	25
100	1.08	0.11	1.77	0.98	1.25**	4 400**	181	-	-	-	151	26
1 000	0.77	0.13	1.68*	0.98	1.19**	3 900**	154	-	-	-	148	26
10 000	0.56**	0.15	1.73*	1.05	1.18**	4 300**	133**	9*	3 200**	68*	225**	35**

Data are means of three replicates, each from two, combined, experimental units.
*, ** = significantly different from the control at $P \leq 0.05$ and 0.01, respectively, by the LSD test.

Table 5. Shoot/root ratios of nutrients in *Amaranthus viridis* and two cultivars of *Hibiscus sabdariffa* from pots infested with *Meloidogyne incognita* race 1 for 6 weeks, at four initial nematode densities.

Initial number of eggs/pot	Shoot/Root ratio											
	Essential macroelements					Essential microelements					Other micro-elements	
	N	P	K	Ca	Mg	Na	Mn	Co	Fe	Zn	Al	V
					A. viridis[y]							
0	1.03	1.30	1.55	1.53	1.41	0.20	0.41	0.50	0.12	1.17	0.05	0.06
100	0.81	1.20	1.36	2.47	1.90	0.18	0.48	-	-	-	0.05	0.05
1 000	0.98	1.17	1.43	1.86	1.31	0.14	0.50	-	-	-	0.04	0.06
10 000	1.14	1.26	1.56	1.76	1.36	0.06*	0.74*	0.30	0.10	1.18	0.04	0.05
					H. sabdariffa cv. White[z]							
0	2.20	1.70	0.56	1.78	0.28	0.05	2.14	0.12	0.25	0.82	1.91	0.05
100	1.80	1.64	0.55	1.64	0.26	0.09	1.70	-	-	-	1.89	0.05
1 000	1.84	1.54	0.61	1.66	0.29	0.05	1.67	-	-	-	2.50	0.09
10 000	1.84	1.54	0.70	1.75	0.27	0.08	1.58	0.10	0.22	0.70	2.07	0.08
					H. sabdariffa cv. Red[z]							
0	1.40	1.45	0.59	1.57	0.26	0.44	1.50	0.08	0.07	1.36	0.81	0.06
100	1.33	1.45	0.70	1.71	0.37	0.46	1.37	-	-	-	0.83	0.06
1 000	1.64	1.23	0.76	1.74	0.36	0.37	1.65	-	-	-	0.83	0.06
10 000	1.39	0.93	0.71	1.57	0.43	0.47	1.40	0.11	0.09	1.45	0.53	0.05

* = significantly different from the control at $P \leq 0.05$ by the LSD test.

[y]Means of six replicates.

[z]Means of three replicates, each from two, combined, experimental units.

Nematode-influenced changes in nutrient concentrations varied with the plant organ. Roots exhibited differences more frequently than shoots. Differences in nutrient concentrations between infested and noninfested plants similar to those noted in these studies have been reported for nitrogen (21), phosphorus (5) potassium (6, 12, 19), calcium (13, 14, 15), magnesium (8), sodium (21), manganese (18), and iron (12).

Translocation of manganese may have been increased in infested plants as the shoot/root ratio was higher at Pi = 10 000 than at Pi = 0. At the highest Pi, sodium translocation appeared to be inhibited, as indicated by the significantly low shoot/root ratio. However, as the ratios for all other elements were unaffected by nematode infestation, *M. incognita* may have caused alterations mainly in the nutrient concentrations of the plants rather than in the translocation of the elements.

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