The Histochemical Localization of Several Enzymes of Soybeans Infected with the Root-Knot Nematode *Meloidogyne incognita acrita*

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Abstract: The sites of activity of alkaline phosphatase, acid phosphatase, esterase, peroxidase, adenosine triphosphatase, and cytochrome oxidase were demonstrated histochemically in fresh sections of 'Lee' soybeans infected by the root-knot nematode *Meloidogyne incognita acrita*. Each of the six enzymes was more active at the sites of infection than in adjacent non-infected tissue. During the early stages of infection, an increase in enzyme activity was observed in several cells in the proximity of the lip region of the nematode. However, when definite syncytia were observed, increased enzyme activity was confined primarily within the limits of the syncytium. Increased activity paralleled syncytial development and nematode maturation.

In a previous paper (8) the authors described the histochemical localization of several oxidoreductases in soybean (*Glycines max* L.) roots infected with the root-knot nematode *Meloidogyne incognita acrita* Chitwood & Oteifa. The activities of these enzymes (5 dehydrogenases and 2 diphorases) were increased at the feeding site of the nematode. Furthermore, stimulation of these enzymes occurred prior to the development of the syncytium and probably began shortly after the nematode stylet penetrated the host cell. In later stages of infection a similar host enzyme response was observed at the posterior region of the nematode; apparently, the response was initiated by excretory products of sedentary larvae. Simulation of oxidoreductases in the host cells adjacent to the anus of larvae preceded hyperplasia. Host-cell division induced at the posterior region of the larvae resulted in the formation of a tissue resembling a lateral root primordium (8).

There are other reports of increased metabolic activity in syncytia induced by nematodes. Owens and Novotny (11) stressed the rate of ribosomal RNA synthesis in nematode-induced tomato syncytia. Rubinstein and Owens (13) employed microautoradiography techniques to show increased RNA and DNA synthesis in syncytia induced in tomato roots by *M. incognita acrita*. They concluded that nucleic acid synthesis was influenced by the developmental stages of the parasite and syncytium. Bird (2) also observed a direct correlation between giant-cell protein synthesis and the growth rate of the nematode. Myuge (10) reported a three-fold increase in the rate of respiration of cucumber root gall induced by *Meloidogyne*. On the other hand, the induction of galls by *Meloidogyne* infecting tomato roots has been reported to occur without concomitant respiratory increases (3).

Earlier studies on the histochemical localization of oxidoreductases in soybean root tissue infected by root-knot nematode prompted the investigation of other enzymes that might be stimulated during the infection process. The selection of the foregoing enzymes was based on readily demonstrable histochemical procedures for enzymes that could represent various metabolic pathways in affected host tissues. Thus, the following enzymes were histochemically demonstrated in soybean roots infected by *M. incognita acrita*: cytochrome oxidase, alkaline phosphatase, acid phosphatase, peroxidase, aden-

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osine triphosphatase, and a non-specific esterase.

Materials and Methods

'Lee' soybean roots infected by the root-knot nematode *M. incognita acrita* were used for all histochemical procedures. Cultural procedures, method of inoculation, and the technique for sectioning the infection sites have been described in detail previously (8). Briefly, seeds were germinated in the greenhouse in fine vermiculite and the roots inoculated during the cotyledonary stage with an aqueous suspension of second-stage nematode larvae. After a 24 hr incubation period in moist vermiculite, the roots were washed to remove larvae that had not penetrated. The plants were transplanted into fine vermiculite and grown in the greenhouse until used. Fresh sections, about 100 μ thick, were cut from the infected roots.

Abbreviations: CO = cytochrome oxidase; E = non-specific esterase; A^+P = acid phosphatase; A^-P = alkaline phosphatase; ATPase = adenosine triphosphatase; P = peroxidase; Tris-M = tris(hydroxymethyl) aminomethane-maleate buffer; DMF = N,N'dimethylformamide; HApRA = hexazotized pararosaniline = (tris diazotized pararosaniline).

Histochemistry: Composition of the histochemical localization media, types of controls and other pertinent information are given in the localization procedures for the individual enzymes. Table 1 summarizes the procedures. When the localization reactions produced the desired intensity of color, the reactions were stopped by washing the sections in tap water. Sections, other than those demonstrating A^+P localization, were wet-mounted in 0.05 M Tris-M pH 7.2 on microscope slides. Sections demonstrating A^-P activity were wet-mounted in tap water with the pH adjusted between 8 and 9. The sections were examined and recorded as described previously (8). The techniques used here do not distinguish between the synthesis of new enzyme or an increase in the specific activity of existing enzyme. Therefore, reference to "activity" is meant to imply increased chromophore production; this could be the result of either, or both, higher specific activity or additional enzyme.

(i) *Cytochrome oxidase Localization.*—The demonstration of CO was made with a modification of the procedure given by Burstone (4); a schematic illustration is shown in Fig. 1. The localization medium consisted of 5.0 mg *p*-aminodiphenylamine; 5.0 mg *p*-amino-*m*-methoxy diphenylamine; 1.0 ml ethyl alcohol, 95%; 9.0 ml Tris-M, 0.08 M, pH 7.2. The two diphenylamines were dissolved in the alcohol and added slowly to the buffer with constant stirring. This mixture was filtered through Whatman No. 1 paper, and sections were incubated in the filtrate for 17 min at ca. 20 C. Controls for the demonstration of CO included: inactivation of the enzyme by boiling the sections in 0.1 M Tris-M pH 7.2 for 5 min; enzyme inhibition by the incorporation of 0.2 ml of 0.1 M NaN_3 into the localization medium; and inhibition of color formation by the removal of cytochrome-c from the sections by washing in three changes of 0.01 M NaCl.

(ii) *Esterase Localization.*—The histochemical demonstration of E was accomplished by a modification of Barrnett and Seligman's indigo dye technique (Table 1). A schematic diagram of the reaction is presented by Barka and Anderson (1). The localization medium consisted of 2.5 mg of 5-bromo-4-chloroindoxylacetate dissolved in 0.5 ml of 95% ethyl alcohol; 1.0 ml of 0.01 M potassium ferrocyanide; 1.0 ml of 0.01 M potassium ferricyanide; 4.0 ml of 0.2 M Tris-M, pH 7.0. Sections were incubated in this medium for 9 to 10 min at ca. 20 C. Tissue sections held at 100 C for 5 min served as a control for E.

TABLE 1. Summary of Enzyme Localization by Histological Procedures

Enzyme	Reagents	Incubation (min)	Control	Reaction Results	Type of Reaction
Cytochrome oxidase	5.0 mg *p*-aminodiphenylamine 5.0 mg *p*-amino-*m*-methoxy diphenylamine 1.0 ml ETOH 95% 9.0 ml Tris-meleate buffer, 0.08M, pH 7.2	17	NaN_3; heat sections to 100 C for 5 min	Red precipitate at active sites	Oxidative coupling
Non-specific esterase	2.5 mg 5-bromo-4-chloroindoxylacetate 0.5 ml ETOH 95% 1.0 ml K Ferrocyanide, 0.01M 1.0 ml K Ferricyanide, 0.01M 0.4 ml Tris-maleate buffer, 0.2M, pH 7.0	9	Omit substrate; heat sections	Blue precipitate at active sites	Indigo dye formation
Alkaline phosphatase	5.0 mg Naphthol AS-TR phosphate 1.0 ml N,N′dimethylformamide 2.0 ml Veronal-HCl buffer, 0.1M, pH 9.2 1.0 ml Calcium chloride, 2% 0.4 ml Magnesium chloride, 2% 5.6 ml Distilled water . . . 2.0 ml Cobalt acetate, 2% . . . 5.0 ml Ammonium sulfide, 0.01%	45 1 3	Omit substrate; omit $CaCl_2$	Black-dk. brown precipitate at active sites	Cobalt sulfide precipitation
Acid phosphatase	5.0 mg Naphthol AS-TR phosphate 1.0 ml N,N′dimethylformamide 5.0 ml Veronal acetate buffer 1.6 ml Hexazotized pararosaniline 12.4 ml Distilled water	20	Omit substrate	Red-orange Precipitate at active sites	Azo coupling
Adenosine triphosphatase	4.0 ml Adenosine triphosphate, 0.01M 4.0 ml Tris-maleate buffer, 0.2M, pH 7.0 1.0 ml Lead nitrate, 4% 1.0 ml Magnesium nitrate, 2% . . . 5.0 ml Ammonium sulfide, 0.01%	25 3	Omit substrate; omit $Pb(NO_3)_2$; sulfhydryls	Black-dk. brown precipitate at active sites	Lead sulfide precipitation
Peroxidase	1.0 ml Benzidine•HCl, 0.05M 1.0 ml Tris-maleate buffer, 0.2M, pH 5.5 0.5 ml Hydrogen peroxide, 1% 0.5 ml Nickelous ammonium sulfide, 0.1M	1	Omit H_2O_2; omit benzidine; KCN	Blue-brown precipitate at active sites	Hydrogen-donor oxidation

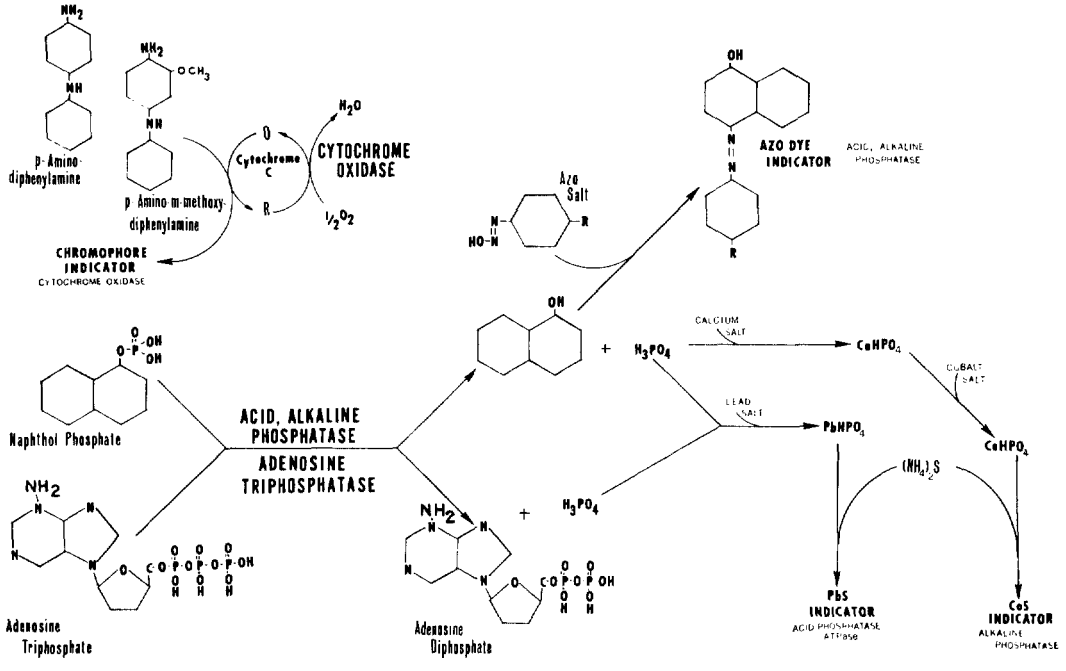


FIG. 1. Schematic illustrations of the localization reactions for cytochrome oxidase, acid phosphatase, alkaline phosphatase, and adenosine triphosphatase. Cytochrome oxidase is localized by a cyclic redox reaction with cytochrome-c (more probably the cytochrome complex). Oxidized cytochrome-c is reduced by acceptance of H from the diphenylamine bases. The oxidized diphenylamines then couple to produce a chromophore. The reduced cytochrome-c is oxidized by cytochrome oxidase, thus, regenerating the oxidized form of the cytochrome and allowing continued chromophore production. Acid phosphatase and alkaline phosphatase can hydrolyze naphthol phosphate substrates. The products of hydrolysis—naphthol and orthophosphate—can be used to produce a chromophore indicator of the enzyme hydrolyzing the substrate. The activities of the two enzymes are separated by the pH at which the reaction is carried out. To obtain contrast at the side of localization of the enzyme in the tissue section, the phosphate product is converted to a sulfide precipitate. Or, if the naphthol product is used for the localization, it is coupled to an azo salt to produce an azo dye chromophore. Adenosine triphosphatase is localized at neutral pH in a fashion similar to the sulfide precipitation procedure used for acid phosphatase. The substrate, however, must be ATP.

(iii) *Acid phosphatase Localization.*—A+P (see Fig. 1) was localized by a modification of the azo coupling technique developed by Burstone (4), and modified by Barka and Anderson (1) and Davis and Ornstein (5). The localization medium consisted of 8.0 mg naphthol AS-TR phosphate dissolved in 1.0 ml DMF. This was added slowly to a mixture of 5.0 ml veronal acetate [97.1 mg sodium acetate + 147.1 mg sodium barbital in 5.0 ml H_2O (1)]; 1.6 ml HApRA [0.8 ml 4% pararosanaline + 0.8 ml 4% sodium nitrate (4)]; distilled water to a final volume of 20.0 ml. The mixture was filtered through Whatman No. 1 paper and the pH of the filtrate was adjusted to 4.8 with 0.1 N HCl or 0.1 N NaOH. Root sections were incubated in the localization medium for 20–25 min at room temperature. Gomori type reactions (see 1) were also used to demonstrate A+P activity. With this type reaction β-glycerol phosphate, glucose-6-phosphate, and 3′adenosine monophosphate were used as the substrates, the latter was most suitable.

(iv) *Alkaline phosphatase Localization.*—The localization procedure for A^-P is a modification of that given by Jensen (9) and is essentially a Gomori cobalt sulfide precipitation method (see Fig. 1). The localization medium was prepared by dissolving 10.0 mg naphthol AS-TR phosphate in 1.0 ml DMF; 2.0 ml of 0.1 M veronal-HCl pH 9.2; 1.0 ml of 2% $CaCl_2$; 0.4 ml of 2% $MgCl_2$; and 5.6 ml H_2O. The mixture was filtered through Whatman No. 1 paper, and root sections were incubated in the filtrate for 45 min at ca. 20 C. After incubation the sections were washed in three changes of water (pH 8 to 9) and transferred into a 2% cobalt acetate solution for 1 min. The sections were again washed in three changes of water (pH 8 to 9) and transferred into ammonium sulfide, ca. 0.01%. With some sections, A^-P was demonstrated by a simultaneous azo coupling technique as described for A^+P and outlined in Fig. 1. This procedure was the same as that used for A^+P except that the A^-P localization medium was buffered at pH 9.2 with 0.1 M veronal-HCl.

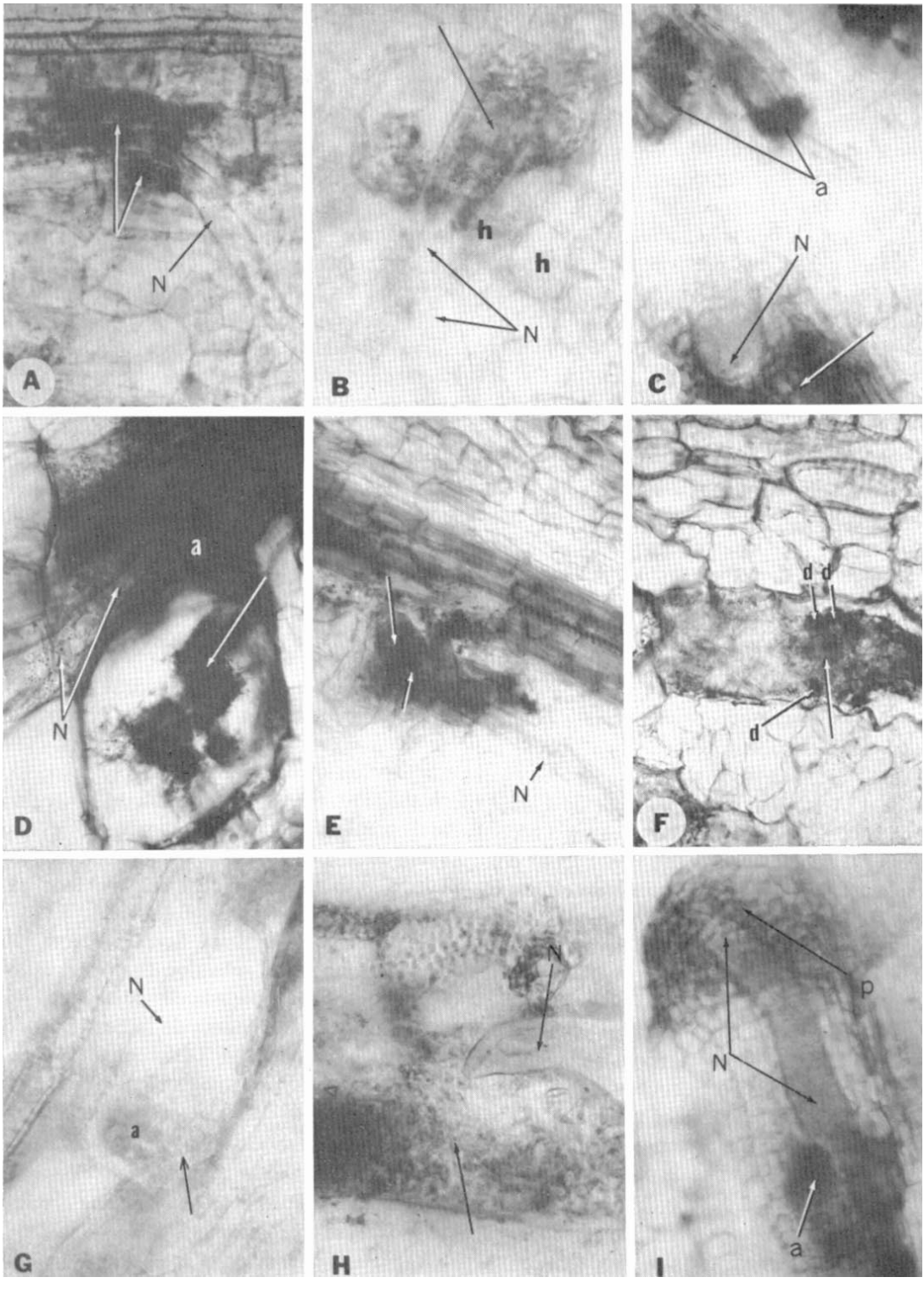
(v) *Adenosine triphosphatase Localization.*—The localization technique for ATPase is modeled after the system used by Gomori (see 12) to demonstrate A^+P (see Fig. 1) and is basically that of Wachstein and Meisel (see 1). The reaction (see Fig. 1) was conducted at pH 7 to 7.2 to minimize effects by A^+P or A^-P. The localization medium consisted of 4.0 ml of 0.01 M ATP; 4.0 ml of 0.2 M Tris-M pH 7.0; 1.0 ml of 4% $Pb(NO_3)_2$; and 1.0 ml of 1.0% $Mg(NO_3)_2$. Root sections were incubated in this localization medium for 25 min at room temperature. After incubation the root sections were washed in three changes of water, post-incubated for 1 min in ammonium sulfide (ca. 0.01%) and washed again for 10 min in tap water.

(vi) *Peroxidase Localization.*—The P localization technique is a modification of the method presented by Pearse (12). A schematic representation of the reaction was presented in an earlier report (14). The localization medium consisted of 2 volumes of 0.05 M benzidine·HCl; 2 volumes of 0.2 M Tris-M pH 5.5; 1 volume of 1.0% H_2O_2; and 1 volume of 0.1 M $NiSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$ (nickelous ammonium sulphate). The localization reaction was conducted in glass or porcelain spot plates. Generally the H_2O_2 was not added to the localization medium until the root sections had incubated for 5 min. The delay in adding the H_2O_2 served as a control. In some cases sections were placed in 4% formal-calcium for 30 min prior to conducting the localization reaction in a medium lacking nickelous ammonium sulphate. The slight formalin fixation served to stabilize the reaction at the benzidine blue stage. No adverse effect due to formalin fixation was detected in the P localization pattern of the control sections.

RESULTS

The 6 enzymes, histochemically localized, were most active at the feeding site of *M. incognita acrita* in 'Lee' soybean roots. The stimulation of activity was largely restricted to the host cells at the nematode lip region and paralleled both larval maturation and syncytium development. The results obtained with the individual enzyme localization procedures were as follows:

CYTOCHROME OXIDASE LOCALIZATION: In the early stages of infection the intensity of the chromophore in host cells adjacent to the feeding site of the nematode indicated a low degree of CO activity. Differential chromophore deposition in the vascular region of secondary roots was not readily detectable at 5 days after inoculation. However, by 11 days after inoculation, individual cells upon which the nematode had been feeding showed very intense chromophore deposition (Fig. 2A). Parenchyma cells at a slight distance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from the nematode lip region showed hypertrophy but low CO activity, whereas the cells closely associated with the lip region showed high activity (Fig. 2A, 2B). The stimulated CO activity, restricted to the syncytium, is shown 14 days after inoculation (Fig. 2B). A syncytial site in the vascular region had increased CO activity as a result of nematode feeding (Fig. 2C). At the posterior portion of the nematode, the host cells show high CO activity and hyperplasia. The stimulation of enzyme activity and hyperplasia of host cells at the posterior region of the nematode was initiated sometime prior to 18 days after inoculation; this is considerably earlier than previously reported (8).

ALKALINE PHOSPHATASE LOCALIZATION: Cobalt sulfide, used as the chromophore indicator of A^-P activity, appeared as black deposits in the central cytoplasm and walls of the syncytium. Figure 2D shows a nematode in a feeding position between a well defined syncytium and a group of cells with high A^-P activity; the group of cells has relatively the same level of A^-P activity as the syncytium, but it has not coalesced into a typical syncytium. The restricted nature of the stimulated activity of A^-P in the syncytium is shown in Fig. 2E, TF. The A^-P activity of the syncytium is many times that of the adjacent non-stimulated cells. Figure 2E shows the nematode oriented parallel to the long axis of the root. The greatest part of the syncytium is in the pith, but there is some extension into the xylem. Figure 2F shows an enlarged syncytium with an intense localization of chromophore. The nematode that induced the syncytium is not shown.

ACID PHOSPHATASE LOCALIZATION: Figure 2G shows a syncytium 6 days after inoculation. Abundant chromophore was localized in the syncytium near the nematode feeding site. Figure 2H shows a syncytium induced by the nematode 11 days after inoculation. The intense localization of the enzyme within the syncytium is indicated by the dark area. Figure 2I shows both the anterior and posterior regions of an enlarged larva. The syncytium at the anterior region of the nematode displayed high chromophore deposition. A nematode at this stage of development is usually associated with a much larger syncytium; probably the major portion of the syncytium is not shown but was contained in an adjacent section. In the host cells proximal to the posterior region of the same nematode, high A^+P activity and hyperplasia are prominent.

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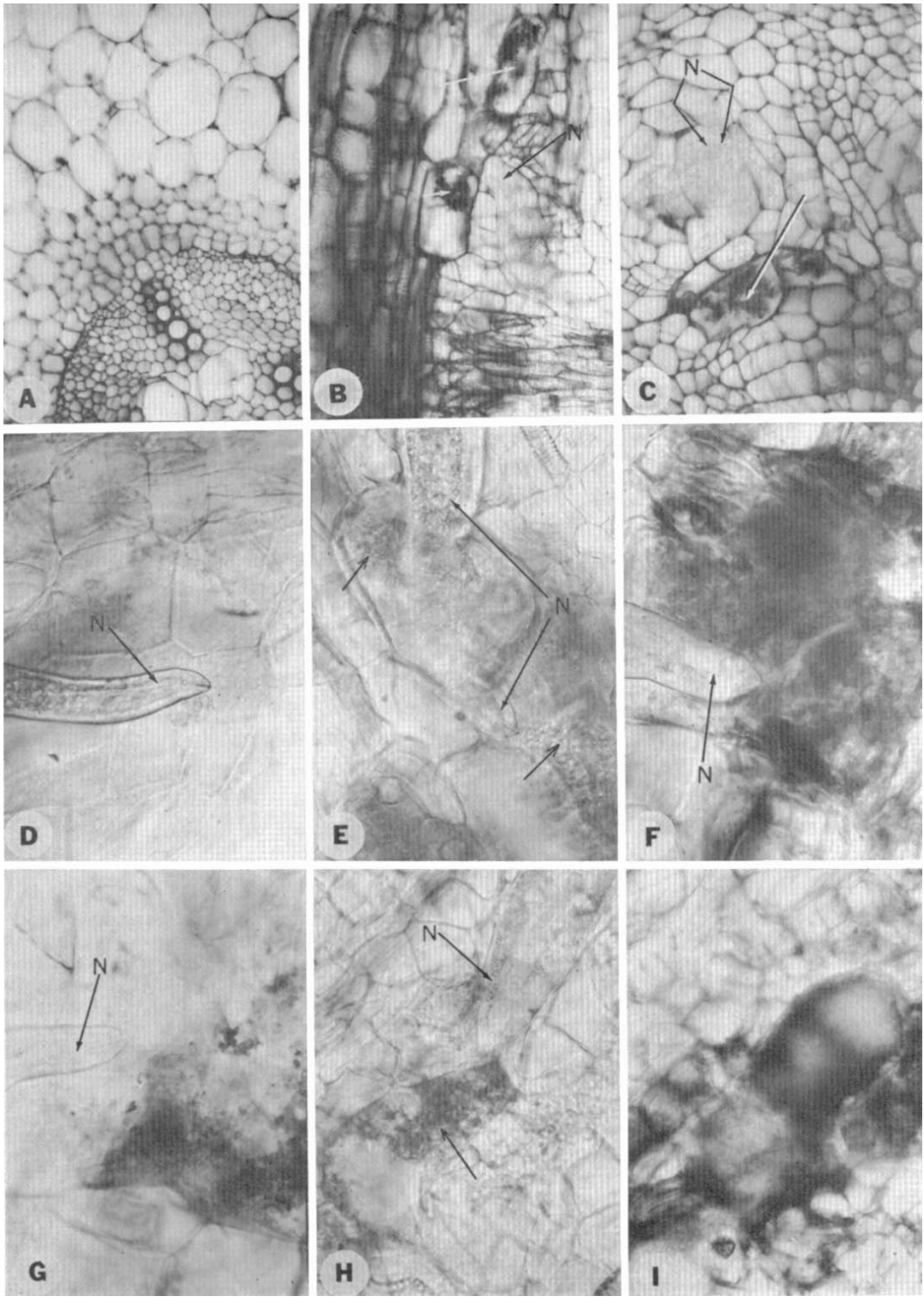
FIG. 2. The localization of cytochrome oxidase (A-C); alkaline phosphatase (D-F); acid phosphatase (G-I), in soybean (*Glycines max*) roots infected by the root-knot nematode (*Meloidogyne incognita acrita*). A. Sites of high activity (arrows) in cells around the lip region of the nematode (N), 11 days after inoculation. B. A syncytium (arrow), 14 days after inoculation, with some stimulation of activity. Note the hypertrophied parenchyma cells (h) a short distance from the nematode (N). C. Hyperplasia and high enzyme activity (arrow) in host cells at the posterior region of the nematode (N), observed 18 days after inoculation. Sites of high enzyme activity of the host cells in the stele at the anterior region of two larvae are also indicated (a). D. A nematode (N) adjacent to a syncytium (arrow) but feeding on a group of cells with high activity (a), 10 days after inoculation. E. A syncytium (arrow) and nematode (N), 10 days after inoculation. Note that the stimulation of activity is concentrated in the syncytium. F. An enlarged view of a syncytium (arrow), 16 days after inoculation. Note the granular nature of the cytoplasm and the highly restricted localization. The exceptionally dense sulfide deposit (d) may be artifacts due to product accumulation around nuclei. G. A syncytium (arrow), 6 days after inoculation. The activity (a) in the syncytium is highest near the stylet of the nematode (N). H. A syncytium (arrow), 11 days after inoculation, with fairly high activity. The nematode (N) is enlarged and is approaching the adult stage. I. The anterior region (a) and posterior region (p) of an advanced larval stage (N). The reaction in the posterior region shows moderate hyperplasia. The section was made 15 days after inoculation.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PEROXIDASE LOCALIZATION: Heavy deposits of the P reaction product (benzidine blue) were differentially accumulated in syncytia induced by the nematode. The nature and pattern of P localization in non-inoculated roots are shown in Fig. 3A. The highest P activity appeared to be appressed to cell walls, especially those located in the stele; the cortex and epidermis showed lower activity. After infection the cytoplasm of affected cells becomes very dense as described by Bird (2), and P localization in these cells becomes more granular in nature (Fig. 3B). Figure 3B represents a section 6 days after inoculation. A transverse section of a syncytium 16 days after inoculation is shown in Fig. 3C. General hypertrophy of stelar tissues was observed in the area where the nematode was located. The clusters of lipid-like droplets in the region of hypertrophy are the internal contents of the excised nematode.

NON-SPECIFIC ESTERASE LOCALIZATION: Prior to syncytium formation, while the larva was in the transitory phase, there was very little detectable stimulation of E activity. As late as 8 days after inoculation second stage larvae were observed in parenchymatous tissue (Fig. 3D). The slight enlargement of the larva indicates that it obtained some nourishment for growth; however, there was no indication that the activity of E was increased in the root tissue near the lip region of this nematode. Fig. 3E shows another root section with a syncytium 9 days after inoculation. Although the intensity of the chromophore is not at its peak during this stage of infection, the enzyme localization showed greater intensity than in Fig. 3D. There is only a one-day difference in the incubation periods for the infections shown in Fig. 3D and 3E. The difference in the stages of infection of these two sections is evident in the size of the larvae and the degree of host cell response. Consequently, it appears that the number of days after inoculation is not always an ideal parameter for judging the developmental stage of infection. Fig. 3F shows an extensive syncytium 23 days after inoculation. The activity of E is quite high throughout the syncytium.

ADENOSINE TRIPHOSPHATASE LOCALIZATION: ATPase is evident in host cells immediately adjacent to the lip region of a nematode 6 days after inoculation (Fig. 3G). The apparent polynuclear condition of the

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FIG. 3. The localization of peroxidase (A-C); esterase (D-F); adenosine triphosphatase (G-I), in soybean (*Glycines max*) roots infected by the root-knot nematode (*Meloidogyne incognita acrita*). A. A transverse section showing the sites of localization in a non-inoculated root. The endodermis and vascular parenchyma are high in enzyme activity; the sites of localization are appressed to the cell wall. B. An extensive syncytium (arrows) induced by a nematode (N) within 6 days after inoculation. Note the intense cytoplasmic localization in the infected cells. C. A transverse section through a root 16 days after inoculation. The nematode (N) was cut as a result of its orientation in the root. The syncytium (arrow) associated with the nematode is shown. D. A parasitic second stage larva (N) in parenchymatous tissue, 8 days after inoculation. There is very little activity associated with the host cells at the lip region of the nematode at this stage of infection. E. Nine days after inoculation an early stage of syncytial development was observed. The syncytium (arrows), induced by the nematode (N), showed increased enzyme activity. It is not likely that the one-day-longer incubation for disease development would account for the difference in the stage of infection (cf. Fig. 3D and 3E). F. The increased activity is quite apparent 23 days after inoculation. The nematode (N) has induced an extensive syncytium and is approaching maturity. G. An infection site 6 days after inoculation. The limits of the syncytium induced by the nematode (N) are not clearly defined; the stimulated activity, however, is apparent. H. Nematode (N) at feeding site, 13 days after inoculation. The syncytium (arrow) has high activity and a typical granular appearance. I. Intense localization was observed in sections 21 days after inoculation. Note the strict localization of activity within the confines of the syncytium.

affected area indicates that the cells are developing into a typical syncytium. Scattered islands of high ATPase activity within syncytia are shown in Fig. 3G, 3H, and 3I. As the nematode developed, 13 and 21 days after inoculation (Fig. 3H and 3I, respectively), the cytoplasm became more dense, and ATPase activity increased.

Discussion

In keeping with the practice established earlier, a brief discussion of the mechanisms of the localization reactions follow:

The histochemical localization of CO (see Fig. 1) was accomplished through the coupling of two diphenylamines which are oxidized by cytochrome-c. Because cytochrome-c is not abundant in the sections, it is doubtful that much chromophore could be produced if reduced cytochrome-c (reduced as a result of oxidation of the bases) were not returned to the oxidized state. Thus, reaction specificity is obtained because cytochrome-c is reozixized by CO. Dixon and Webb (6) point out that a number of cytochromes may be involved in producing the active oxidase; the localization reaction, however, would remain essentially the same. The degree of solubility of cytochrome-c lends itself to an excellent control reaction. By washing sections in saline prior to incubation in the localization medium, a large portion of the cytochrome-c is removed. Thus, when these sections are incubated in the localization medium, the rate of chromophore production is reduced. The velocity of the reaction can be restored, however, by adding cytochrome-c to the localization medium.

The histochemical localization of both A^+P and A^-P can be achieved using the same substrate and either of two localization procedures (see Fig. 1). The hydrolysis of napthol-AS-phosphates of both A^+P and A^-P yields an orthophosphate and a naphthol moiety, each of which can be used to localize the sites of the enzyme. The naphthol group can be simultaneously coupled to an azo group to produce an insoluble azo dye, or the orthophosphate can be precipitated with calcium (for A^-P) or lead (for A^+P) and be eventually converted to a black sulfide precipitate, which is easily noticed in contrast to the rest of the tissue.

Since poor coupling occurs at acid pH with most diazonium salts, hexazotized pararosaniline was used in the azo coupling technique for A^+P localization. In the sulfide precipitation technique calcium is incorporated into the A^-P localization medium to precipitate the phosphate product of hydrolysis. However, because calcium phosphate is soluble at acid pH, it was replaced by a salt that precipitates the phosphate at the pH required for A^+P activity. Therefore, when A^+P was localized by the sulfide precipitation technique, lead was incorporated into the localization medium. Since neither lead phosphate (for A^+P) nor calcium phosphate (for A^-P) are easily observed with a light microscope, they were replaced with black sulfides which provide greater contrast.

ATPase was localized by the sulfide precipitation method, using ATP as the substrate, and while holding the localization medium near neutrality. The neutral pH reduces the activity of both A^+P and A^-P while allowing ATPase to function.

The localization of E was achieved through the enzymatic hydrolysis of a substrate (substituted indoxyl acetate) to a leuco dye (indoxyl). The leuco dye is immediately oxidized to the chromophore dye (indigo) by ferricyanide. The supply of oxidant (ferricyanide) is maintained by incorporating equimolar volumes of ferricyanide and ferrocyanide in the localization medium.

The localization procedure for P has been discussed previously (14).

The root-knot nematode *M. incognita*

acrita is an obligate parasite and needs a living host to fulfill its nutritional requirements and complete its life cycle. Therefore, changes in the nature of the plant tissues infected by the nematode affect the development of the parasite. The induction of syncytia by nematodes is, therefore, a vital aspect of this host-parasite relationship. Bird (2) recently demonstrated the close interdependence of the nematode and the syncytium it induces. A comparable relationship exists between Lee soybeans and *M. incognita acrita* larvae; *i.e.*, larvae maturation paralleled syncytial development.

The cause of increased enzyme activity within the syncytium may be attributed to the probing of the nematode stylet into host cells, or, to the secretion of products through the nematode stylet into the syncytium. The histochemical localization of specific factors which induce or inhibit this host-parasite response would be helpful in further elucidating the physiology of root-knot infections. Since root-knot larvae readily penetrate both susceptible and resistant plant roots (7), the physiological response of the resistant plant could affect the inhibition of syncytial development. Histological studies of resistant plants infected by syncytia-inducing nematodes show that resistant plants are not induced to produce syncytia to the same extent as are susceptible plants (7). A histochemical study of the response of soybean plants resistant to the root-knot nematode should help in determining some of the relationships between host and parasite.

The microscopic histochemical approach to this problem confers an advantage not available through standard biochemical techniques. With the application of enzyme histochemical procedures we have demonstrated that nematode stimulation of certain enzymes in host cells occurs predominately within the syncytium. Noticeable increase in enzyme activity were not observed outside the confines of the syncytium. Although the results were not quantitative, chromophore production per unit of time indicates that the stimulation of enzyme activity within syncytia was considerable.

Histochemical techniques provide a way of observing host-enzyme responses in living tissues and cells closely related to the site of nematode action. From this vantage point, it would be interesting to compare enzyme alteration in infected-resistant and infected-susceptible hosts. While information is available on morphological (7) and biochemical (11) changes that occur in resistant plants invaded by endoparasitic nematodes, little work has been done with a technique that combines the two disciplines. Since the anatomical area that is biochemically affected by such nematode infections may be quite small, it is imperative that histochemical techniques be employed, lest the effects of infection be lost by dilution from non-affected cells.

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