Morphology and Histochemistry of Soybean Roots Infected with Heterodera glycines

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ABSTRACT

Morphological and histochemical observations were made on roots of susceptible, Lee, and resistant, Pickett, soybeans infected with the soybean cyst nematode, *Heterodera glycines*. Certain host enzymes were localized near the nematode stylet during penetration and migration of the infective larvae. Increased enzyme activity was observed in syncytia of both susceptible and resistant hosts during the early stages of disease development. As syncytia development progressed in the susceptible host, high enzyme levels were demonstrated in the dense cytoplasm. In the resistant host, syncytia were initiated and showed a slight increase in enzyme activity, but the syncytia shortly became necrotic and deteriorated. Cortical necrosis was common in both susceptible and resistant hosts as a result of intracellular migration of larvae.

Enzyme stimulation and necrosis associated with cyst nematode infections are discussed in relation to similar studies previously reported for root-knot infections of the same host. Phytopathology 60:1493-1498.

The histopathology of the host response to Heterodera infection has been described for a number of species (3, 10, 11, 16, 21). A syncytium induced by the cyst nematode develops through progressive dissolution of cell walls adjacent to the syncytium. As nuclei and cellular contents are incorporated into the syncytium, the syncytial protoplasm increases in density and appears granular in texture.

Host-parasite relationships of various plants infected by the root-knot nematode *Meloidogyne* spp. have also been studied (7, 9, 19). Similarities and differences in host response to infection by these sedentary endoparasites have been documented. A major feature characterizing the susceptible-host response to root-knot and cyst nematode infection is the induction of an abnormal symplast. This structure, called by various names [giant cell (22), syncytia (18), lysigenoma (8)], has been extensively reviewed (12). Syncytial formation and morphology have been described by a number of researchers (15, 19).

Histochemical investigations have provided some insight into the function of the syncytium (4, 5, 17). Rubinstein & Owens (20) showed an increase in nucleic acids in root knot-induced syncytia which suggests a preconditioning of the syncytium for hyperactive metabolism. Enzyme histochemical investigations on the response of soybeans to infection by the root-knot nematode reinforce the concept that the syncytium is metabolically hyperactive (13, 23, 24). Comparable enzyme histochemical studies of syncytia induced by the soybean cyst nematode have not been made; therefore, comparisons of the enzyme histochemical response of a single species of host parasitized by two genera of nematodes is precluded.

The purpose of this paper, therefore, is to (i) histochemically demonstrate certain oxidoreductive and hydrolytic enzymes that are induced in the host by the soybean cyst nematode; (ii) compare these responses in susceptible and resistant soybean roots during penetration and growth of the nematode; and (iii) discuss some of the similarities and differences in the responses of soybean plants infected by *Heterodera glycines* and *Meloidogyne incognita*.

MATERIALS AND METHODS.—Lee (susceptible) and Pickett (resistant) (6) soybeans (*Glycine max* [L.] Merr.) were inoculated with measured suspensions of the soybean cyst nematode (*Heterodera glycines* Ichinohe) as described previously for root-knot infections (13). The inoculated plants were grown in the greenhouse in pots containing fine vermiculite. Fresh sections, about 100 μ thick, were cut from infected roots 1 to 21 days after inoculation. The nonfixed sections were placed in a buffered holding medium (13) until used; the holding time did not exceed 10 min. Localization reactions for the visual demonstration of glucose-6-phosphate dehydrogenase, malate dehydrogenase, iso-citrate dehydrogenase, DPN-dependent diaphorase, alkaline phosphatase, acid phosphatase, esterase, and peroxidase were run by procedures described previously (13, 23). Four additional enzymes were investigated: aldolase, ethanol and lactate dehydrogenases, and phenolase. The procedures for identifying these enzymes were as follows. Ethanol and lactate dehydrogenases were localized by the dehydrogenase procedure described earlier (13), but with the respective substrates. Aldolase was localized by the procedure of Allen & Bourne (1) without further modification. Phenolase was localized using 0.1 M D, L-dihydroxyphenyl alanine (dopa) as a substrate, buffered at pH 7.2 with 0.05 M Tris [tris(hydroxymethyl) amino methane] buffer.

RESULTS AND DISCUSSION.—The soybean cyst nematode, *H. glycines*, induced a slight increase in enzyme activity in host cells pierced by the stylet during penetration of the root. Both susceptible and resistant cultivars initially responded in the same way. Subsequent intracellular migration of the larvae caused cell wall destruction and extensive necrosis in both cultivars.

Syncytia were common and extensive in the susceptible host, less frequent and of limited size in the resistant host. During the later stages of disease development, necrosis was associated with the syncytia in resistant hosts; the susceptible host became necrotic as a result of the intracellular migration of the larvae.

All of the enzymes investigated, except phenolase, were hyperactive at newly established feeding sites of

the nematode. The syncytia supported the highest levels of activity, but in the resistant host the increase in activity stopped shortly after syncytia were initiated.

Susceptible host response.—Larvae were observed migrating inter- and intracellularly toward the stele within 1 day after inoculation (Fig. 1-A). Host cells that were probed, but not entered, by the larvae had slightly increased levels of enzyme activity; however, cells that the nematode penetrated tended to become necrotic. The necrosis was probably a response to the mechanical injury sustained by the cell when the nematode entered. Transverse sections (Fig. 1-B) cut 1 day after inoculation show a larva in nearly the same position as that shown in the previous figure. Cells entered by the nematode were destroyed, but adjacent cells were stimulated. A syncytium did not form, but the nematode appeared to have probed cells near its lip region. An extensive syncytium supporting very high enzyme activity developed 8 days after inoculation (Fig. 1-C). The nematode that induced the syncytium enlarged substantially.

The progressive enlargement of soybean syncytia induced by *H. glycines* has been discussed by Endo (10, 11). Host cell walls nearest the syncytium are broken down, and a coalescence of cytoplasm takes place. Figure 1-D shows this type of progressive extension of the syncytium. Eleven days after inoculation, a well-defined syncytium with high enzyme activity was observed. Host cells somewhat removed from the syncytia also supported increased enzyme activity. These cells appeared to be merging with the syncytium through a process of cell wall breakdown and subsequent coalescence.

Transverse sections of roots with syncytia induced by advanced stages of the nematode often showed lower levels of enzyme activity than did longitudinal sections. Much of the syncytial content of nonfixed transverse sections was lost during sectioning and handling. A transverse section of a root 11 days after inoculation (Fig. 1-E) shows a nematode associated with a syncytium and some necrosis associated with cell destruction. A determination of enzyme activity within the syncytium was impossible because the cytoplasm was lost. However, intrasyncytial cell wall fragments and aberrations were apparent.

Resistant host response.—Increased enzyme activity in response to soybean cyst nematode infection was limited. Some increased activity was observed, however, during penetration and migration of the larvae, and substantial increases in enzyme activity were associated with syncytia. Syncytial development was usually restricted in the resistant host.

Figure 1-F (1 day after inoculation) shows an active site near the stele where substantially high enzyme activity would usually be interpreted as a sign of syncytium formation; however, extensive necrosis was closely associated with the cells supporting increased enzyme activity. Perhaps the necrosis accounts for a previous observation that syncytia are often initiated but seldom develop appreciably in resistant soybeans (11).

Necrosis of cortical cells in the path of the migrating nematode was a common feature associated with resistant plants (Fig. 1-G). The dense cytoplasm of the affected cells indicates the larva probed and probably fed upon the cell long enough to induce necrosis. The detail of the cytoplasm and the recognition of subcellular components indicates that necrosis did not progress far.

Figure 1-H represents a typical resistant response to the soybean cyst nematode during the later stage of disease development (16 days after inoculation). Extensive cortical cell disruption and necrosis was brought about by the intracellular migration of the nematode. Some cortical cells apparently were only probed by the larva. They showed increased cytoplasmic density and elevated enzyme activity; however, some of these cells were also associated with necrosis. A syncytium was initiated but did not develop and eventually became necrotic. The necrotic syncytium precluded further nematode development; thus, a type of resistance was achieved.

Transverse and longitudinal sections (Fig. 2-A and 2-B, respectively) show the morphology of the network of modified cell walls that result when syncytia are induced by the soybean cyst nematode. Whether

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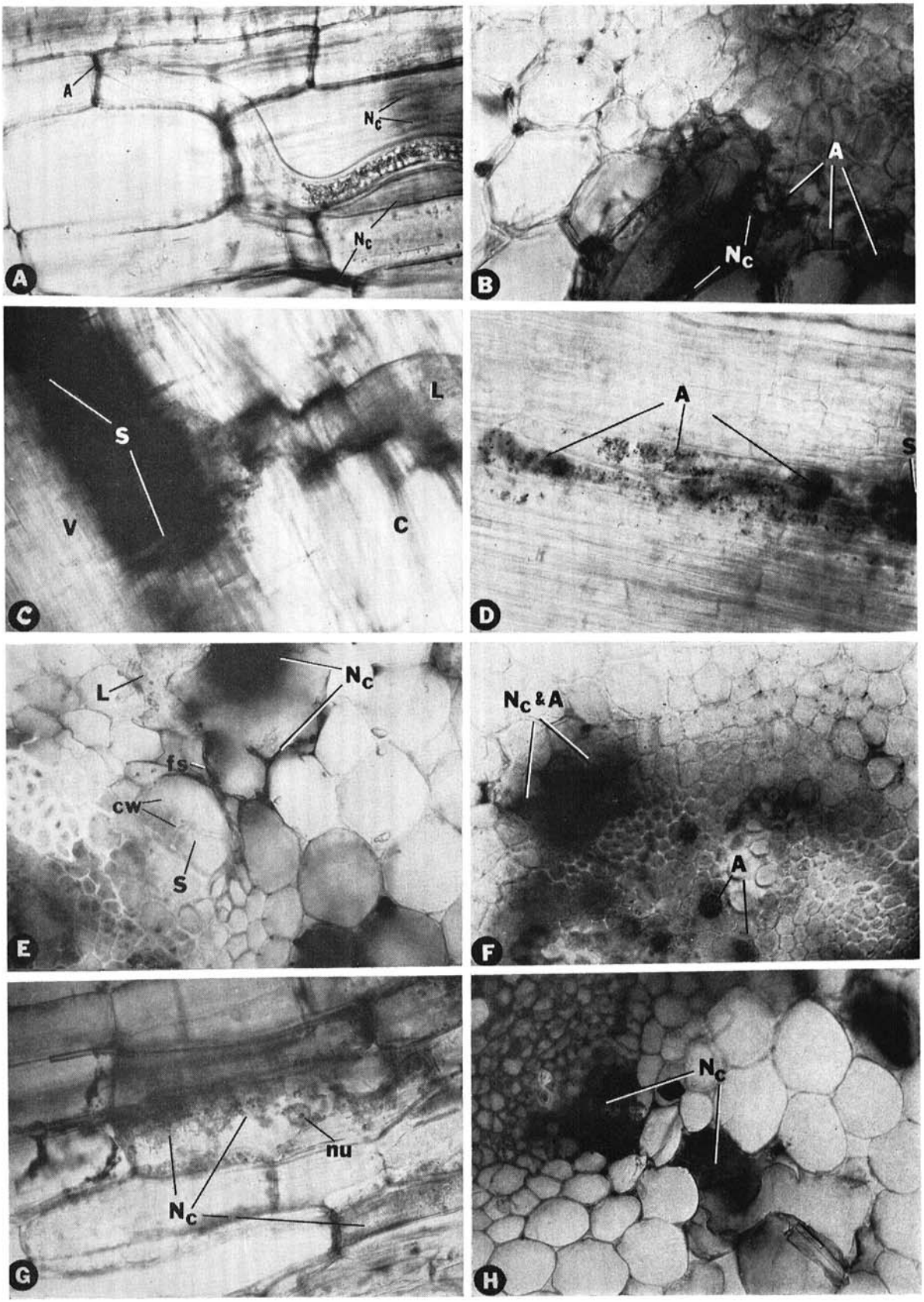
Fig. 1. The histochemical localization of various enzymes in the susceptible (Lee, A-E) and resistant (Pickett, F-H) cultivars of soybean infected by the soybean cyst nematode (*Heterodera glycines*). **A)** A longitudinal section, 1 day after inoculation, showed slight necrosis (Nc) in host cells along the path of the migrating larva. A small but intense zone of DPN-dependent diaphorase activity (A) was observed in host cells probed by the larva. **B)** A transverse section, 1 day after inoculation, showing increased DPN-dependent diaphorase activity (A) in host cells near the larva stylet. The cortical cell necrosis (Nc) was a result of the intracellular migration of the larva. **C)** Eight days after inoculation, an extensive syncytium (s) supporting very intense 6-phosphogluconate dehydrogenase activity was observed in longitudinal section. The larva (L) that induced the syncytium within the vascular system (V) had enlarged substantially and was sedentary in a radial plane across the cortex (C). **D)** A syncytium (s) was observed 11 days after inoculation. The host cells at the edge of the syncytium supported high glucose-6-phosphate dehydrogenase activity (A) and appeared to be merging with the syncytium. The cells nearest the syncytium are the first to coalesce, helping to account for syncytium expansion. **E)** A transverse section, 11 days after inoculation, showed cortical cell necrosis (Nc) resulting from intracellular larva migration. The larva (L) was excised during sectioning, but its feeding site (fs) was recognizable. Within the syncytium (s) associated with the feeding site were cell wall fragments or aberrations (cw). **F)** A section from a resistant plant 1 day after inoculation showed increased ethanol dehydrogenase activity in some cells in the midst of many cells that were necrotic (Nc, A). The necrosis was probably a resistance response, and not induced by intracellular migration of the larva. **G)** A longitudinal section shows a cortical cell (Nc) that was probed by a larva and is becoming necrotic. Some subcellular organelles are still intact as evidenced by the prominent nucleus (nu). **H)** A transverse section 16 days after inoculation typifies the resistance response at this stage of disease development. Extensive necrosis (Nc) was observed; some of the necrosis was a host resistance response, while some was obviously the result of intracellular larva migration.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the modified walls are in the process of decomposition or synthesis has not been adequately determined.

The relatively high enzyme activity and the dense cytoplasm observed in the syncytia in susceptible hosts tended to emphasize the metabolically active process of tissue alteration during syncytial development and nematode growth. In resistant plants, the localization of increased enzyme activity was limited to the early stages of syncytial development. In the absence of continued feeding by the nematode, deterioration of the syncytium and diminished enzyme activity occurred (or vice versa). Nevertheless, the transitions to the state of reduced enzyme activity and the induction of necrosis could be used as a means of determining the effective period of syncytial function and nematode feeding. Bird (4) demonstrated the interdependence of syncytia and *Meloidogyne* development in tomato roots by selectively destroying the nematodes. In the absence of living nematodes, the syncytia deteriorated.

Lee soybeans were used as the susceptible cultivar in earlier studies with the root-knot nematode (13, 23, 24). The same cultivar was used here as the susceptible host for the soybean cyst nematode. Therefore, meaningful comparisons of the susceptible response of one host to two genera of nematodes can be made. For studies on the resistant response to root-knot and soybean cyst nematode, different hosts were used. Hence, comparisons of the resistant response to two genera of nematode are not as valid.

During the early stages of infection, syncytia induced by *Heterodera glycines* had lower levels of enzyme activity than the syncytia of comparable soybean plants infected by the root-knot nematode, *M. incognita*. The lower levels of enzyme activity perhaps were due to the more generalized response of root tissues to stimulation by *H. glycines*. When a larva of *H. glycines* becomes sedentary at a feeding site, a single syncytium usually forms in response to the feeding action. As the nematode grows and the syncytium develops, extensive cell wall dissolution takes place which results in the merging of cytoplasm and constituent nuclei. The cell-to-cell transition of normal tissue into syncytia and the vacuolate condition of the syncytial contents in the early stages of infection may account for the lower enzyme activity.

In *Meloidogyne* infections, similar cell-wall dissolution processes have been reported (7, 9, 15), but it has also been shown that a syncytium can develop from a single cell which is stimulated by the root-knot nematode. Evidence for this type of multinucleation was recently described in detail by Huang & Maggenti (14). They found that synchronous mitosis and polyploidization can occur in *M. javanica*-induced syncytia

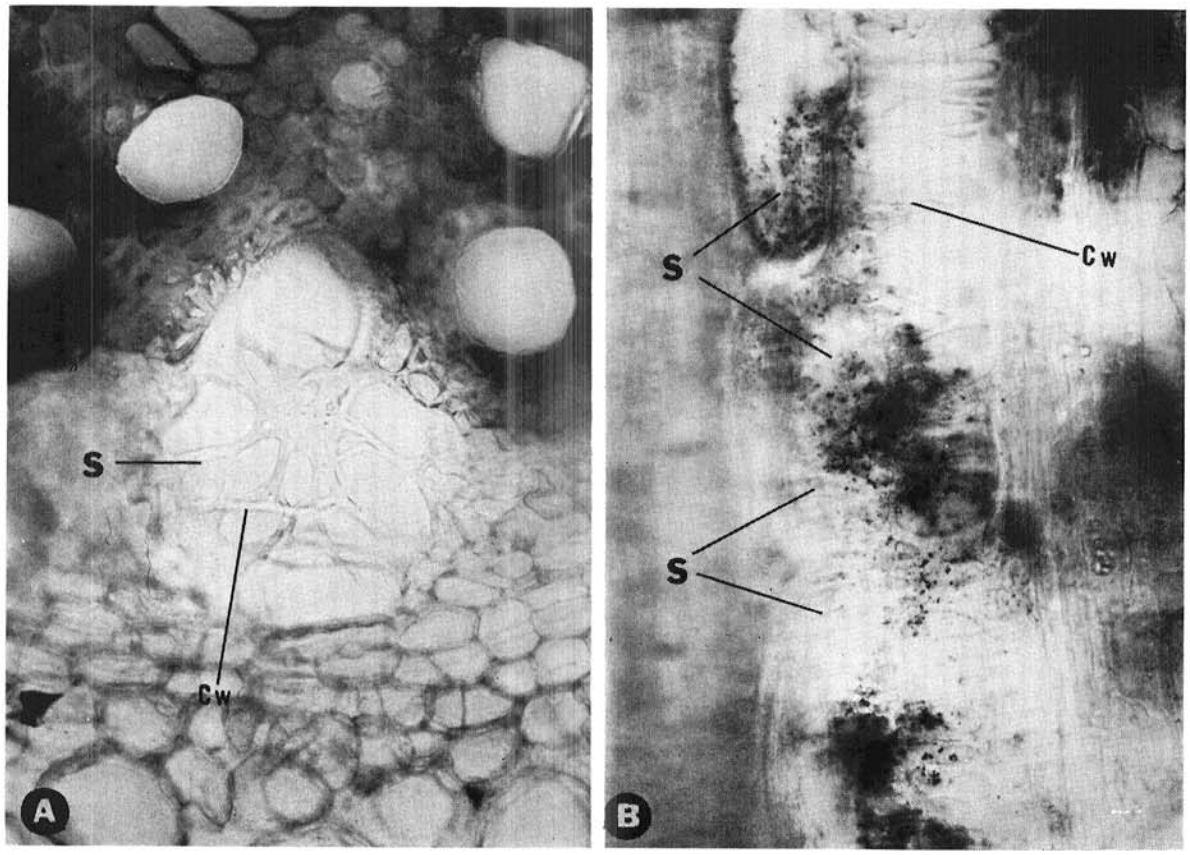

Fig. 2. Transverse and longitudinal sections (A and B, respectively) of a syncytium (s). The cell wall fragments or aberrations are apparent.

of *Vicia faba* roots in the absence of cell wall dissolution. These nucleic acid changes in conjunction with the increased cytoplasmic density confined in these syncytia may account for the relatively high enzyme localization observed during the early stages of root-knot infection. Differences in the degree of enzyme localization in *Heterodera*- and *Meloidogyne*-induced syncytia in soybeans were diminished as the infection period was extended. In both types of infections, relatively high levels of enzyme activity were detected in the syncytia.

The most obvious difference between soybeans infected by *H. glycines* and *M. incognita* was in the necrotic response. Extensive necrosis was observed in both the susceptible and resistant hosts infected by *H. glycines*. Some of the necrosis associated with soybean cyst nematode infections was a result of the intracellular migration of the larvae. For the most part this necrosis occurred in the cortex of both cultivars and was not thought to be an active host response to restrict syncytial formation. The necrosis associated with syncytia formed in the resistant cultivar infected by *H. glycines* was thought to be an active mechanism to limit the development of the syncytia; hence, nematode maturation would be prevented. Root-knot infections generally did not show cortical necrosis in either cultivar because larval penetration occurred near the apical meristem and subsequent migration was intercellular. Necrosis was, however, often observed in the resistant host as a response to *M. incognita* feeding.

In some respects, the host responses were the same for infections by *H. glycines* and *M. incognita*. Slight stimulation of enzyme activity occurred in the susceptible and resistant cultivars during the early stage of disease development. The development of syncytia in resistant hosts was arrested by necrosis produced in response to both types of nematodes. However, syncytial arrest may be more effective in the root-knot infections where syncytia were seldom initiated before necrosis occurred.

Except for cortical necrosis induced by *H. glycines*, the susceptible host responded essentially the same to root-knot and cyst nematode infections. Increased host enzyme activity was associated with nematode feeding and syncytial development.

Aldolase was the only enzyme studied in the glycolytic pathway. However, it would appear from the aldolase localization that glycolysis is activated at early stages of disease development, as activity of this enzyme becomes quite intense in the syncytia of susceptible plants. The extensive necrosis and the brownish color associated with necrosis in the resistant host are too easily confused with the chromophore indicator of aldolase activity. Therefore, while glycolysis in the resistant host may be stimulated, at least initially, the brown necrotic color prevented us from obtaining clear histochemical evidence of increased activity.

Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, enzymes of the hexose shunt pathway, appeared to be stimulated by the nematode in both susceptible and resistant hosts. In the susceptible host, these enzymes became most pronounced in the syncytium. In the resistant host, the activity of these enzymes increased in the syncytium, but did not reach the level attained in the susceptible host; the development of the syncytium was arrested by necrosis before a substantial increase in enzyme activity could occur. Enzymes of other major respiratory pathways (malate dehydrogenase represented the tricarboxylic acid cycle, a diaphorase represented the step between the tricarboxylic acid cycle and terminal oxidation, cytochrome oxidase represented aerobic terminal oxidation, and ethanol and lactate dehydrogenases represented anaerobic respiration) essentially responded to infection as did the hexose shunt enzymes.

Data indicate that the activities of various respiratory enzymes are substantially increased in susceptible plant syncytia. One possible explanation for this stimulation is that the specific activities of the enzymes within syncytia are increased. Quantitative determinations of enzyme activity of syncytia require the extraction of syncytial cytoplasm apart from normal tissue. Due to the limited size and location of syncytia, extracting the enzymes from only the syncytial fluids would be very difficult. An induced increase in the amount of enzyme within the syncytia is another possible explanation.

Avers & King (2) showed that all mitochondria in a cell do not support all the enzymes usually associated with mitochondria; i.e., mitochondrial heterogeneity exists in healthy plants. Some of our studies suggest that there is either an increase in the total number of mitochondria per unit volume, or that mitochondria which normally do not participate in particular reactions become active in syncytia. Perhaps the balance in the heterogeneity is upset by infection; this prospect is currently being considered.

Phenolase (dopa oxidase) was the only enzyme that was not localized in either the susceptible or resistant hosts before or after infection. The inability to localize this enzyme was not due to unreactive reagents, since good localization was obtained within the nematode at the cyst stage. The lack of localization of phenolase is peculiar if one considers the amount of browning that occurs in host tissues as a result of infection.

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