

RESEARCH NOTES

Amino Acids and Carbohydrates Secreted by *Meloidogyne incognita*

E. L. H. WANG and G. B. BERGESON¹

There is clear evidence that plant-parasitic nematodes secrete numerous organic substances (4, 5). As a part of our studies of root exudates in *Meloidogyne incognita*-infected and uninfected tomato plants (8), a qualitative and quantitative identification was made of the amino acids and carbohydrates secreted by larvae of *M. incognita*.

Second-stage larvae of *M. incognita* (Kofoid and White) Chitwood, collected from galled roots of gnotobiotic cultures of *Lycopersicon esculentum* Mill. 'Tecumseh,' were surface-sterilized. Flasks, each containing 10⁶ larvae in 300 ml sterile deionized water, were incubated in a shaker at 25 C for 48 h under monoxenic conditions. Water in which larvae had been incubated was passed through three layers of filter paper in a sterile sintered-glass filter to remove the larvae. Sterility was tested by plating 1 ml of incubation solution on PDA medium (8). Incubation solutions were further passed through a sterile Millipore (Bedford, Mass) filter (0.45 and 0.22 μ m), concentrated in a rotary evaporator at 45 C, adjusted to pH 7, and passed sequentially through a column (1 \times 4 cm) of Dowex 50 resin H⁺ form and a column (1 \times 4 cm) of Dowex 1 resin in formate form at a flow rate of 1 ml per 2 min. (8). The effluent from the Dowex 1 column contained the neutral fraction (carbohydrates). The cationic fraction (amino acids) was obtained by eluting a Dowex 50 column with 2N NH₄OH. Both fractions were evaporated to dryness at 45 C. The cationic fraction was redissolved in citrate buffer, pH 2.2, and placed in an amino acid analyzer (Beckman, model 120C) for qualitative and quantitative analysis of amino acids. The neutral fraction was redissolved in 38% isopropanol for two-dimensional

thin-layer chromatography (3). Carbohydrates, identified qualitatively by R_f values, color, and comparison with known samples, were estimated quantitatively by the method of Purdy and Truter (6).

The test for sterility indicated fewer than 10 microbial colonies/ml of incubation solution, which we believe made a negligible chemical contribution to the solution. Fifteen amino acids and six carbohydrates were identified from the water in which larvae of *M. incognita* were incubated (Table 1).

Since the activity of microflora in the rhizosphere of root-knot-nematode-infected plants is strongly influenced by the host exudate (1, 8), and since root-knot larvae are a part of the constituents of the galled root rhizosphere, amino acids and carbohydrates probably contribute a part of the exogenous nutrients for enhancement of microflora in the rhizosphere.

TABLE 1. Amino acids and carbohydrates secreted by surface-sterilized larvae of *Meloidogyne incognita* into sterile deionized water at 25 C.

Amino acids	μ g/10 ⁶ larvae /48 h ^a	Carbo- hydrates	μ g/10 ⁶ larvae /48 h ^b
Glutamic acid	33.4	Sucrose	210.4
Alanine	19.6	Mannose	173.2
Aspartic acid	18.2	Raffinose	142.9
Arginine	17.3	Melzitose	105.7
Serine	15.4	Sorbose	103.8
Lysine	15.0	Melibiose	65.0
Threonine	14.3	Total	801.0
Leucine	12.7		
Glycine	11.2		
Isoleucine	9.6		
Proline	7.9		
Tyrosine	7.4		
Valine	7.3		
Phenylalanine	7.3		
Histidine	5.6		
Total	202.2		

Received for publication 21 November 1977.

¹Nematologist, Department of Agriculture and Fisheries, Box 834, Hamilton 5, Bermuda; and Associate Professor of Nematology, Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907.

^aEach value is the average of two replications.

^bEach value is the average of four replications.

LITERATURE CITED

1. BERGESON, G. B., S. D. VAN GUNDY, and I. J. THOMASON. 1970. Effect of *Meloidogyne javanica* on rhizosphere microflora and Fusarium wilt of tomato. *Phytopathology* 60:1245-1249.
2. CANVIN, D. T., and H. BEEVERS. 1961. Sucrose synthesis from acetate in the germinating castor bean: kinetics and pathway. *J. Biol. Chem.* 236:955-988.
3. LATO, M., B. BRUNELLI, G. CIUFFINI, and T. MEZZETTI. 1968. Bidimensional thin-layer chromatography of carbohydrates on silica gel impregnated with boric acid. *J. Chromatogr.* 34:26-34.
4. MYERS, R. F., and L. R. KRUSBERG. 1965. Organic substances discharged by plant parasitic nematodes. *Phytopathology* 55:429-437.
5. AIST, S., and R. D. RIGGS. 1969. Amino acids from *Heterodera glycines*. *J. Nematol.* 3:254-259.
6. PURDY, S. J., and E. V. TRUTER. 1962. Quantitative analysis by thin-film chromatography. *Analyst* 87:802-807.
7. THOMPSON, J. F., C. J. MORRIS, and R. K. GERING. 1959. Purification of plant amino acids for paper chromatography. *Anal. Chem.* 31:1028-1031.
8. WANG, E. L. H., and G. B. BERGESON. 1974. Biochemical changes in root exudate and xylem sap of tomato plants infected with *Meloidogyne incognita*. *J. Nematol.* 6:194-202.