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Peroxidase Isozymes from *Meloidogyne* spp. and Their Origin[1]

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Abstract: Two peroxidase isozymes (E_f 0.43 and 0.53) were detected by electrophoretic analysis in homogenates of *Meloidogyne arenaria, M. hapla, M. javanica,* and *M. incognita* females reared on tomato. No peroxidase isozymes were detected electrophoretically in homogenates of adult males, preparasitic larvae, or eggs. Peroxidase isozymes from females reared on bean, eggplant, or tobacco differed from those from females reared on tomato. Bean and eggplant populations had a single peroxidase isozyme each, respectively E_f 0.21 and 0.28. No peroxidase isozymes were detected in tobacco populations under the conditions used, although total activity assays did reveal low levels of peroxidase activity in homogenates of tobacco populations. The peroxidase isozymes detected in females reared on tomato or bean appear similar to the peroxidase isozymes present in root-knot galls, adjacent ungalled roots, and roots from uninoculated plants of the corresponding hosts. The probability is discussed that most of the peroxidase activity associated with *Meloidogyne* spp. females is of host origin. *Key Words:* root-knot nematodes, enzymes, microgel electrophoresis, host-parasite relation.

Huang and co-workers (2) reported that *Meloidogyne incognita* infections of tomato resulted in increased peroxidase activity in the host tissue during the development of second-stage larvae and adult females, but not during the development of the third and fourth larval stages. Huang and co-workers (3) also reported that *M. incognita* infections of tomato stems, but not of roots, resulted in the appearance of peroxidase isozymes that were not detectable in the absence of the nematode. Hussey and co-workers (5, 6) reported the presence of two peroxidase isozymes in the homogenates of *M. incognita* and *M. arenaria* females that had been reared on tomatoes, and subsequently (6) reported the presence of peroxidase activity in the stylet secretions of *M. incognita* females. They also reported that the host plant affected the number of peroxidase isozymes detectable in homogenates of adult female nematodes (5, 6).

To explore further the involvement of peroxidases in the *Meloidogyne*-host interaction, the present study was undertaken: to determine the level of peroxidase activity in different developmental stages of *Meloidogyne* spp.; 2) to reexamine the effect of host plant on the peroxidase isozyme profile of *Meloidogyne* spp. females; and 3) to compare the peroxidase isozyme profile from the nematode with the peroxidase isozyme profile of the host plant. A preliminary report has been published (9).

MATERIALS AND METHODS

Nematode populations were reared routinely on *Lycopersicon esculentum* Mill. (cv 'Rutgers') under greenhouse conditions (24 ± 5 C). Forty days after inoculation, adult females were extracted from root tissue after treatment with Pectinol 59-L (Rohm and Haas, Philadelphia, PA 19105) as described by Hussey (4). Nematode egg masses separated from root debris were treated with 1.05% NaClO for 4 min. Eggs freed from the gelatinous matrix in this manner were collected on a 26-μm screen. This procedure resulted in a very clean preparation of eggs. Preparasitic second-stage larvae (L_2) were collected by placing a suspension of eggs on a 26-μm screen and incubating for several days at 24 C as described by Vrain (10). Adult males were collected from plants inoculated with 20,000 eggs per 10-cm-diam pot. To increase the number of males developing, about two-thirds of the top growth of each plant was

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removed 10 days after inoculation. After an additional 18-day incubation period the roots were harvested, washed free of soil, and placed in moisture chambers. Males emerging from the roots during a four-day incubation period were hand-picked from the root washings.

Crude homogenates of nematode samples were prepared as described by Hussey *et al.* (5). The nematodes were surface-sterilized by treating with 0.1% chlorhexidine acetate for 15 min, then rinsed three times with cold 0.01 M phosphate buffer (pH 7.5) containing 0.85% NaCl and 0.001 M $MgCl_2$. The samples were then homogenized at 4 C in 1.5 volumes of the same buffer with Potter Elvehjem tissue grinders; homogenates were centrifuged for 20 min at 3000 g at 4 C. The resulting supernatant was used for all assays of peroxidase activity; protein concentrations of the supernatants were determined by the Lowry method (7).

Peroxidase isozymes present in the nematode samples were determined in an anionic "micro"-gel electrophoresis system (8). Seven percent polyacrylamide separating gels with 2% stacking gels were cast in 50-μl micropipettes; samples of 5 to 10 μg protein (ca. 5 μl) were layered over the gels. Electrophoresis was at 4 C with 140 volts until the tracking dye had migrated 15 mm. The microgels were collected for assay by gently breaking the glass micropipettes. Sites of peroxidase activity were determined by incubating the gels in 0.01 M phosphate buffer (pH 6.0) containing 0.03% H_2O_2; O-dianisidine was used as the co-substrate (1). Total peroxidase activity in different samples was measured colorimetrically as the change in absorption at 460 nm (ΔA_{460nm} min^{-1} mg $protein^{-1}$) using the same reaction mixture at pH 5.0.

Host tissues analyzed for peroxidase activity consisted of whole root-knot galls, adjacent ungalled root tissue, and roots from uninoculated plants. Homogenates of host tissues were prepared as described above.

Data presented are from tests repeated at least once on different nematode and plant samples.

RESULTS

Electrophoretic analysis of peroxidase isozymes present in different developmental stages of *Meloidogyne* spp. revealed two isozymes in the homogenates of adult females reared on tomato (Table 1). Those isozymes had mean E_f values of 0.43 and 0.53 and appeared to be similar for each species tested (Fig. 1). No peroxidase isozymes were detected electrophoretically in homogenates of adult males, eggs, or preparasitic L_2 from any of the species tested (Table 1).

Assays for total peroxidase activity revealed very low levels of activity in homogenates of eggs and preparasitic L_2 of each *Meloidogyne* species tested (Table 2). Total peroxidase activity was two to three orders of magnitude higher in homogenates of adult females than that detected in the eggs or preparasitic L_2. Homogenates of *M. hapla* females had the greatest peroxidase activity when O-dianisidine was the co-substrate (H-donor), with reduced levels of activity with similar concentrations of other co-substrates. Homogenates of *M. hapla* eggs had no detectable activity when H-donors other than O-dianisidine were used as co-substrates.

When homogenates prepared from *M. hapla* reared on *Solanum melongena* L. (cv

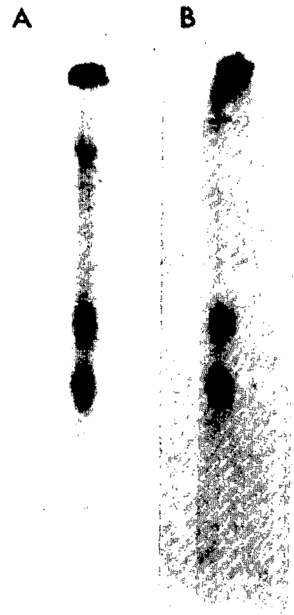

FIG. 1. Peroxidase isozyme profiles obtained by electrophoretic separation with a micro-gel system of proteins from: A) *Meloidogyne javanica* females; and B) *M. hapla* females. Both populations reared on tomato.

TABLE 1. Peroxidase isozymes detected by micro-gel electrophoresis in different populations of *Meloidogyne* spp. reared on tomato.

Species and origin	Number of peroxidase isozymes			
	Adult females[a]	Adult males	Eggs	Preparasitic L_2
M. arenaria				
Florida, USA (#352)	2	–	–	–
Ohio, USA (#79-2)	2	–	0	0
Japan (#168)	2	–	–	–
M. hapla				
North Carolina, USA (#14)	2	–	–	–
Chile (#171)	2	–	–	–
Kenya (#348)	2	0	0	0
M. javanica				
North Carolina, USA (#7-2)	2	–	–	–
Ethiopia (#313)	2	–	0	0
Kenya (#349)	2	–	–	–
M. incognita				
North Carolina, USA (#19)	2	–	–	–
Belgium (#89)	2	0	0	0

[a]Mean E_f values for the two isozymes detected in adult females are 0.43 and 0.53.

'Long Purple'), *Nicotiana tabacum* L. (cv 'NC-2363'), or *Phaseolus vulgaris* L. (cv 'Red Kidney') were examined electrophoretically for peroxidase isozymes and compared with the isozyme profile from females reared on tomato, the isozyme profile differed for each host (Fig. 2). Females reared on tomato had two peroxidase isozymes of E_f 0.43 and 0.53, while females reared on eggplant and bean had one peroxidase isozyme each (respective E_f values of 0.28 and 0.21). Peroxidase isozymes were not detected in homogenates of females reared on tobacco. A total activity assay, however, revealed peroxidase activity of ΔA_{460nm} min^{-1} mg protein^{-1} = 0.16. Tests with *M. incognita* and *M. javanica* females reared on the same hosts gave peroxidase isozyme profiles identical to those observed with *M. hapla* females.

TABLE 2. Total peroxidase activity in the different developmental stages of *Meloidogyne arenaria, M. hapla, M. javanica,* and *M. incognita.*

Species	ΔA_{460nm} min^{-1} mg protein^{-1}[a]	KCN inhibition[b]
M. arenaria #79-2		
Adult females	1.43	+
Eggs	0.007	+
preparasitic L_2	0.004	+
M. hapla #348		
Adult females	1.08	+
Eggs	0.002	+
M. javanica #313		
Adult females	1.44	+
Eggs	0.014	+
M. incognita #89		
Adult females	1.54	+
Preparasitic L_2	0.024	+

[a]O-dianisidine used as the co-substrate (H-donor).
[b](+) denotes complete inhibition of activity in the presence of 0.75 μm KCN.

Comparison of peroxidase isozymes of *M. hapla* females reared on tomato or bean with the isozymes of the host tissues revealed some similarities. For both host-pathogen systems, the peroxidase isozymes routinely detected in the nematode were also present in the galls, adjacent ungalled tissue, and roots from uninoculated plants of the corresponding host (Fig. 3 and 4). The host root tissues also had additional peroxidase isozymes that were not detected in the nematodes. Additionally, the gall tissue of both hosts had peroxidase isozyme profiles that differed slightly from those of the adjacent ungalled roots and roots from uninoculated plants.

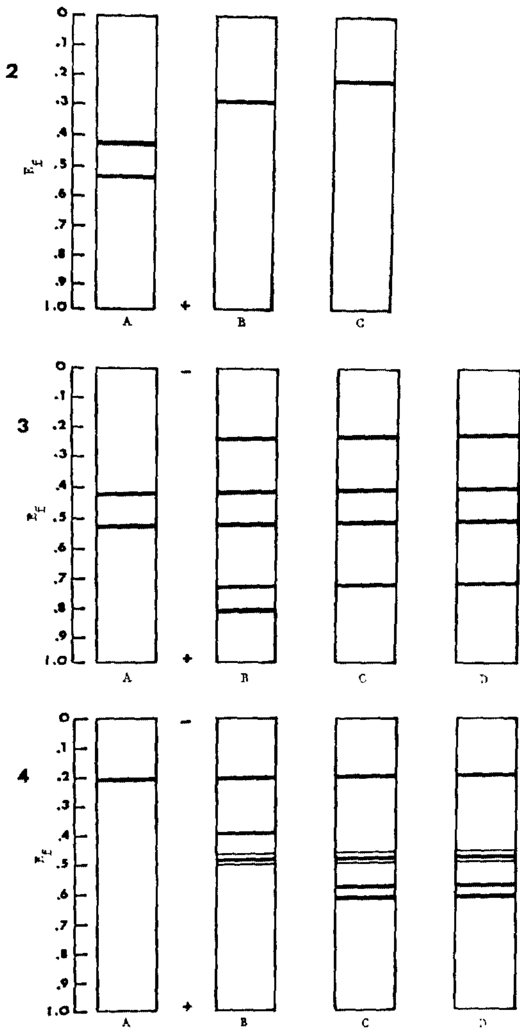

FIG. 2-4. Schematic representation of peroxidase isozyme profiles from *Meloidogyne hapla* females and different host tissues. 2) Isozyme profiles from nematodes reared on: A) tomato; B) eggplant; and C) bean. 3 & 4) Comparison of isozyme profiles from nematodes reared on tomato or bean, respectively, with tissues from the corresponding host. A) *M. hapla* females; B) *M. hapla* root galls; C) adjacent ungalled root-tissue; and D) root tissue from uninoculated plants.

DISCUSSION

Of the different developmental stages of *Meloidogyne* species tested only the adult females appear to have significant levels of peroxidase activity. The peroxidase isozymes detected in the females appear to be identical to those reported in *M. incognita* females by Hussey and Sasser (6). The low levels of peroxidase activity detected in homogenates of *Meloidogyne* spp. eggs and preparasitic L_2 may be due to nonspecific oxidases. Differences in peroxidase activity between the adult females and the eggs appear to be unrelated to co-substrate specificity.

It is interesting that, of the developmental stages of *Meloidogyne* spp. tested for peroxidase activity, only the adult females have fed on host tissue and only they have significant peroxidase activity. Also, as reported by Hussey and co-workers (5, 6), the host plant has a major effect on the peroxidase isozymes detectable in homogenates of adult females. These observations lead to the conclusions either that the host influences the peroxidases produced by the nematode, or that the peroxidases isolated from the nematode are of host origin, ingested during feeding. The apparent similarity in electrophoretic mobility of peroxidase isozymes from *M. hapla* females reared on tomato or bean to peroxidase isozymes isolated from different host tissues lends support to the latter hypothesis.

From these data, most of the peroxidase activity detectable in the homogenates of *Meloidogyne* spp. females appears to be of host origin and, therefore, may not play an important role in the host-parasite interaction. The data provide no insight, however, on the origin of the peroxidase activity detected by Hussey and Sasser (6) in the stylet secretions of *M. incognita* females. The origin of this peroxidase activity must be determined before any role can be assigned peroxidases in the *Meloidogyne*-host interaction.

Contrary to the earlier report of Huang and co-workers (3), *M. hapla* infections of tomato and bean did induce slight modifications in the peroxidase isozyme profiles of the root-gall tissue as compared with the isozyme profiles from roots of inoculated plants. Changes in the peroxidase isozymes are apparently restricted to the gall tissue since the isozymes in the adjacent ungalled root tissue were identical to those in the uninoculated roots. The significance, if any, of this modification of the peroxidase isozyme profile in response to *M. hapla* infections is not known.

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