Histopathology of and Oxidative Enzyme Patterns in Wando Peas Infected with Two Populations of Ditylenchus dipsaci

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ABSTRACT

Nematodes of the Waynesville, North Carolina (WNC), population of *Ditylenchus dipsaci* induced a hypersensitive reaction in epidermal tissues of Wando pea seedlings, and did not further penetrate the tissues. Cortical cells adjacent to the necrotic lesions divided and gave the appearance of cork formation. Nematodes of the Raleigh, North Carolina (RNC), population of *D. dipsaci* penetrated Wando pea and caused tissue disruption characteristic of the susceptible response observed in other host species. The resistant response (necrosis) was preferentially expressed over the susceptible response (galling), as demonstrated by cross-inoculation.

Oxidative enzyme patterns from RNC nematode-inoculated, WNC nematode-inoculated, and noninoculated Wando pea seedlings were compared. Differences in peroxidase isoenzymes were detected between inoculated and noninoculated tissues. No differences were detected between RNC nematode-inoculated and WNC nematode-inoculated tissues. Peroxidase activity, determined histochemically, was greater in inoculated than in noninoculated tissues. In RNC nematode-inoculated stems, high peroxidase activity was localized in parenchymatous cells directly associated with nematode-induced cavities in the cortex. In WNC nematode-inoculated stems, high peroxidase activity was associated with cortical cells adjacent to necrotic lesions in the epidermis. Phytopathology 60:1818-1825.

Little is known about the biochemical basis for resistance in plants to parasitic nematodes. Van Gundy & Kirkpatrick (22) showed that resistance in certain citrus rootstocks to the citrus nematode, *Tylenchulus semipenetrans,* was associated with a toxin in expressed root juice. Anatomical differences occurred in root tissues from the various rootstock plants. Root juice from plants of a highly resistant cultivar was more toxic to nematodes than juice from moderately resistant plants. The difference in resistance was attributed to the unidentified toxin, as roots of both cultivars exhibited the same degree of morphological resistance to *T. semipenetrans.* Two plants, asparagus and marigold, are known to secrete into soil compounds toxic to nematodes (19).

Only one study has directly associated nematode resistance in plants with an enzyme secreted by a nematode. Mountain & Patrick (16) showed that the lesion nematode, *Pratylenchus penetrans,* feeding in the cortex of peach roots, secreted the enzyme, β-glucosidase, which hydrolyzed amygdalin to yield prussic acid (HCN). Nematode injury to plant cells during penetration and colonization of root tissues also induced release of plant β-glucosidase which contributed to destruction of root cells. Prussic acid was toxic to both host and parasite. Rootstocks which were resistant to *P. penetrans* contained higher concn of amygdalin than did susceptible rootstocks.

Formation of phytoalexins (3) and accumulation of phenols (6) are important responses in many plants resistant to bacteria and fungi. Changes in either enzyme synthesis, activity, or both were demonstrated to accompany infections by several pathogens (10, 20, 21, 23). Oxidative enzymes such as peroxidase and polyphenoloxidase appear to be important, especially in hypersensitive reactions. For example, tobacco leaves infected with *Pseudomonas tabaci* contained increased peroxidase activity and produced new isoenzymes of peroxidase (13).

Macko et al. (15) compared proteins and enzymes of urediospores from two physiological races of *Puccinia graminis* var. *tritici.* Although several enzymes were studied, differences were detected only in their peroxidase and polyphenoloxidase enzymes. Extracts of urediospores from the less virulent 111 physiological race following polyacrylamide gel electrophoresis separated into more bands with peroxidase and polyphenoloxidase activity than did similar extracts from the more virulent 21 race. Slight differences also existed in the soluble protein patterns.

In plants, resistance to the stem nematode, *Ditylenchus dipsaci,* is often associated with a hypersensitive reaction. Barker & Sasser (1) differentiated between two North Carolina populations of *D. dipsaci* by the response they induced in the garden pea, *Pisum sativum* 'Wando'. Wando pea was susceptible to nematodes of the Raleigh population (Wake County), as indicated by separation of cells, galling, and reproduction in the tissues. Nematodes of the Waynesville population (Haywood County) induced a hypersensitive reaction in tissues of Wando pea, and nematode reproduction was inhibited. Howell & Krusberg (7) compared free- and protein-amino acids of Raleigh nematode population-inoculated and noninoculated Wando pea tissues. Both

fractions of amino acids were much greater in inoculated than in noninoculated plants, suggesting that protein synthesis was stimulated in galled tissues.

The objectives of this study were (i) to study the histopathology of Wando pea tissue infected with nematodes of the Waynesville and Raleigh populations of *D. dipsaci;* and (ii) to compare oxidative enzymes produced by Wando pea seedlings at different time intervals after inoculation with the Raleigh nematode population (susceptible reaction) and Waynesville nematode population (resistant reaction) with those produced by similar noninoculated plants.

MATERIALS AND METHODS.—Nematodes of the Waynesville, N. C. (WNC), and Raleigh, N. C. (RNC), populations of *Ditylenchus dipsaci* (Kühn) Filipjev were propagated on alfalfa callus tissue (11). Nematodes were separated from 6- to 8 week-old cultures by the Baermann funnel technique.

Inoculation procedures and incubation conditions were as previously described (8). Histopathological procedures were as described (8), with the following exceptions. Seedlings inoculated with WNC nematodes were harvested 2, 3, 5, 7, and 14 days after inoculation; and those inoculated with RNC nematodes, after 7 and 14 days.

Counts of nematodes in host tissues were made at each harvest. Five infected shoots were homogenized for 20 sec in a Waring Blendor, concd on a 325-mesh screen, and washed onto a Baermann funnel. Nematodes were collected after 24 hr and counted.

Cross-inoculation studies.—Wando pea seedlings were inoculated with either RNC or WNC nematodes as described (8), except that 7.26 cm × 7.26 cm plastic pots were used instead of flats, and each pot contained 5 seedlings. There were four replications of each treatment. After 12, 18, 24, and 72 hr, the seedlings were inoculated with the opposite population applied to the surface of the sand. Pots were covered with cellophane for 48 hr. Growing conditions were as described (8). Plants were indexed for susceptible (galled) or resistant (necrotic) responses 14 days after initial inoculation.

Enzyme analyses of inoculated and noninoculated pea shoots.—Waynesville, N. C., nematode-inoculated (resistant reaction) and RNC nematode-inoculated (susceptible reaction) pea plants were harvested 7, 14, and 28 days after inoculation. Noninoculated plants of the same ages served as controls. Shoots of plants (1 g) were ground 2 min in a cooled mortar with the aid of powdered glass in 1.25 ml (v/w) of cold buffer at pH 8.0 freshly prepared from 0.1 M Tris[tris(hydroxymethyl)aminomethane]-HCl, 0.5 M sucrose, 0.1% ascorbic acid, and 0.1% cysteine-HCl (22). The resulting slurry was squeezed through a double layer of cheesecloth, and the residue discarded. The suspension was centrifuged at 20,000 *g* for 30 min, and the supernatant fluid was the source of proteins.

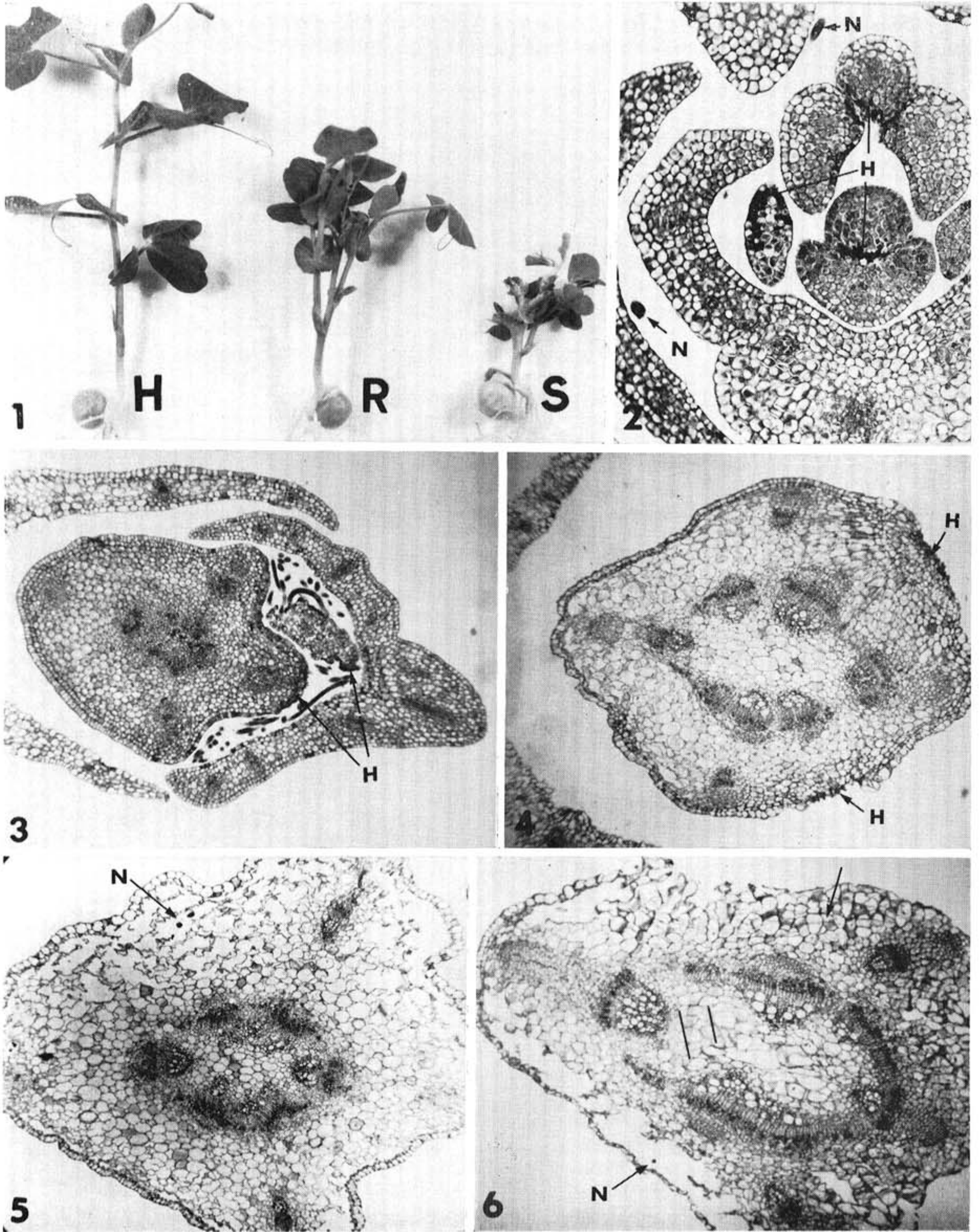
Enzymes in the preparations were separated by disc electrophoresis on polyacrylamide gels (4, 18). Instead of using a sample gel, a fraction of the preparation (approx protein concn of 0.15 mg obtained by dilution with extracting buffer) was applied directly on top of the spacer gel. Protein determinations were made by the method of Lowry et al. (14) on trichloroacetic acid precipitate dissolved in 0.1 N NaOH. Electrophoresis was conducted at 4 C with 2.5 ma/column in an anionic system. The gels were incubated in freshly prepared substrate solutions following electrophoresis to visually demonstrate the location of specific enzymes. Controls consisted of heating gels in test tubes in boiling water for 10 min or incubating gels in a solution minus the substrate. Peroxidases were demonstrated with the procedure of Staples & Stahmann (21), but with catechol, guaiacol, or benzidine-HCl in 0.1 M phosphate buffer at pH 6.8 as cosubstrates. Commercial horseradish peroxidase (Nutritional Biochemical Corp., Cleveland, Ohio) was used as a standard. Gels were incubated in 0.3% DL-dihydroxyphenylalanine (DOPA) in 0.05 M potassium phosphate buffer at pH 7.0 (21) to detect polyphenoloxidase (PPO) activity. Comparisons were made with commercial mushroom tyrosinase (NBC, Cleveland, Ohio).

Prior to electrophoresis, 0.25% soluble starch was added to the separating gel to aid in locating catalase activity. Following incubation in a substrate medium (2), gels were rinsed with distilled water and immersed in a solution of 1.5% potassium iodide (acidified with acetic acid immediately prior to use) until gels were dark blue with unstained bands indicating catalase activity.

Relative peroxidatic activity in the enzyme preparations was measured spectrophotometrically by following oxidation of pyrogallol. Enzyme preparations were diluted 1:10 with the extracting buffer prior to assaying for peroxidatic activity. The reaction mixture consisted of 5 ml of 0.05 M pyrogallol in 0.06 M phosphate buffer at pH 6.0, 0.1 ml of diluted enzyme preparation, and 0.5 ml of 1% hydrogen peroxide (12). Null point was adjusted to zero at 420 nm on a Coleman universal spectrophotometer prior to adding hydrogen peroxide. The time required for change in OD between 0.10 and 0.30 was recorded.

Sites of peroxidase activity were demonstrated histochemically in sections of RNC nematode-infected and WNC nematode-infected Wando pea seedlings 12 days after inoculation. Cross sections (200 μ thick) were cut with a sliding microtome from fresh plant material mounted in pith. Sections were transferred to a collecting medium of 4% Formalin and incubated in a localization medium that consisted of 1.0 ml of 0.05% benzidine-HCl and 0.5 ml of 0.3% hydrogen peroxide (24). After incubation for 1 min, sections were mounted on microscope slides in distilled water for observation.

RESULTS.—*Histopathological investigations.*—The gross symptoms of Wando peas infected with nematodes of the WNC population of *D. dipsaci* differed greatly from those of plants infected with RNC nematodes (Fig. 1). Symptoms of RNC nematode-infected plants were characterized by galling and distortion of the infected tissues, and severe stunting of the entire plant (Fig. 1). Symptoms were first apparent in the subapical region of the shoot, and later extended into young leaves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and basipetally into the stem. Necrosis was not apparent until the later stages of infection. In WNC nematode-infected plants, necrosis was apparent in the shoot apex 2 days after inoculation, and apical growth was inhibited (Fig. 1). Lateral shoots developed from lateral buds and became dominant. There was no galling of infected tissue. Nematodes were located among the embryonic leaves of the shoot apices 2 days after inoculation. Damage to embryonic leaves was erratic, occurring as scattered groups of necrotic cells in the epidermis and occasionally in contiguous parenchymatous mesophyll cells (Fig. 2). Nematodes also attacked apical meristems, lateral buds, and stipules. Only stylets of nematodes penetrated the plant tissue, inducing the necrotic areas. Necrotic cells contained granular cytoplasm that stained deep red with safranin. Although walls of affected cells collapsed, the cells remained intact. Nuclei were shrunken and also stained red, and nucleoli were indistinguishable. Similar staining reactions were observed in shoot apices 3 days after inoculation. None of these symptoms was observed in sections of noninoculated tissue.

Tissue damage in WNC nematode-infected seedlings 5 and 7 days after inoculation differed little from plants harvested earlier. In general, necrosis in the older plants was more extensive, although still restricted to the epidermis and adjacent tissue; nematodes still had not entered the plant tissue. Several nematodes were found among the leaves, but necrotic cells occurred only in scattered groups in the epidermis (Fig. 3). These cells contained granular cytoplasm and stained deep red. Nematodes also damaged lateral buds by inducing necrosis and mechanically disrupting cellular organization. Even 14 days after inoculation, necrotic cells were still mostly confined to the epidermis, but in some sections, adjacent cortical cells were also affected (Fig. 4). These cortical cells had undergone mitotic divisions, giving the appearance of cork formation. Cells in the hyperplastic areas were much smaller than adjacent unaffected cortical cells, and the hyperplasia was directed toward the necrotic cells.

In pea seedlings inoculated with nematodes of RNC population, tissue disruption characteristic of the susceptible response in other host species was observed. Large cavities bordered by greatly misshapen cells occurred in the cortex and parenchymatous mesophyll tissue of infected seedlings 7 days after inoculation (Fig. 5). The large cavities, resulting from destruction of parenchymatous cells, extended acropetally into the embryonic leaves of shoot apices and basipetally into the stem. Epidermis adjacent to infected tissue was hyperplastic and convoluted. Nematode damage to stipules resembled that in embryonic leaves. Damage was limited to tissue composed of parenchymatous cells whereas vascular tissue remained unaffected. Necrosis was not observed in tissues galled by RNC nematodes at this harvest.

Similar but more extensive damage was observed in RNC nematode-infected tissues 14 days after inoculation. Nematodes had penetrated basipetally far into stems, causing general destruction of the cortex. In several heavily infected seedlings, damage to tissues was severe (Fig. 6). Portions of the epidermis were destroyed, exposing the cortex. Outer layers of cortex were necrotic and partially sloughed, with the adjacent cortical cells dividing and giving the appearance of cork formation. No symptoms were observed in sections of noninoculated plants.

The number of WNC nematodes associated with the host tissues consistently ranged from one to six/plant. The number of RNC nematodes varied with the severity of the infection. At 7 days after inoculation, the number of RNC nematodes ranged from 8 to 15; at 28 days, the number ranged from 225 to 450.

Cross-inoculation studies.—The resistant response was preferentially expressed over the susceptible response regardless of which population of nematode was used as the initial inoculum (Table 1). Even when inoculation with WNC nematodes followed inoculation with RNC nematodes by 72 hr, approx 55% of the infected plants exhibited the resistant reaction (necrosis), whereas only 45% exhibited the susceptible reaction (galling). At intervals between inoculations less than 48 hr, approx 75% of the infected plants exhibited the resistant response. Some plants had no symptoms, and therefore were considered to be escapes. When the initial inoculum was WNC nematodes, resistance was expressed in approx 80% of the plants.

Comparison of enzyme patterns of noninoculated and inoculated plants.—Multiple forms of oxidative enzymes occurred in the soluble protein fractions of noninoculated and inoculated plants. Although these enzymes were different in noninoculated and inoculated plants (Fig. 7), no differences were detected between RNC nematode-inoculated and WNC nematode-inoculated plants.

With DOPA as the substrate, PPO activity was detected at sites where peroxidase activity was detected. Polyphenoloxidase activity was stronger at certain sites.

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Fig. 1-6. Histopathology of Wando pea seedlings following inoculation with Raleigh or Waynesville, N. C., populations of *Ditylenchus dipsaci*. **1)** Wando pea seedlings, 18 days after inoculation, showing noninoculated plant (H), WNC nematode-inoculated plant (R), and RNC nematode-inoculated plant (S) (×0.6). **2)** Cross section of a shoot apex 2 days after inoculation with the WNC population showing a nematode (N) present among the embryonic leaves and scattered groups of necrotic epidermal cells (H) (×100). **3)** Cross section of a shoot apex, 5 days after inoculation with the WNC population, showing several nematodes located in the leaf axil and groups of necrotic cells (H) in the epidermis (×40). **4)** Cross section of a stem, 14 days after inoculation with the WNC population, showing necrotic cells (H) in the outer layer of cortex with contiguous dividing cells (×18). **5)** Cross section of a stem, 7 days after inoculation with the RNC population showing a nematode (N) located in a large cavity bordered by greatly misshapen cortical cells. The epidermis adjacent to the damaged cortex is convoluted (×35). **6)** Cross section of a severely infected stem, 14 days after inoculation with the RNC population, showing a nematode (N) located in a large cavity in the cortex and cork formation (arrow) in the area of stem where the epidermis was destroyed (×23).

TABLE 1. Effects of cross-inoculations with nematodes of the Raleigh and Waynesville, N. C., populations of *Ditylenchus dipsaci* on symptom expression in Wando pea seedlings

	Initial inoculum					
	RNC			WNC		
Time[a]	Sus[b]	Res[c]	Esc[d]	Sus[b]	Res[c]	Esc[d]
12	8	10	2	6	13	1
18	4	11	5	1	16	3
24	4	14	2	3	13	4
48	7	8	5	3	12	5
72	7	8	5	6	13	1
Control[e]	7		3		7	3

[a] Hours after initial inoculation when plants were inoculated with nematodes of the other population.
[b] Sus = Number of plants out of 20 inoculated, with susceptible response (galling).
[c] Res = Number of plants out of 20 inoculated, with resistant response (necrosis).
[d] Esc = Number of plants out of 20 inoculated, without apparent symptoms (escapes).
[e] Plants inoculated only with initial inoculum.

Bands *a* and *b* had weak PPO activity, whereas, band *h* was intense (Fig. 7).

Although multiple forms of peroxidase were detected, the number of sites varied with the cosubstrate. Band *h*, the most consistent, occurred with all cosubstrates. Four sites (*a*, *c*, *d*, and *h*) of enzymic activity were detected with extracts from noninoculated plants with benzidine-HCl. Five sites of peroxidase activity were detected with extracts from diseased plants. Enzymic activity occurred at band *e* in addition to bands *b*, *c*, *d*, and *h*. With catechol as a cosubstrate, strong enzymic activity occurred at bands *a* and *h*, but only a weak reaction occurred at band *g*. Activity at band *g* was detected in all three preparations of plants in approx 20% of the experiments.

An additional site of peroxidase activity, band *f*, was detected only when guaiacol was the cosubstrate. Activity at band *f* occurred in approx 10% of the preparations from nematode-infected tissues, but was not detected in preparations from noninoculated plants.

Band *a* had strong catalatic activity based on a positive starch-iodine test. A thin band with catalatic activity also consistently developed near the tops of the gels. Catalatic activity of the three extracts from plants 7 and 14 days after inoculation were identical. In one experiment with extracts from RNC nematode-infected tissues 28 days after inoculation, the faster migrating band resolved into two bands of catalatic activity.

The over-all peroxidatic activity, using pyrogallol as a cosubstrate, was higher in extracts from inoculated tissues than from noninoculated tissues (Table 2). Greatest peroxidatic activity occurred in extracts of WNC nematode-infected tissues 7 days after inoculation. Extracts from RNC nematode-infected tissues also had greater peroxidatic activity than extracts from noninoculated tissues, with greatest activity 28 days after inoculation when tissue degradation was greatest.

Peroxidase localization.—Peroxidase activity, determined histochemically by a benzidine-blue reaction, was greater in inoculated than in noninoculated tissues. High peroxidase activity (dark color) was associated with certain tissues; most notably the epidermis (Fig. 8). Cortical parenchyma and xylem cells showed less activity. Peroxidase activity was greatest next to the cell walls of these tissues in noninoculated seedlings.

In RNC nematode-infected tissue, high peroxidase activity was localized in parenchymatous cells directly associated with nematode-induced cavities in the cortex (Fig. 9). A netlike pattern of distorted, misshapen cortical cells apparently resulted from hypertrophy and malformation following destruction of surrounding cells.

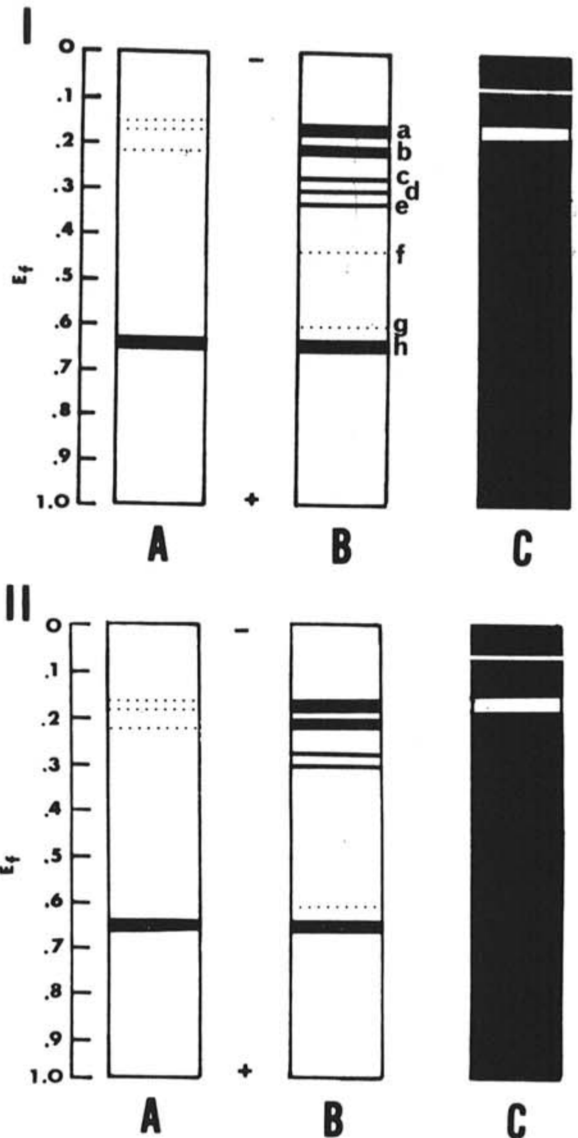


Fig. 7. Zymograms obtained from soluble protein fractions of inoculated (I) and noninoculated (II) Wando pea seedlings. Zymograms show sites of polyphenoloxidase (A), peroxidatic (B), and catalatic (C) activities. Certain peroxidatic bands developed with specific cosubstrates.

TABLE 2. Relative peroxidase activity in extracts from inoculated and noninoculated Wando pea shoots. Plants were inoculated with either the Raleigh or Waynesville, N. C., population of *Ditylenchus dipsaci*. Values are based on the oxidation of pyrogallol[a]

	Time[b]		
Age[c]	WNC Nematode-inoculated	RNC Nematode-inoculated	Non-inoculated
7		16.4	24.8
		8.5	13.2
	4.2	11.5	13.3
	3.9		12.8
	5.0		22.9
14		18.5	31.4
		10.6	18.4
	7.2	11.9	14.0
	9.6		21.4
	8.5		31.0
28		6.3	22.3
		7.6	17.8
		16.3	25.7

[a] Data from experiments on different groups of plants and is mean of two replications.
[b] Seconds required for change in OD from 0.10 to 0.30 at 420 nm.
[c] Days after inoculation.

These misshapen cells within cavities and cells bordering the cavities had high peroxidase activity. Cells which were collapsed and apparently devoid of cytoplasm also had an intense localized peroxidase reaction. Peroxidase localization in other tissues was similar to that observed in sections of noninoculated plants.

Peroxidase activity was also high in sections of WNC nematode-infected tissues (Fig. 10). Strong peroxidase activity was associated with parenchymatous cells of cork formed in the cortex adjacent to necrotic lesions. This increase in peroxidase activity was confined mostly to the cortical cells adjacent to necrotic epidermal cells.

DISCUSSION.—The resistant response in the Wando cultivar of pea to WNC nematodes differs from the resistant reaction elicited in the Alaska cultivar of pea by this nematode (8). The WNC nematodes induce a hypersensitive reaction in the epidermis of Wando pea seedlings which apparently inhibits further penetration. When Alaska pea was the host, WNC nematodes penetrated the tissues 24 to 48 hr after inoculation (8). A necrotic reaction was induced in the embryonic leaves of Alaska pea 6 hr after inoculation. As the leaves matured and nematodes entered the leaf tissue, however, a susceptible response developed, but necrosis soon followed. When Alaska seedlings were inoculated with RNC nematodes, symptoms similar to those induced by WNC nematodes developed.

In Wando seedlings, RNC nematodes successfully parasitized the host, destroying parenchymatous cortical cells and inducing galling. Collapsed, misshapen cells are characteristic of susceptible reactions elicited in plant tissues by the stem nematode (5). These misshapen cells appear to be alive, but confirmation should be made by histochemical demonstration of activity of enzymes involved in intermediary metabolism. Such studies would also establish whether or not the metabolism of cells in proximity to nematodes had been stimulated. Howell & Krusberg (7) reported an increase in free- and protein-amino acids, suggesting an increase in protein synthesis in galled tissue of Wando pea infected by RNC nematodes compared to noninoculated pea tissue. Histochemical studies of Alaska pea seedlings infected by *D. dipsaci* should indicate what enzyme changes occur in tissues of a moderately resistant host.

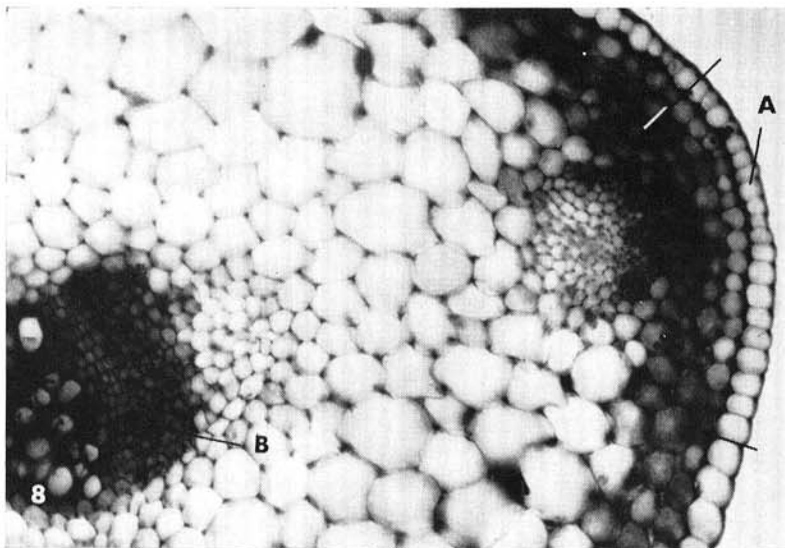
The possible role of pectolytic and cellulolytic enzymes in pathogensis of Wando pea by RNC and WNC nematodes was investigated by Muse et al. (17). Although activity of certain of these enzymes differed between nematodes of the two populations, no correlation could be established between enzyme activity and the differential response of Wando pea. WNC nematodes produce sufficient levels of pectolytic and cellulolytic enzymes to facilitate their entrance into Alaska pea, Dupuits alfalfa, and other hosts. The failure of WNC nematodes to enter Wando seedlings, however, is apparently related to the sensitivity of the host to secretions by this nematode.

The resistant response in Wando pea to WNC nematodes is preferentially expressed over the susceptible response to RNC nematodes. Destruction of the apical meristem and the consequent probable inhibition of synthesis of necessary growth-promoting substances required for the susceptible response could account for the dominance of the resistant reaction.

Although polyphenoloxidase and catalase enzymes from noninoculated tissues were similar to those from diseased tissues (RNC nematode-inoculated and WNC nematode-inoculated), additional peroxidase isoenzymes were present in diseased tissues, and could be the result of de novo or increased synthesis. These isoenzymes had to have originated in the plant tissues, as peroxidases were not detected in extracts of the nematodes (9). Differences in cosubstrate specificity of the peroxidase isoenzymes demonstrated the necessity of using several cosubstrates when testing for sites of peroxidase activity in polyacrylamide gels. More peroxidase isoenzymes were detected using benzidine-HCl than guaiacol or catechol as cosubstrates. Benzidine-HCl appeared to be the most general cosubstrate for peroxidase isoenzymes. The top band with peroxidatic activity was established as a catalase because of the positive starch-iodine test, and no detectable activity with guaiacol or benzidine-HCl.

The additional peroxidase isoenzymes could account for the high over-all peroxidatic activity observed with diseased tissues. The term "peroxidatic" is used in preference to peroxidase because both peroxidases and catalases can utilize pyrogallol as a cosubstrate in the presence of hydrogen peroxide. The lower peroxidatic activity associated with RNC nematode-inoculated tissues was probably related to some terminal growth and galling. Therefore, in protein preparations from RNC nematode-inoculated shoots, the higher concn of soluble protein would dilute the peroxidase and catalase enzymes.

The histochemical localization of sites of peroxidase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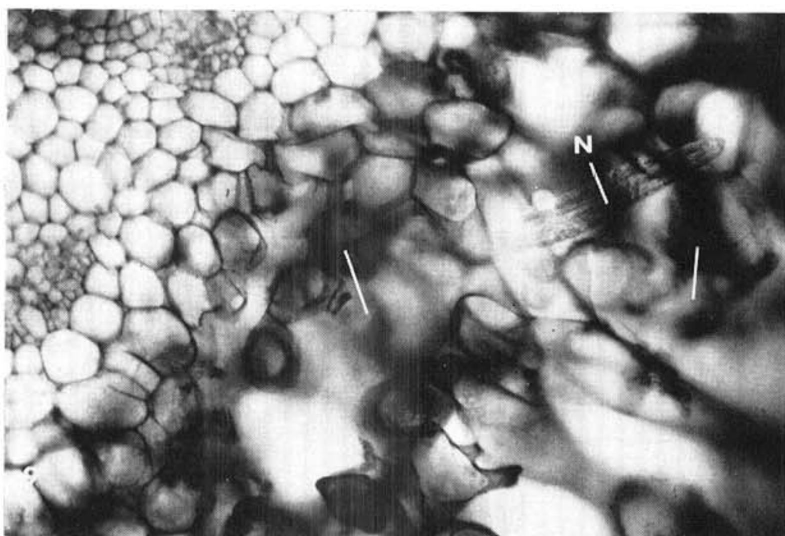
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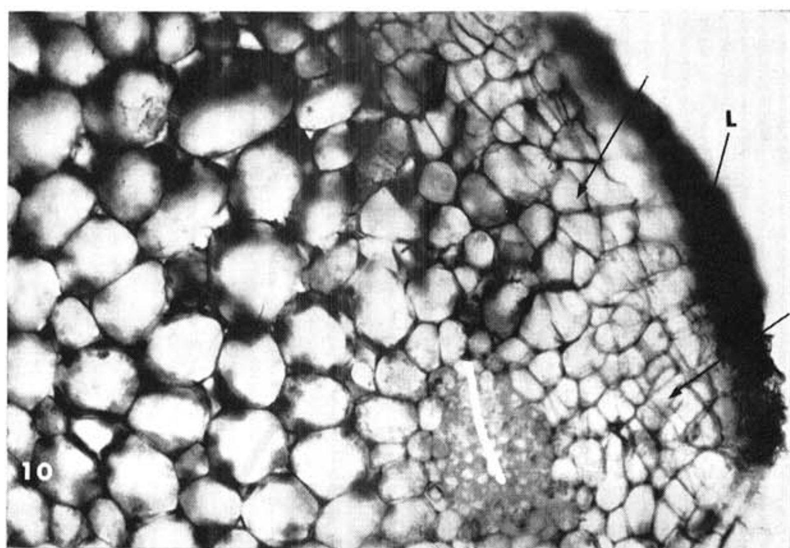
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Fig. 8-10. Histochemical localization of sites of peroxidase activity in cross sections of fresh stems of Wando pea 12 days after inoculation with Waynesville (WNC) or Raleigh (RNC), N. C., populations of *Ditylenchus dipsaci* and in sections of noninoculated plants. **8)** A section of a noninoculated plant showing high peroxidase activity (dark cells) associated with epidermal (A) and vascular (B) tissues. The dark color zone (arrows) in the outer periphery of cortex is attributed to chloroplasts in these cells. **9)** A section of RNC nematode-inoculated tissue showing high peroxidase activity localized in parenchymatous cells (arrows) directly associated with nematode-induced cavities in the cortex. A nematode (N) is present among the misshapened parenchymatous cells. **10)** A section of WNC nematode-inoculated tissue with strong peroxidase activity associated with parenchymatous cells (arrows) of cork formed in the cortex adjacent to a necrotic lesion (L).

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activity in diseased tissue also demonstrated high peroxidase activity associated with the infected tissue. Since more cellular destruction occurred in the RNC nematode-inoculated tissue, the peroxidase activity was more extensive in this tissue. Host peroxidases are probably not important in the differential response of Wando pea to the two populations of *D. dipsaci,* as high peroxidase activity occurred in both diseased tissues.

Future studies on resistance of Wando pea to WNC nematodes should be concerned with determining exactly what is secreted by this nematode that induces the hypersensitive response in the host. Isolation and assay of substances secreted under aseptic conditions would elucidate what substance(s) is responsible for inducing the resistant response.

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