RESEARCH NOTES

Beta-Glycosidases from Meloidogyne incognita and M. javanica[1]

J. L. Starr[2]

B-glycosidases have been implicated in plant pathogenesis by numerous authors (6,8,9,10). B-glucosidase from *Pratylenchus penetrans* was reported to hydrolyse HCN from a cyanogenic glucoside (amygdalin) during pathogenesis of peach roots (7), and the activity of B-glucosidase from preparasitic larvae of *Heterodera rostochiensis* has been correlated with the ability to parasitize resistant potato lines (13). Currently, data is not available on the types and amounts of B-glycosidase activity associated with root-knot nematodes. Objectives of this study, therefore, were to determine the types of B-glycosidase activities associated with *Meloidogyne incognita* (Kofoid and White 1919) Chitwood 1949 and *M. javanica* (Treub 1895) Chitwood 1949 and to compare these enzyme activities to those associated with host root tissues.

Nematode populations were reared on *Lycopersicon esculentum* Mill. 'Rutgers' under greenhouse conditions (24 ± 4 C). Adult females were obtained from root tissue 40 d after inoculation as described by Hussey (2). Preparasitic second-stage larvae were collected as described by Vrain (12). Root tissue consisted of excised root-knot galls from tomato 35 d after inoculation of *M. javanica* and root apices (terminal 2 cm) from uninoculated plants of similar age.

Crude homogenates of nematode samples were prepared as described by Hussey et al. (3). Nematodes were surface sterilized with 0.1% chlorhexidine acetate then rinsed three times with cold, sterile 0.01 M PO_4 buffer (pH 7.5) containing 0.85 NaCl and 0.001 M $MgCl_2$. Samples were homogenized at 4 C with 1.5 volumes of the same PO_4 buffer and centrifuged for 20 min at 8000 × g at 4 C. The supernatant was used for B-glycosidase assays. Protein concentrations were determined by the method of Lowery et al. (5). Root tissue homogenates were prepared similarly to nematode homogenates.

Enzyme activities were determined according to the Worthington procedure (1) using 100 µl of homogenate (50- to 200-µg protein) and p-nitrophenyl-B-D-galactopyranoside, p-nitrophenyl-B-D-glucopyranoside, and p-nitrophenol-B-D-fucoside as substrates. Controls consisted of heat inactivated enzyme and reaction mixtures minus either substrate or homogenate.

Adult females of *M. incognita* and *M. javanica* had high B-galactosidase activity and low B-glucosidase activity (Table 1). Only traces of B-fucosidase activity were detected in *M. incognita;* no B-fucosidase activity was detected in *M. javanica.* Enzyme activity with all three substrates was optimum between pH 4.0-5.0; no activity was detected at pH 8.0. Adult females had higher specific activity for each enzyme than did preparasitic larvae (Table 1). For these species the specific B-galactosidase activity was consistantly greater than the specific B-glucosidase activity; the reverse situation was reported for *H. rostochiensis* (13).

B-galactosidase and B-glucosidase activ-

Table 1. B-Glycosidase activities associated with adult females and preparasitic larvae (L_2) of *Meloidogyne incognita* and *M. javanica.*

	µM p-nitrophenol released min^{-1} · mg protein^{-1}*		
	B-galactoside	B-glucoside	B-fucoside
M. incognita—			
females	35.4	11.4	4.5
L_2	9.7	6.0	3.8
M. javanica—			
females	39.5	5.7	0
L_2	9.4	2.7	0

*Data are means of three separate tests.

Received for publication 30 June 1980.

[1]Paper No. 6455 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27650. This work was supported by a grant from the Agency of Internation Development to J. N. Sasser, Contract No. AID/ta-1234. The use of trade names does not imply endorsement of the products named or criticism of similar ones not mentioned.

[2]Former Research Associate, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27650. Current address Nematode Advisory Section, Agronomic Division, North Carolina Department of Agriculture, Blue Ridge Road Center, Raleigh, NC 27611.

ities were detected at pH 4.0 in root gall tissue and in uninoculated roots (Table 2); no activity was detected in root tissue at pH 7.5. Additionally, it was noted that specific B-galactosidase activity in the gall tissue was greater than in the uninoculated tissue and in both tissues specific B-galactosidase activity was greater than B-glucosidase activity.

Recent studies (4,11) on the origin of peroxidase activity associated with root-knot nematodes presented evidence that most, or all, of the enzyme activity was of host and not nematode origin; this does not appear to be true of B-glycosidases. The presence of B-glycosidases in preparasitic larvae of both species, which have not fed on host tissue, and in uninoculated root tissue are indicative of constitutive synthesis of these enzymes.

LITERATURE CITED

1. Anonymous. 1972. B-Galactosidase (3.2.1.23) P. 106 *in* The Worthington manual, Worthington Biochemical Corp., Freehold, New Jersey.

2. Hussey, R. S. 1971. A technique for obtaining quantities of living Meloidogyne females. J. Nematol. 3:99-100.

3. Hussey, R. S., J. N. Sasser, and D. Huisingh. 1972. Disc-electrophoretic studies of soluble proteins and enzymes of Meloidogyne incognita and M. arenaria. J. Nematol. 4:183-189.

4. Jones, M. G. 1980. Micro-gel electrophoretic examination of soluble proteins in giant transfer cells and associated root-knot nematodes (Meloidogyne javanica) in balsam roots. Physiol. Plant Pathol. 16:359-367.

5. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chemistry 193: 265-275.

6. Mace, M. E. 1973. Histochemistry of beta-glucosidase in isolines of Zea mays susceptible or resistant to northern corn leaf blight. Phytopathology 63:243-245.

7. Mountain, W. B., and Z. A. Patrick. 1959. The peach replant problem in Ontario VII. The pathogenicity of Pratylenchus penetrans (Cobb 1917) Filip & Stek. 1941. Can. J. Bot. 37:459-470.

8. Myers, D. F., and W. E. Fry. 1978. Enzymatic release and metabolism of hydrogen cyanide in sorghum infected by Gloeocercospora sorghi. Phytopathology 68:1717-1722.

9. Olah, A. F., and R. T. Sherwood. 1973. Glycosidase activity and flavanoid accumulation in alfalfa infected by Ascochyta imperfecta. Phytopathology 63:739-742.

10. Reddy, M. N., P. A. Peterson, and C. L. Tipton. 1973. Activity of some hydrolytic enzymes in maize during development of southern corn leaf blight disease caused by Helminthosporium maydis Race T. Phytopath. Z. 78:264-273.

11. Starr, J. L. 1979. Peroxidase isozymes from Meloidogyne spp. and their origin. J. Nematol. 11:1-5.

12. Vrain, T. C. 1977. A technique for the collection of larvae of Meloidogyne spp. and a comparison of eggs and larvae as inocula. J. Nematol. 9:249-251.

13. Wilski, A., and J. Giebel. 1966. B-Glucosidase in Heterodera rostochiensis and its significance in the resistance of potato to this nematode. Nematologica 12:219-244.

Table 2. B-Glycosidase activities associated with tomato roots infected by *Meloidogyne javanica* and uninoculated tomato roots.

		μM p-nitrophenol released $min^{-1} \cdot mg\ protein^{-1}$*	
	pH	B-galactoside	B-glucoside
M. javanica—gall tissue	4.0	18.8	3.9
	7.5	0	0
Uninoculated roots	4.0	7.9	2.3
	7.5	0	0

*Data are means of two separate tests.