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Meloidogyne incognita and Tomato Response to Thiamine, Ascorbic Acid, L-arginine, and L-glutamic Acid

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Abstract: The influence of solutions of ascorbic acid, thiamine, L-arginine, and L-glutamic acid on egg hatch, juvenile survival, and development and reproduction of *Meloidogyne incognita* in susceptible and resistant tomatoes was studied. Maximum inhibition of egg hatch occurred at 2,000, 4,000, and 2,000 ppm for ascorbic acid, L-arginine, and L-glutamic acid, respectively. Larval survival was significantly reduced by concentrations of 2,000 ppm ascorbic acid and 1,000 ppm of L-arginine. Maximum inhibition of egg hatch and mortality of juveniles was achieved at a concentration of 4,000 ppm of ascorbic acid and L-arginine. L-glutamic acid and thiamine had respective moderate and minimal toxic effects. Foliar sprays of ascorbic acid, L-arginine, or L-glutamic acid suppressed the numbers of root galls, females, and egg masses on the susceptible tomato cultivar Tropic. Ascorbic acid and L-arginine had highly significant effects when applied to foliage before inoculation with nematodes. Thiamine had little effect. All sprays suppressed the numbers of root galls and females in roots of the resistant cultivar VFN8 when treatments were applied before inoculation. They were not, however, effective as post-inoculation treatments. Growth of a susceptible cultivar was improved by post-inoculation and pre-inoculation treatments when compared with the control plants which had neither nematode infection nor chemical treatment. No positive growth response to chemical treatment was seen in resistant control plants.
Key words: amino acid, *Meloidogyne incognita*, tomato, vitamin.

Amino acids reduce nematode populations and damage on various hosts. Efficacy differs according to amino acid isomers and the host plant, as well as nematode species (6,7,13,14,17). Prasad and Webster (12) suggested that *Heterodera* and *Nacobbus* were affected by D-amino acids because these antimetabolites were concentrated at root tips which are the initial feeding sites of the nematodes. Some amino acids have been found to inhibit egg hatch, juvenile survival, and root galling by *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 (11,15,16).

There are few reports suggesting a role for ascorbic acid in the plant's defense mechanism (4,10). The amount of ascorbic acid in susceptible tomato (*Lycopersicon esculentum* Mill) cultivars was reported to be lower than in resistant cultivars (4). A decrease in plant ascorbic acid content can reduce the resistance of tomatoes to nematode infection (2–4,10). The role played by ascorbic acid in biological defense mechanisms was postulated to be control of the cyanide-resistance respiration in plant tissues (4).

The objectives of this study were to determine the effect of different concentrations of selected amino acids and vitamins on 1) *M. incognita* egg hatch and juvenile mortality, 2) development of *M. incognita* in susceptible and resistant tomatoes, and 3) growth of tomato plants.

MATERIALS AND METHODS

In vitro studies: Stock cultures of *M. incognita* race 1 were maintained in the greenhouse on susceptible tomato cultivar Tropic. To test the effect of two vitamins and two amino acids on egg hatch, 10 egg masses of uniform size were placed on a small piece of foam (2 × 2 × 1 cm) immersed in 40 ml of each concentration of ascorbic acid, thiamine, L-arginine, and L-glutamic acid (0, 100, 200, 500, 1,000, 2,000, and 4,000 ppm) dissolved in sterile distilled water. Each treatment was contained in a 10-cm-d petri dish, and all treatments were replicated four times. The test was conducted at room temperature (20 C). Hatched juveniles were withdrawn and

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TABLE 1. Accumulated hatch (numbers of juveniles) of *M. incognita* eggs in solutions of vitamins and amino acids.

Concentration (ppm)	Ascorbic acid	Thiamine	L-arginine	L-glutamic acid
0	835	970	949	859
100	820	925	788	824
200	752	1,027	764	766
500	736	1,142	704	736
1,000	587	910	797	772
2,000	474	937	600	633
4,000	436	903	261	630
LSD at 5% level	102	114	57	93
LSD at 1% level	140	157	78	127

Numbers of juveniles recovered in 15 days from single egg masses, average of four replicates.

counted at intervals of 1, 3, 6, 9, 12, and 15 days. Fresh solution was added after each withdrawal of juveniles.

To test the effect on juvenile survival, 1,000 freshly hatched juveniles were placed in 40 ml of the same concentrations of amino acids or vitamins that were used in the egg hatch test. Each chemical concentration was replicated four times. The test was conducted at room temperature (20 C). Dead and live juveniles were counted at the same intervals (days) as the egg hatch test.

In vivo studies: Preliminary studies had indicated that a spray concentration of 1,000 ppm of ascorbic acid, thiamine, L-arginine, or L-glutamic acid was not phytotoxic to tomato. Three-week-old tomato seedlings of susceptible (Tropic) and resistant (VFN8) cultivars were planted in 15-cm-d pots containing 500 g steamed loamy sand (3% clay, 4% silt, 93% sand).

One week after plants were established, eight uniform seedlings in each group were sprayed 20 times with one of the chemicals at a concentration of 1,000 ppm in distilled water. Each plant was sprayed with 15 ml solution on alternate days with an atomizer taking care that the chemical did not enter the soil. The day following the last spray each plant was inoculated with 1,000 eggs of *M. incognita.* One set of eight plants at each concentration served as a noninoculated control. The experimental design was a randomized complete block replicated eight times. Growing conditions were the same for all treatments. Observations on plant growth and development were taken 42 days after inoculation.

One-month-old tomato seedlings of both cultivars were inoculated with 1,000 eggs of *M. incognita.* Starting 10 days later the plants were sprayed on alternate days for 10 treatments with either ascorbic acid, thiamine, L-glutamic acid, or L-arginine at concentrations of 1,000 ppm. Data on plant growth, root-knot galling, and nematode development were taken 42 days after inoculation.

RESULTS

Effect on egg hatch: L-arginine at 100 ppm or more and ascorbic acid and L-glutamic acid at 200 ppm or more significantly ($P \leq$

TABLE 2. Inhibition (I) of egg hatch and mortality (M) of *M. incognita* juveniles in solutions of vitamins and amino acids.

Concentration (ppm)	Ascorbic acid		Thiamine		L-arginine		L-glutamic acid	
	I	M	I	M	I	M	I	M
0								
100	1.7	1.3	4.6	1.7	17.0	4.2	4.0	0.8
200	9.9	16.0		1.3	1.3	7.1	10.8	1.5
500	11.9	7.1		3.3	25.8	12.2	14.3	1.8
1,000	29.7	13.2	6.1	6.3	16.0	33.6	10.1	1.0
2,000	43.2	28.1	3.4	6.8	31.6	44.2	26.3	10.0
4,000	47.8	55.0	6.9	14.2	72.5	47.2	26.6	19.0

Percentages are average of four replications. Inhibition (I) was calculated using the following formula:
I = [(No. of juveniles from eggs in check − juveniles from eggs in treatment)/(No. of juveniles from eggs in check)] × 100.

0.05) inhibited hatch of *M. incognita* eggs (Table 1). No concentrations of thiamine suppressed hatch, but at 500 ppm thiamine stimulated hatch ($P \leq 0.01$). Thus, L-arginine and ascorbic acid, in that order, were the most inhibitory to hatch of *M. incognita* eggs in vitro (Table 2). Maximum hatch in thiamine and L-glutamic acid solutions occurred at 6 days. In ascorbic acid and L-arginine solutions, the hatch was much more variable and occurred over a period of 6–9 days depending on chemical concentration.

Effect on juvenile survival: Juvenile survival in solutions of ascorbic acid and L-arginine was inversely proportional to the chemical concentration and time (Table 3). In ascorbic acid and L-arginine solutions, few juveniles survived longer than 6 days at concentrations of 500 ppm. At 200 ppm ascorbic acid or L-arginine, no juveniles were alive at 15 days. The higher doses of both ascorbic acid and L-arginine resulted in 100% mortality after 9 days exposure. L-glutamic acid was toxic to second-stage juveniles, especially at the higher concentrations. Thiamine had little toxicity to juveniles even at high concentration.

Effect on females and eggs: Amino acids and ascorbic acid, when applied as pre-inoculation or post-inoculation treatments on Tropic tomato significantly reduced female and egg numbers (Table 4). Thiamine applied post-infection suppressed egg numbers with a trend toward fewer females, but the reverse was not true with pre-inoculation application. Fewer galls formed on roots of all treated plants. Accordingly, the reproduction rate of *M. incognita* as measured by Pf/Pi ratios was sharply decreased.

Fewer mature *M. incognita* were found on the resistant tomato cultivar VFN8, and no eggs were produced (Table 5). Despite the fact that the root-knot nematode was not able to complete its life cycle in 6 weeks in any of the treatments, these chemicals significantly affected the number of galls formed and adult females in the pre-inoculation treatments. The inhibitory effect of these chemicals was greatly influenced

TABLE 3. Survival in percent of 1,000 *M. incognita* juveniles at 1, 3, 6, 9, 12, and 15 days as influenced by selected vitamins and amino acids.

Concentration (ppm)	Ascorbic acid						Thiamine						L-arginine						L-glutamic acid					
	1	3	6	9	12	15	1	3	6	9	12	15	1	3	6	9	12	15	1	3	6	9	12	15
0	100	99	96	86	80	60	100	99	96	86	80	60	100	98	96	84	81	64	100	98	96	84	81	64
100	91	83	74	68	55	50	100	95	87	77	73	57	96	95	67	53	41	40	100	97	80	68	42	31
200	93	86	21	15	0	0	98	98	78	66	64	55	99	96	72	42	17	0	100	98	62	60	53	38
500	84	68	12	2	0	0	97	95	68	59	54	52	98	87	12	4	0	0	94	73	73	61	39	24
1,000	79	69	22	0	0	0	97	92	78	66	57	51	73	49	10	0	0	0	71	71	67	63	25	21
2,000	54	47	0	0	0	0	95	95	77	67	51	45	65	54	15	0	0	0	76	73	70	45	31	21
4,000	19	0	0	0	0	0	94	88	80	65	56	42	65	55	22	0	0	0	72	54	54	34	32	26

Percentages are averages of four replications.

TABLE 4. Development and reproduction of *M. incognita* infecting susceptible Tropic tomato treated with vitamins and amino acids.

	Pre-inoculation				Post-inoculation			
Treatment	Galls/ plant	Mature females	Egg masses	Pf/Pi	Galls/ plant	Mature females	Egg masses	Pf/Pi
Control	403	300	155	8.4	434	295	146	6.3
Thiamine	261	177	139	5.1	383	273	102	5.6
Ascorbic acid	236	168	78	5.0	313	259	70	3.1
L-arginine	231	169	66	3.7	335	212	96	3.8
L-glutamic acid	257	176	108	6.4	372	245	107	4.0
LSD at 5% level	40	25	35	1.3	55	35	11	1.0
LSD at 1% level	57	35	49	1.9	77	49	15	1.4

Numbers are averages of four replications.

by the time of application relative to the establishment of the nematodes in the root system.

Effect on host plant growth: Most of the chemicals increased plant growth in both post-inoculation and pre-inoculation treatments (Table 6). The plants reacted differently, however, according to the specific chemicals, time of application, and whether infected or noninfected. Thiamine, ascorbic acid, L-arginine, and L-glutamic acid tended to increase dry shoot weight of noninfected plants treated early; however, growth of infected plants treated pre-inoculation with ascorbic acid and L-glutamic acid was significantly ($P \leq 0.05$) greater than the check. In the post-inoculation treatments, the increase was more pronounced and dry weight of shoots was significantly greater ($P \leq 0.01$) than infected plants treated with thiamine, ascorbic acid, and L-glutamic acid (Table 6). The tested chemicals did not improve the growth of the resistant cultivar VFN8. Dry weights of shoots of plants pre-inoculation-treated with ascorbic acid and L-arginine were higher than those of nematode-infected nontreated plants; however, these differences were not significant.

DISCUSSION

Effects of amino acids and vitamins on *M. incognita* may be due to direct toxicity or the nematodes may be affected by the pH and (or) osmotic pressure of the solution (1,5,9). The pH of L-arginine at 500 and 4,000 ppm is 10.4 and 10.8, respectively, whereas that of ascorbic acid is 3.1 and 2.7. Eggs and second-stage juveniles of *M. incognita* may not tolerate these extremes. The respective pH of thiamine at 500 ppm and 4,000 ppm is 3.7 and 3.2, however, and this had little effect on hatch of *M. incognita* eggs or survival of second-

TABLE 5. Development of *M. incognita* infecting resistant VFN8 tomato treated with vitamins and amino acids.

	Pre-inoculation			Post-inoculation		
Treatment	Galls/plant	Mature females	Egg masses	Galls/plant	Mature females	Egg masses
Control	34	14	0	29	12	0
Thiamine	18	8	0	22	12	0
Ascorbic acid	21	8	0	23	9	0
L-arginine	13	5	0	19	9	0
L-glutamic acid	20	9	0	24	12	0
LSD at 5% level	12	4		12	4	
LSD at 1% level	17	6				

Numbers are averages of four replications.

TABLE 6. Host growth response of susceptible Tropic and resistant VFN8 tomato cultivars in *M. incognita* (Mi) infested (+) and noninfested (−) soil and treated with vitamins and amino acids.

		Tropic dry wt. (g)				VFN8 dry wt. (g)			
		Pre-inoculation		Post-inoculation		Pre-inoculation		Post-inoculation	
Treatment	Mi	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control	−	6.8	1.3	6.3	1.4	7.9	1.8	5.5	1.3
	+	6.2	1.1	5.3	1.4	6.8	1.7	6.0	1.3
Thiamine	−	7.3	1.0	6.8	2.0	6.6	2.0	4.3	1.0
	+	6.7	1.7	8.2	1.8	6.0	1.2	5.8	1.7
Ascorbic acid	−	7.8	1.5	7.9	2.9	7.5	1.5	5.5	1.3
	+	9.6	2.1	8.1	2.0	7.6	1.8	5.7	1.4
L-arginine	−	8.1	1.1	7.6	2.3	7.7	1.9	5.7	1.2
	+	8.5	1.9	7.6	2.2	8.1	1.6	6.7	1.5
L-glutamic acid	−	7.5	1.9	6.8	1.9	7.3	1.7	6.2	1.3
	+	9.0	1.9	8.5	2.3	6.8	1.7	6.4	1.4
LSD at 5% level		2.1	0.4	1.3	0.5	1.7	0.5	1.4	0.4
LSD at 1% level		2.8	0.6	1.8	0.7	2.3	0.7	1.9	0.5

Dry weights (g) are averages of four replications.

stage juveniles. The osmotic pressure of ascorbic acid and L-arginine solutions at higher concentration approaches levels that may be toxic to nematodes with prolonged exposure.

The observed effects on nematode development in tomato roots following application of ascorbic acid and L-arginine to the foliage are probably not related to their direct toxicity either to eggs or juveniles of *M. incognita.* Ascorbic acid applied in this manner probably does not significantly alter either the pH or the osmotic pressure of the cell sap. Alteration of the inherent susceptibility of the host plant is more likely. This is supported by the greater effectiveness of ascorbic acid or L-arginine sprays applied to foliage before inoculating the roots with juveniles, compared with post-infection sprays.

Our results on numbers of juveniles developing into females and the average fecundity per female supports the hypothesis of Arrigoni et al. (4) that ascorbic acid plays a key role in the defense mechanism of plants to pathogens such as the root-knot nematodes. To date, all of the evidence is indirect, but large increases in ascorbic acid have been demonstrated in nematode resistant tomato roots following infection by nematodes (4). Melillo et al. (10) showed that plants treated with ascorbic acid had fewer galls and the galls contained fewer giant cells. Ascorbic acid does not increase in susceptible roots.

Prasad and Webster (12) and Evans and Trudgill (6) suggested that antimetabolites, such as some DL-amino acids, might be used as nematicides. Ascorbic acid and L-arginine show some promise; however, Evans and Trudgill (6) found that DL-methionine was not cost effective. Although ascorbic acid and L-arginine show some promise, their utility will be known only after extensive field tests determine their nematicidal properties and cost effectiveness.

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