

The Effect of the Sugarbeet Nematode *Heterodera schachtii* on the Free Amino Acids in Resistant and Susceptible Beta Species

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ABSTRACT

The concentration of free amino acids was unchanged in the fibrous roots of *Beta patellaris* (a resistant species) in nematode-infected plants. In the fibrous roots of nematode-infected plants of *B.*

vulgaris (a susceptible species), concentrations of total amino acids, aspartic acid, glutamic acid, and glutamine were significantly increased. *Phytopathology* 60:1727-1729.

The infection of sugarbeet by the sugarbeet nematode (*Heterodera schachtii* Schm.) causes considerable stunting and sometimes death of the plants. With other plant parasitic nematodes, these stunting effects cannot be accounted for by mechanical injury alone (1, 14, 15).

It is reported that certain nematodes secrete specific proteolytic enzymes into the host, causing a breakdown of host material and the accumulation of free amino acids (11, 12). Changes in the composition of amino acids in the host plant as affected by virus (4) infections have been reported. Several nematode species have also been reported to cause an accumulation of amino acids in the host (3, 7, 8, 9, 11, 13). Myuge (12) reported that *Heterodera* did not appear to cause an accumulation of free amino acids in the host plant.

This study was initiated in an attempt to find a suitable selection scheme for breeding purposes, since the present conventional methods have not been successful (2). Since amino acids play an important role in sugarbeet yellows resistance (6), they may also play an important role in sugarbeet nematode resistance. Therefore, it was desirable to determine if *H. schachtii* larvae attacking the roots of the sugarbeet cause a change in the free amino acids in the host plant.

MATERIALS AND METHODS.—*Experiment A.*—Twenty sugarbeet seedlings each of *Beta patellaris* Moq. (a resistant species) and two *B. vulgaris* L. breeding lines, US 41 (a susceptible cultivar) and 063 (a tolerant selection), were transplanted into 3-inch pots containing steam-treated sand and watered with Hoagland's solution. Two weeks after transplanting, 10 plants of each entry were inoculated with 2,000 surface-sterilized *H. schachtii* larvae. The nematode larvae were hatched and surface-sterilized in large quantities by a technique developed by Whitney & Doney (16). Inoculation was accomplished by pipetting the desired number of larvae around the base of the plant and watering very lightly. One week after inoculation, root diffusate was collected from each plant by leaching 100 ml of distilled water through each pot in an 8 hr period. Two weeks after inoculation, fibrous roots and leaf samples were collected from each plant and frozen. Samples were thawed later, and juice was expressed in a hydraulic press under 5,000 lb. pressure from each sample for amino acid analysis.

Experiment B.—Seventy plants of 62-9134 (a uniform hybrid of *B. vulgaris*) were transplanted into

185-ml plastic vials. Two weeks after transplanting, 35 plants were each inoculated with 8,000 surface-sterilized nematode larvae. Six weeks after inoculation, a fibrous root sample was taken from each plant and frozen for subsequent amino acid analysis.

Experiment C.—Sixty plants each of *B. patellaris* and 11 cultivars of *B. vulgaris* were transplanted into plastic vials. Two weeks after transplanting, 30 plants of each cultivar were inoculated with 4,000 surface-sterilized nematode larvae. Six weeks after inoculation, a fibrous root sample was taken from each plant and frozen for subsequent amino acid testing.

A completely randomized design was used in experiments A and B; whereas, experiment C was in a split-plot design.

Root diffusate was analyzed only for total free amino acids. Leaf and fibrous root juice samples were analyzed for total free amino acids, aspartic acid, glutamic acid, and glutamine. The concn of total amino acids was determined by spreading, with drops of water, 5 μ liters of expressed juice a distance of 2.5 inches on a strip of filter paper 0.5 inch wide. The strips were dried and dipped in ninhydrin reagent, and the color was developed under carefully controlled conditions. Glutamic acid standards were run concomitantly. The color was eluted from the strips and the intensity determined using a Spectronic 20 at 410 m μ . For the three individual amino acids, 2.5 μ liters of expressed juice was spotted in duplicate on chromatography paper and developed for 5.5 hr in redistilled phenol saturated with water. The papers were dried, and the density of the spots was determined using a photovolt transmission densitometer. Standard curves were prepared from a series of dilutions of each amino acid. The concn of the amino acids in the expressed juice were determined from these curves (4, 5).

RESULTS.—In experiment C, an actual count revealed an average of 160 white females at the soil-vial interface 4 weeks after inoculation. In a similar study, 30 to 40% of the total nematodes found on a plant were at the soil-vial interface. From this information it was estimated that between 20 to 25% of the nematode larvae added in experiment C invaded the plant roots. An infection rate of 18 to 20% was found in similar studies in which roots were stained in lactophenol acid fuchsin (10) 1 week after inoculation.

The concn of total amino acids in root diffusate, 1 week after inoculation, was very small. The concn of

TABLE 1. Concentration in μg per g of root juice of amino acids in fibrous roots of healthy and *Heterodera schachtii*-infected plants

	Aspartic acid		Glutamic acid		Glutamine	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
Experiment A						
<i>Beta patellaris</i>	35	23	56	58	210	226
<i>B. vulgaris</i>	49	66**a	119	171**	453	616
(US 41 and 063)						
Experiment B						
<i>B. vulgaris</i>	19	28**	21	37**	26	47**b
(uniform hybrid)						
Experiment C						
<i>B. patellaris</i>	36	38	73	72	46	63
<i>B. vulgaris</i>	48	62**	72	108**	63	117**

a ** = Significant increase at $P = .01$.

b * = Significant increase at $P = .05$.

total amino acids in root diffusate was increased slightly in nematode-infected plants in both *B. patellaris* and *B. vulgaris*, but in neither species was there a significant increase.

There was a slight increase of total amino acids in the leaves of infected plants in *B. patellaris* and a slight decrease in *B. vulgaris*, but differences were small and not significant. In the two *B. vulgaris* cultivars, US 41 showed a decrease and 063 increased in total amino acids in the leaves when infected with nematodes; but neither difference was significant.

In the fibrous roots of nematode-infected plants, a significant increase in total amino acids was observed in *B. vulgaris*, whereas little or no effect was obtained in *B. patellaris*.

There was little or no change in the concn of aspartic acid, glutamic acid, and glutamine in the leaves as a result of nematode infection in either *B. patellaris* or *B. vulgaris*. Small differences were observed, but in no case were these differences significant.

Table 1 shows the concn of aspartic acid, glutamic acid, and glutamine in the fibrous roots of healthy and infected plants for all three experiments. For *B. patellaris* (experiments A and C), a significant increase was not observed in the concn of any of the three amino acids as a result of nematode infection. But highly significant increases in the concn of aspartic acid and glutamic acid in the fibrous root of the nematode-infected plants of *B. vulgaris* were observed in all three experiments. Significant increases in the concn of glutamine in the fibrous roots of infected plants of *B. vulgaris* were obtained in experiments B and C. An

increase in glutamine was also observed in experiment A.

These results indicate a significant increase in the three amino acids studied in the fibrous roots of infected *B. vulgaris*, but no effect on the amino acid levels in the fibrous roots of infected *B. patellaris* plants. This difference in effect was tested in the analysis of variance.

The F tests of the analysis of variance for experiments A and C are presented in Table 2. The species \times nematode versus healthy line in the analysis of variance tests the reaction of the two species to nematode invasion. A significant interaction indicates that *B. vulgaris* was affected differently than *B. patellaris* by nematode infection. A significant interaction was obtained for aspartic and glutamic acid in experiment C. A significant interaction for aspartic acid was also obtained in experiment A. There was a significant difference between species in all tests, and a significant difference between nematode-infected and healthy plants in all tests except glutamine in experiment A.

Plant wt were taken in experiments B and C (Table 3). There appeared to be very little nematode effect on the fibrous root wt at this stage of infection. A significant reduction in tap-root wt was found in experiment B. In experiment C, the tap roots of healthy plants weighed more than those of nematode-inoculated plants; however, this difference was not large enough to be significant.

DISCUSSION.—The root diffusate was collected and analyzed because it plays such an important role in the life cycle of the sugarbeet nematode. The concn of

TABLE 2. F tests of analysis of variance for aspartic acid, glutamic acid, and glutamine in Experiments A and C

	Aspartic acid		Glutamic acid		Glutamine	
	Exp. A	Exp. C	Exp. A	Exp. C	Exp. A	Exp. C
Nematode vs. healthy	7.20*a	11.98**b	7.49*	49.11**	1.83	16.94**
Species	15.98**	6.02**	21.37**	2.45**	6.77**	3.53**
Species times (nema vs. healthy)	3.96*	5.69*	2.00	7.91**	0.69	1.97

a * = Significant at $P = .05$.

b ** = Significant at $P = .01$.

TABLE 3. Means of fresh wt of fibrous and tap roots of healthy and *Heterodera schachtii*-inoculated plants

	Fibrous roots (g)		Tap roots (g)	
	Nematode-inoculated	Healthy	Nematode-inoculated	Healthy
Experiment B	1.30	1.49	1.93	2.47***
Experiment C	1.85	1.70	2.81	2.96

a ** = Significantly greater than nematode-inoculated plants at $P = .01$.

amino acids was so small in root diffusate that the analysis of root diffusate was discontinued after experiment A.

No significant effects due to nematode invasion were found in the concn of amino acids in the leaves 2 weeks after inoculation. Therefore, no leaf samples were taken in experiments B and C.

Aspartic acid, glutamic acid, and glutamine made up a large portion of the total amino acids. In addition, greater effects were observed in these three amino acids than the remaining amino acids as a group. Therefore, analyses were confined to the concn of aspartic acid, glutamic acid, and glutamine in experiments B and C.

The average number of nematodes in the samples was not enough to effect amino acid concn, since most of the white females were washed from the roots prior to sampling. Separate studies involving similar nematode populations indicated that the amount of free amino acids in the samples contributed by the nematodes was insignificant.

An upset in plant metabolism would precede the stunting or visual effects and, therefore, be an earlier and perhaps a more reliable measure of the nematode effect. Additional work is necessary to determine the reliability of a selection criterion based on the nematode effect on the concn of free amino acids.

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