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Antioxidant Enzymes in Phytoparasitic Nematodes[1]

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Abstract: Presence of different antioxidant enzymes, such as superoxide dismutase (SOD), catalase, and ascorbate, *p*-phenilendiamine-pyrocathecol (PPD-PC), *o*-dianisidine, and guaiacol isoperoxidases, was shown in the phytoparasitic nematode species *Meloidogyne incognita, M. hapla, Globodera rostochiensis, G. pallida, Heterodera schachtii, H. carotae,* and *Xiphinema index.* The activity of the enzymes tested differed among the life stages examined. SOD was present in cysts but was not detected in *Meloidogyne* egg masses. Catalase activity of *Meloidogyne* females was higher than that of preparasitic stages and cyst-nematode females. For the first time, ascorbate peroxidase was found to occur commonly in phytoparasitic nematodes, with the highest activity in the invading life-stages. In all the life stages examined, the antioxidant enzyme activities of *M. hapla* were markedly higher than those of *M. incognita.* Glutathione peroxidase was not found in the species examined.

Key words: antioxidant enzyme, catalase, *Globodera pallida, Globodera rostochiensis, Heterodera carotae, Heterodera schachtii, Meloidogyne hapla, Meloidogyne incognita,* peroxidase, SOD, *Xiphinema index.*

Phytoparasitic nematodes are aerobic organisms (Atkinson, 1980). Aerobes must deal with the high reactivity of oxygen, which can sequentially acquire four electrons, forming toxic intermediates in the process; several of these are free radicals. Moreover, production of active oxygen (AO) species occurs in the plant-pathogen interaction (Kauss, 1990; Dixon et al., 1994; Baker and Orlandi, 1995). Therefore, nematodes must deal not only with AO generated by their own metabolism but also with those produced by the host in response to their attack, thus suggesting the need for antioxidant mechanisms in such parasites. A preliminary investigation of second-stage juveniles (J2) of *Meloidogyne incognita* and *M. hapla* revealed that they may have an enzymatic system of superoxide dismutase (SOD) and ascorbate peroxidase, which neutralize superoxide anions (O_2^-) to H_2O through H_2O_2 (Molinari, 1995).

AO production in plants is particularly important in the defense mechanism as well as in the recognition of incompatible pathogens (Baker and Orlandi, 1995). A relatively long-lived AO generation occurring 1.5 to 3 hours after inoculation of incompatible plant suspension cells with *Pseudomonas syringae* pathovars has been depicted as the earliest detectable specific reaction of plant cells to incompatible pathogens (Orlandi et al., 1992).

Direct evidence of AO production in the plant-nematode incompatible interaction was found in an investigation of the later stages of *Meloidogyne*-tomato interaction (Zacheo and Bleve-Zacheo, 1988), although an early AO production comparable to that described for *Pseudomonas*-plant cells interaction may be presumed to occur as a response of plants to the feeding action of endoparasitic J2 (Molinari, 1996).

Plants have an efficient enzymatic system for AO detoxification that includes SOD, ascorbate peroxidase, catalase, and glutathione peroxidase, and that allows plants to tolerate O_2^--generating stress, such as air pollutants, pathogen attacks, chilling, and heating (Molinari, 1991a; Baker and Orlandi, 1995). Oxidative-stress defense and potential life span have been closely correlated in the free-living nematode *Caenorhabditis elegans* (Vanfleteren, 1993). Furthermore, numerous investigations have confirmed that successful parasitism of animals by helminths depends on efficiency of the antioxidant defenses of the parasites against AO generated by the cells of the host immune system (Clark et al., 1986). It is likely that such antioxidant defenses may represent the biochemical means required to overcome

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plant defenses, thereby enabling endoparasitic nematodes to establish and maintain permanent feeding sites for their development and reproduction.

Investigations undertaken to detect the presence and level of antioxidant enzymes in phytoparasitic nematodes are scarce; SOD was found in mature females of *M. incognita* and *Heterodera glycines* (Radice et al., 1988; Vanderspool et al., 1994), and peroxidase was found in females and juveniles of *Meloidogyne* spp. and *Globodera* spp. (Starr, 1979; Ibrahim, 1991; Fox and Atkinson, 1985), but few reports of other enzymes such as catalase, ascorbate, or glutathione peroxidases exist (Hussey and Krusberg, 1971).

This paper reports qualitative and quantitative analyses of SOD, catalase, and ascorbate, glutathione, *p*-phenylendiamine-pyrocathecol (PPD-PC), *o*-dianisidine, guaiacol, and ferricyanide isoperoxidases in cyst-forming, root-knot, and dagger nematodes.

Analyses are reported for cysts, egg masses, swollen females, and vermiform stages of seven species (*M. incognita, M. hapla, G. rostochiensis, G. pallida, H. carotae, H. schachtii, Xiphinema index*).

Materials and Methods

Nematodes: Populations of root-knot nematodes were collected from different infested soils of Apulia (southern Italy) and maintained on susceptible *Lycopersicon esculentum* cv. Roma VF in a glasshouse. Egg masses and mature females of *Meloidogyne* spp. were hand-picked from infected tomato plants. Cysts of *G. rostochiensis* and *G. pallida* were obtained from infested soils in the vicinity of Trento (northern Italy) and Polignano (Apulia), respectively. Potato plants were planted in these soils in the glasshouse and mature females later were collected. Infested soil from Avezzano (southern Italy) was planted with sugar beet in the glasshouse; cysts and mature females of *H. schachtii* were hand-picked from infected plants. The cysts were then used to develop glasshouse cultures. Cysts and females of *H. carotae* were obtained from infested soil and from carrot plants, respectively, at Margherita di Savoia (Apulia). Cysts were extracted with a Fenwick apparatus (Fenwick, 1940). Specimens of *X. index* were obtained from cultures maintained on fig in a glasshouse by extraction with the Cobb wet-sieving technique. *Meloidogyne* J2 were obtained by incubating egg masses in distilled water at 27 °C.

Enzyme isolation: Nematodes were thoroughly washed with distilled water, surface-sterilized by incubation for 1 hour in a chilled solution of 5 µg/ml amphotericin B and 1 mg/ml streptomycin sulphate, and finally rinsed with sterilized water before placing them in the grinding buffer. Samples of *X. index* generally consisted of 40 to 60 hand-picked nematodes in 0.5 ml of 0.1 M sodium-potassium phosphate (Na-K P_i), buffered at pH 6.0. Suspensions of *Meloidogyne* J2 were filtered through 1µm pore cellulose nitrate filters and then collected in 0.5 ml of 0.1 M Na-K P_i, pH 6.0, to concentrate nematodes. White adult cyst nematodes and gravid females and egg masses of root-knot nematodes, 20 to 30 each, were placed in 0.5 ml of 0.1 M Na-K P_i, pH 6.0. Samples of 400 to 500 cysts in 0.5 ml of 0.1 M Na-K P_i, pH 6.0, also were used. Samples were homogenized in a chilled 2-ml glass mortar with a pestle connected to a motor drive. The highest speed was used to grind vermiform nematodes with the time required for homogenization based on the absence of large particles when observed with a light microscope. Coarse homogenates were clarified twice by centrifugation at 12,000*g* for 5 minutes in a bench centrifuge. After centrifugation, samples were immediately used for enzyme activity tests or kept at –80 °C until tested. Protein content was determined with the Lowry procedure with bovine serum albumin as the standard (Lowry et al., 1951).

Determination of enzyme activities: All measurements were carried out spectroscopically with a 557 Perkin-Elmer double-beam spectrophotometer. SOD activity was assayed as the percentage of inhibition on the reduction of cytochrome *c* (80 µm) by the xanthine (1 mM)-xanthine oxidase (20 mU) system in 1 ml assay medium of 0.1 M Na-K

P_i, pH 7.8, 20 mM NaN_3 and 1 mM EDTA. Standard reactions were carried out with 150–200 µl of extraction buffer, which was substituted with an identical amount of sample suspension in the enzyme activity tests. A high concentration of NaN_3 was included to completely inhibit the cytochrome oxidase present in the crude extracts. Reactions were started by adding xanthine oxidase and were monitored at 550/540 nm. One unit of SOD activity represented the amount of enzyme able to produce a 50% inhibition in a standard reaction (Furusawa et al., 1984).

Catalase activity was measured as the initial rate of disappearance of hydrogen peroxide (Chance and Mahley, 1955), using 20 mM H_2O_2 and 50 µl sample extract in 0.1 M Na-P_i, pH 7.0 (0.5 ml final volume). The rate of H_2O_2 disappearance was followed as the decrease in absorbance at 240 nm, and oxidation of 1 µmole H_2O_2/minute ($\epsilon = 0.038$ $mM^{-1} \cdot cm^{-1}$) represented one unit of enzyme. Ascorbate peroxidase was assayed in a 0.5-ml final volume according to Gerbling et al. (1984). The reaction mixture contained 0.1 N-Tris hydroxymethyl-2-aminoethanesulfonic acid (TES), pH 7.0, 0.1 mM EDTA, 1 mM ascorbate from fresh stock solution (100 mM), 0.13 mM H_2O_2, and 50 µl sample extract. The decrease in absorbance at 298 nm was followed as ascorbate was oxidized, and one unit of enzyme was defined as the ability to oxidize 1 µmole ascorbate per minute ($\epsilon = 0.8$ $mM^{-1} \cdot cm^{-1}$). Glutathione peroxidase activity was monitored in a 1-ml final volume at 340 nm as oxidation of NAPDH (0.2 mM) by glutathione reductase (240 mU) and oxidized glutathione (GSSG), which was produced by the reaction between reduced glutathione (GSH, 1 mM) and H_2O_2 (2 mM) catalyzed by glutathione peroxidase; the assay medium consisted of 50 mM phosphate buffer, pH 7.0, 1 mM EDTA, and 1 mM NaN_3 (Sies and Moss, 1978).

The assay mixture (1 ml final volume) for determination of PPD-PC-peroxidase contained 0.42 mM PPD, 4 mM PC and 50–150 µl sample extract in 100 mM Tris-HCl, pH 7.5. The reaction was started by addition of 1.3 mM H_2O_2 and recorded at 557 nm (Imberty et al., 1985); activity was expressed as $\Delta A_{557} \cdot min^{-1} \cdot mg^{-1}$ prot. For guaiacol oxidation, 5 mM guaiacol and 1.9 mM H_2O_2 in 1 ml of 100 mM Na-K P_i, pH 6.0, were used, and the reaction was monitored at 470 nm (activity expressed as $\Delta A_{470} \cdot min^{-1} \cdot mg^{-1}$). For *o*-dianisidine oxidation, 2 mM *o*-dianisidine and 3.8 mM H_2O_2 in 1 ml 0.1 M sodium acetate buffer, pH 4.5, were used, and the reaction was monitored at 460 nm (activity expressed as $\Delta A_{460} \cdot^{-1} \cdot mg^{-1}$). Finally, ferricyanide-peroxidase was assayed with 1 mM $Fe(KCN)_6$ and 0.13 mM H_2O_2 in 1 ml of Na-K P_i, pH 6.0, and recorded at 420 nm (Brooks, 1986).

Each assay was independently repeated at least four times and results given as means plus or minus the standard deviation. Species of the same genus (*G. rostochiensis/G. pallida, H. schachtii/H. carotae, M. incognita/M. hapla*) were tested for significant differences in each antioxidant enzyme activity with a *t*-test. Comparisons between species of the same genus were carried out by using the sets of data obtained from cysts and females of cyst nematodes and from egg masses, females, and J2 of root-knot nematodes.

Results

SOD activity was present in cysts of *Globodera* and *Heterodera* spp. but was undetected in *Meloidogyne* egg masses (Table 1). In contrast, SOD activity was readily detected in *Meloidogyne* females but was low in cyst nematode females (Table 2). Egg masses, females, and J2 of *Meloidogyne hapla* showed a markedly higher activity of SOD, catalase, and ascorbate peroxidase compared with *M. incognita* counterparts (Tables 1–3). Furthermore, ascorbate peroxidase activity was barely detectable in cysts and *M. incognita* egg masses but was consistent in *M. hapla* egg masses (Table 1).

The ectoparasite *Xiphinema index* showed antioxidant enzyme activities comparable to those of *M. incognita* J2, except for *o*-dianisidine isoperoxidase, which was undetected in *X. index* (Table 3).

TABLE 1. Antioxidant enzyme activities of cysts of *Globodera rostochiensis* (Gr), *G. pallida* (Gp), *Heterodera carotae* (Hc), and *H. schachtii* (Hs), and egg masses of *Meloidogyne incognita* (Mi) and *M. hapla* (Mh).

	Cysts				Egg masses	
Enzyme activity[a]	Gr	Gp	Hc	Hs	Mi	Mh
SOD	4.3 ± 0.8	13.7 ± 4.5	16.0 ± 7.5	2.7 ± 1.2	0.0	0.0
Catalase	11.0 ± 7.3	12.4 ± 5.3	6.7 ± 1.4	14.2 ± 9.6	15.9 ± 12.2	33.2 ± 22.4
Ascorbate isoperoxidase	0.1 ± 0.1	0.2 ± 0.1	0.0	0.3 ± 0.1	0.3 ± 0.2	1.7 ± 1.0
PPD-PC isoperoxidase	0.15 ± 0.05	0.15 ± 0.05	0.15 ± 0.05	0.4 ± 0.05	7.2 ± 5.7	18.8 ± 6.9
o-dianisidine isoperoxidase	0.0	0.0	0.2 ± 0.1	0.4 ± 0.2	42.3 ± 30.5	45.0 ± 26.0
Guaiacol isoperoxidase	0.0	0.0	0.0	0.0	8.9 ± 2.7	8.2 ± 1.2

[a] Data are expressed for SOD, catalase, and ascorbate peroxidase as units · mg^{-1} protein, PPD-PC peroxidase as $\Delta A_{557} \cdot min^{-1} \cdot mg^{-1}$ protein, *o*-dianisidine peroxidase as $\Delta A_{460} \cdot min^{-1} \cdot mg^{-1}$ protein, and guaiacol peroxidase as $\Delta A_{470} \cdot min^{-1} \cdot mg^{-1}$ protein, ± standard deviation.

The activity of three peroxidase isozymes (PPD-PC-, *o*-dianisidine-, and guaiacol-isoperoxidases) varied among the nematode species and life stages tested. Generally, cysts lacked isoperoxidase activity; although vermiform nematodes lacked guaiacol-peroxidase, they showed modest PPD-PC- and *o*-dianisidine-peroxidase activities. Extracts of *H. carotae* and *H. schachtii* females did not contain detectable amounts of *o*-dianisidine- and guaiacol-isoperoxidase activity, whereas *H. schachtii* females showed markedly higher PPD-PC-peroxidase than *H. carotae* females. *Meloidogyne* egg masses were generally rich in isoperoxidase, and *M. hapla* was much richer than *M. incognita* in PPD-PC peroxidases (Tables 1–3). Ferricyanide- and glutathione-peroxidases were undetectable in any of the samples tested (results not shown).

To determine the significance of differences in antioxidant enzyme activities observed between different species in the same genus, data were subjected to an LSD test (Table 4). *Globodera* species were not distinguished by any of the enzyme activities tested. Values of ascorbate and PPD-PC-peroxidase activity of *H. schachtii* were significantly higher than those of *H. carotae*. It was possible to distinguish *M. hapla* from *M. incognita* by means of SOD, catalase, and ascorbate and PPD-PC- isoperoxidase activities.

DISCUSSION

Data presented here demonstrate the presence in *Meloidogyne* preparasitic stages of a SOD/ascorbate peroxidase enzymatic system; such a system should efficiently neutralize superoxide and hydrogen peroxide into water by the oxidation of ascorbate. Our results are in accord with the electrophoretic demonstration of SOD in preparasitic juveniles and females of *M. incognita* (Vanderspool et al., 1994) and further demonstrate the presence of ascorbate peroxidase.

TABLE 2. Antioxidant enzyme activities of females of *Globodera rostochiensis* (Gr), *G. pallida* (Gp), *Heterodera carotae* (Hc), *H. schachtii* (Hs), *Meloidogyne incognita* (Mi), and *M. hapla* (Mh).

Enzyme activity[a]	Gr	Gp	Hc	Hs	Mi	Mh
SOD	2.7 ± 2.4	0.0	0.0	2.4 ± 2.5	14.5 ± 7.9	44.1 ± 15.4
Catalase	10.6 ± 2.1	12.5 ± 4.0	29.2 ± 1.4	12.1 ± 9.7	54.5 ± 40.8	75.8 ± 66.4
Ascorbate isoperoxidase	0.1 ± 0.1	0.1 ± 0.04	0.1 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.6 ± 0.1
PPD-PC isoperoxidase	2.8 ± 2.4	4.8 ± 2.4	0.4 ± 0.2	2.0 ± 1.4	5.6 ± 2.5	8.0 ± 3.0
o-dianisidine isoperoxidase	2.6 ± 1.3	2.9 ± 1.3	0.1 ± 0.1	0.9 ± 0.6	9.1 ± 2.4	13.4 ± 3.9
Guaiacol isoperoxidase	0.1 ± 0.1	0.1 ± 0.1	0.0	0.05 ± 0.05	0.7 ± 0.5	5.0 ± 3.0

[a] Data are expressed for SOD, catalase, and ascorbate peroxidase as units · mg^{-1} protein, PPD-PC peroxidase as $\Delta A_{557} \cdot min^{-1} \cdot mg^{-1}$ protein, *o*-dianisidine peroxidase as $\Delta A_{460} \cdot min^{-1} \cdot mg^{-1}$ protein, and guaiacol peroxidase as $\Delta A_{470} \cdot min^{-1} \cdot mg^{-1}$ protein, ± standard deviation.

TABLE 3. Antioxidant enzyme activities of second-stage juveniles (J2) of *Meloidogyne incognita* (Mi) and *M. hapla* (Mh), and specimens of *Xiphinema index* (Xi).

Enzyme activity[a]	Mi	Mh	Xi
SOD	8.8 ± 3.8	19.3 ± 9.0	8.7 ± 5.5
Catalase	5.6 ± 2.6	25.2 ± 8.2	10.4 ± 7.9
Ascorbate isoperoxidase	0.5 ± 0.4	1.3 ± 0.7	0.6 ± 0.2
PPD-PC isoperoxidase	0.9 ± 0.3	1.5 ± 1.1	3.1 ± 2.6
o-dianisidine isoperoxidase	4.1 ± 2.3	1.5 ± 1.0	0.0
Guaiacol isoperoxidase	0.0	0.0	0.0

[a] Data are expressed for SOD, catalase, and ascorbate peroxidase as units · mg^{-1} protein, PPD-PC peroxidase as $\Delta A_{557} \cdot min^{-1} \cdot mg^{-1}$ protein, *o*-dianisidine peroxidase as $\Delta A_{460} \cdot min^{-1} \cdot mg^{-1}$ protein, and guaiacol peroxidase as $\Delta A_{470} \cdot min^{-1} \cdot mg^{-1}$ protein, ± standard deviation.

Resistance to root-knot nematodes in tomato is conferred by the *Mi* gene (Williamson et al., 1994) and has been correlated with an increase of AO production by the plant (Kaplan and Davis, 1987; Zacheo and Bleve-Zacheo, 1988). Generally, the antioxidant enzyme activities of *M. hapla* J2 were much higher than those of *M. incognita* J2, thus suggesting a major capability of the invading juveniles of *M. hapla* in scavenging the active oxygen species produced by the resistant roots penetrated by nematodes. It is a tantalizing possibility that the antioxidant defenses of *M. hapla* may play a role in its unique ability to break resistance among the most widely known *Meloidogyne* species (Molinari, 1996).

Considering the relative amounts of catalase and ascorbate peroxidase in J2 and females of root-knot nematodes, it seems that ascorbate peroxidase is more prominent in preparasitic stages while catalase activity is much higher in adult females. Catalase, due to its high K_m, is inefficient at scavenging the low levels of H_2O_2 produced in plant cells during early plant-pathogen interactions (Baker and Orlandi, 1995). Therefore, the association of SOD with ascorbate peroxidase would be a more suitable scavenging system than catalase in the biochemical environment that the invading parasite encounters in the early stages of root penetration. On the other hand, the major presence of catalase in adult females suggests that nematodes developing in the root and actively feeding may be challenged by higher amounts of H_2O_2 produced by the host and their own metabolism. In this respect, SOD appears to be less important and does not occur uniformly.

However, SOD activity was readily detected in cysts. The presence of different SOD isozymes has been confirmed in cysts of six *Heterodera* spp., and the isoenzymes are useful for species discrimination (Molinari et al., 1996). Cysts contain catalase, but ascorbate peroxidase and other isoperoxidase activity is negligible. The activity of antioxidant enzymes is completely different in egg masses, which show significant activity of all the enzymes tested but apparently lack SOD activity. However, when native PAGE of homogenates of egg masses was carried out in an automated electrophoresis system (PhastSystem, Pharmacia) and gels stained for SOD activity (Molinari et al., 1996), faint

TABLE 4. Comparison of antioxidant enzyme activities of *Globodera rostochiensis* (Gr), *G. pallida* (Gp), *Heterodera carotae* (Hc), *H. schachtii* (Hs), *Meloidogyne incognita* (Mi), and *M. hapla* (Mh).

Enzyme activity[a]	Gr	Gp	Hc	Hs	Mi	Mh
SOD	2.9	5.8	9.6	6.2	6.6 a	25.0 b
Catalase	8.3	8.7	10.2	15.5	14.7 a	44.7 b
Ascorbate isoperoxidase	0.14	0.25	0.1 a	0.3 b	0.3 a	1.2 b
PPD-PC isoperoxidase	1.1	1.4	0.25 a	1.4 b	4.2 a	8.6 b
o-dianisidine isoperoxidase	3.6	4.5	0.2	0.2	12.8	15.6
Guaiacol isoperoxidase	0.6	1.0	0.0	0.16	7.3	8.3

[a] Data are expressed for SOD, catalase, and ascorbate peroxidase as units · mg^{-1} protein, PPD-PC peroxidase as $\Delta A_{557} \cdot min^{-1} \cdot mg^{-1}$ protein, *o*-dianisidine peroxidase as $\Delta A_{460} \cdot min^{-1} \cdot mg^{-1}$ protein, and guaiacol peroxidase as $\Delta A_{470} \cdot min^{-1} \cdot mg^{-1}$ protein. Means in rows of a genus with different letters are significantly different ($P < 0.05$) according to an LSD test.

but distinguishable bands appeared (not shown); it is apparent that such sensitive methods can detect amounts of SOD activity lower than those detectable by spectroscopy.

Generally, females of cyst-forming nematodes showed antioxidant enzyme activities less consistently than did females of *Meloidogyne*. Only ascorbate peroxidase activity of *H. schachtii* was comparable to that of *M. incognita* females.

The occurrence and significance of peroxidase in *Meloidogyne* spp. have been extensively debated (Hussey and Krusberg, 1971; Hussey et al., 1972; Hussey and Sasser, 1973; Starr, 1979; Veech et al., 1987), and the general opinion is that peroxidase activity found in *Meloidogyne* females is of host origin (Ibrahim, 1991). On the other hand, Starr (1979) found a low level of *o*-dianisidine-peroxidase activity in *M. incognita* J2. In the present study, a low but measurable activity of *o*-dianisidine- and PPD-PC-peroxidase was found in *M. incognita* and *M. hapla* J2, and we have demonstrated even higher PPD-PC-peroxidase activity in *X. index*. These findings suggest that peroxidase might be produced by the nematode itself, in addition to that ingested from plant cell contents, as is likely for the other antioxidant enzymes. Egg masses also contain high peroxidase activity but, in this case, only the gelatinous matrix stained for peroxidase and not the eggs (Ibrahim, 1991).

Whether peroxidase is secreted into the giant cells induced by *Meloidogyne* females has been a subject of debate (Hussey and Sasser, 1973; Veech et al., 1987), although it has been demonstrated that the attack of resistant tomato roots by *M. incognita* enhances host peroxidase activity, primarily in the cell wall fraction (Molinari, 1991b).

Glutathione peroxidase activity was not detectable in any of the samples tested. Similarly, its presence was not demonstrated in *C. elegans* tissue (Vanfleteren, 1993).

The species of phytoparasitic nematodes examined in this study possess at all life-cycle stages, antioxidant enzymes able to convert toxic oxygen species to water. Such toxic species might be produced either by the nematode's own metabolism, as has been demonstrated in *C. elegans* (Vanfleteren, 1993), or by the host plant as a defense mechanism. It is an intriguing possibility that the efficiency of the antioxidant enzymes may be correlated with the degree of virulence of those species. Future research should focus on the relationship between virulence genes of endoparasitic nematodes and their antioxidant defenses.

Literature Cited

Atkinson, H. J. 1980. Respiration in nematodes. Pp. 101–142 *in* B. M. Zuckerman, ed. Nematodes as biological models. New York: Academic Press.

Baker, C. J., and E. W. Orlandi. 1995. Active oxygen in plant pathogenesis. Annual Review of Phytopathology 33:299–321.

Brooks, J. L. 1986. Oxidase reactions of tomato anionic peroxidase. Plant Physiology 80:130–133.

Chance, B., and A. C. Mahley. 1955. Assay of catalases and peroxidases. Pp. 764–775 *in* S. P. Colowick and N. O. Kaplan, eds. Methods in enzymology, vol. 2. New York: Academic Press.

Clark, I. A., N. H. Hunt, and W. B. Cowden. 1986. Oxygen-derived free radicals in the pathogenesis of parasitic disease. Pp. 1–44 *in* J. R. Baker and R. Muller, eds. Advances in parasitology, vol. 25. London: Academic Press.

Dixon, R. A., M. J. Harrison, and C. J. Lamb. 1994. Early events in the activation of plant defense responses. Annual Review of Phytopathology 32:479–501.

Fenwick, D. W. 1940. Methods for the recovery and counting of cysts of *H. schachtii* from soil. Journal of Helminthology 18:155–172.

Fox, P. C., and H. J. Atkinson. 1985. Enzyme variation in pathotypes of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. Parasitology 91:499–506.

Furusawa, I., K. Tanaka, P. Thanutong, A. Mizuguchi, M. Yazaki, and K. Asada. 1984. Paraquat-resistant tobacco calluses with enhanced superoxide dismutase activity. Plant and Cell Physiology 25:1247–1254.

Gerbling, K. P., G. J. Kelly, K. H. Fisher, and E. Latzko. 1984. Partial purification and properties of soluble ascorbate peroxidases from pea leaves. Journal of Plant Pathology 115:59–67.

Hussey, R. S., and L. R. Krusberg. 1971. Disc-electrophoretic pattern of enzymes and soluble proteins of *Ditylenchus dipsaci* and *D. triformis*. Journal of Nematology 3:79–84.

Hussey, R. S., and J. N. Sasser. 1973. Peroxidase from *M. incognita*. Physiological Plant Pathology 3:223–229.

Hussey, R. S., J. N. Sasser, and D. Huisingh. 1972. Disc-electrophoretic studies of soluble proteins and enzymes of *M. incognita* and *M. arenaria*. Journal of Nematology 4:183–189.

Ibrahim, S. K. 1991. Peroxidase isoenzymes from *Meloidogyne* spp. cultured on different hosts. Revue de Nématologie 14:335–344.

Imberty, A., R. Goldberg, and A. M. Catesson. 1985. Isolation and characterization of *Populus* isoperoxidases

involved in the last step of lignin formation. Planta 164: 221–226.

Kaplan, D. T., and E. L. Davis. 1987. Mechanisms of plant incompatibility with nematodes. Pp. 267–276 *in* J. A. Veech and D. W. Dickson, eds. Vistas on nematology. Hyattsville, MD: Society of Nematologists.

Kauss, H. 1990. Role of the plasma membrane in host-pathogen interactions. Pp. 320–350 *in* C. Larsson and I. M. Møller, eds. The plant membrane. Structure, function and molecular biology. Berlin: Springer-Verlag.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193:265–275.

Molinari, S. 1991a. Effect of paraquat on tomato roots cultured in vitro susceptible and resistant to nematodes. Plant and Cell Physiology 32:1129–1135.

Molinari, S. 1991b. Induction of isoperoxidases in resistant and susceptible tomato cultivars by *Meloidogyne incognita*. Journal of Nematology 23:254–258.

Molinari, S. 1995. Role of oxidative and peroxidative processes in the plant-nematode interaction. Nematologia Mediterranea 23:69–73.

Molinari, S. 1996. Molecular aspects of plant-nematode interaction. Nematologia Mediterranea 24: 139–154.

Molinari, S., K. Evans, J. Rowe, M. Russell. 1996. Identification of *Heterodera* cysts by SOD isoenzyme electrophoresis profiles. Annals of Applied Biology 129:in press.

Orlandi, E. W., S. W. Hutchenson, and C. J. Baker. 1992. Early physiological responses associated with race-specific recognition in soybean leaf tissue and cell suspensions treated with *Pseudomonas syringae* pv. *glycinea*. Physiological and Molecular Plant Pathology 40: 173–180.

Radice, A. D., R. D. Riggs, and F. H. Huang. 1988. Detection of intraspecific diversity of *Heterodera glycines* using isozyme phenotypes. Journal of Nematology 20: 29–39.

Sies, H., and K. M. Moss. 1978. A role of mitochondrial glutathione peroxidase in modulating mitochondrial oxidation in liver. European Journal of Biochemistry 84:377–383.

Starr, J. L. 1979. Peroxidase isozymes from *Meloidogyne* spp. and their origin. Journal of Nematology 11: 1–5.

Vanderspool, M. C., D. T. Kaplan, T. G. McCollum, and R. J. Wodzinski. 1994. Partial characterization of cytosolic superoxide dismutase activity in the interaction of *Meloidogyne incognita* with two cultivars of *Glycine max*. Journal of Nematology 26:422–429.

Vanfleteren, J. R. 1993. Oxidative stress and ageing of *Caenorhabditis elegans*. Biochemical Journal 292:605–608.

Veech, J. A., T. L. Starr, and R. M. Nordgren. 1987. Production and partial characterization of stylet exudate from adult females of *M. incognita*. Journal of Nematology 19:463–468.

Williamson, V. M., K. N. Lambert, and I. Kaloshian. 1994. Molecular biology of nematode resistance in tomato. Pp. 211–219 *in* F. Lamberti, C. De Giorgi, and D. McK. Bird, eds. Advances in molecular plant nematology. New York: Plenum Press.

Zacheo, G., and T. Bleve-Zacheo. 1988. Involvement of superoxide dismutases and superoxide radicals in the susceptibility and resistance of tomato plants to infestation by *Meloidogyne incognita*. Physiological and Molecular Plant Pathology 32:313–322.