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Lectin Binding Sites on the Amphidial Exudates of *Meloidogyne*[1]

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Abstract: Lectin binding sites on the surface of *Meloidogyne incognita* Races 1, 2, 3, and 4; *M. javanica*; *M. arenaria* Races 1 and 2; and *M. hapla* Races A and B were determined with lectins conjugated to fluorescein isothiocyanate or colloidal gold. The amphidial exudate, which was demonstrated histochemically to contain carbohydrate, was the principal binding site. Some lectins also bound to the external cuticular surface. Species and race specific binding patterns were observed for both amphidial and cuticular binding sites.

Key words: amphid, electron microscopy, exudate, lectin, ultrastructure.

Lectin binding sites have been characterized on the outer cuticular surface (3,4,10,18) and amphidial exudates (3) of several plant-parasitic and free-living nematodes. In some cases, differences in binding site patterns are race specific as well as species specific (3). Differences in lectin binding site patterns between nematode developmental stages also have been noted (17). Two functions have been suggested for the amphids and (or) amphidial secretions: 1) They may play a role in chemotaxis and host recognition (6,16), and 2) they may elicit phytoalexins in resistant hosts (3). Application of lectins to soil infested with *Meloidogyne incognita* Race 3 reduced galling of tomato plants (7). Thus, a better understanding of the physical and chemical properties of nematode neurosecretory products is needed. Our objectives were 1) to determine if differences in lectin binding sites occur among the common species and races of *Meloidogyne* and 2) to demonstrate histochemically the glycosidic nature of the amphidial secretions. Cuticular binding of lectins was also examined.

MATERIALS AND METHODS

Lectins were obtained from E-Y Laboratories (127 N. Amphlett Blvd., San Mateo, CA 94401). *Canavalia ensiformis* agglutinin (Con A), *Triticum vulgaris* agglutinin (WGA), *Arachis hypogaea* agglutinin (PNA), *Glycine max* agglutinin (SBA), *Maclura pomifera* agglutinin (MPA), *Ulex europaeus* agglutinin-I (UEA), *Griffonia (Bandeiraea) simplicifolia* agglutinins (GS I and GS II), *Ricinus communis* agglutinin (RCA), *Dolichos biflorus* agglutinin (DBA), and *Limulus polyhemus* agglutinin (LPA) were supplied as conjugates of fluorescein isothiocyanate (FITC). UEA was also obtained as a complex with colloidal gold (mean particle diameter, 20 μm). Lectin-FITC conjugates, except those of Con A and LPA, were diluted to a concentration of 0.1 mg/ml with 0.01 M phosphate buffered saline (PBS), pH 7.2–7.4. Con A was diluted to a concentration of 0.1 mg/ml with 0.015 M Tris, pH 7.0, containing 0.015 M NaCl, 0.001 M $CaCl_2$ and 0.001 M $MnCl_2$. LPA was diluted to 0.1mg/ml with 0.05 M Tris, pH 8.0, containing 0.01 M $CaCl_2$ and 0.15 M NaCl. UEA-gold was used without dilution at a concentration of 0.018 mg/ml.

Egg masses of *Meloidogyne* populations (2) were obtained from J. N. Sasser, Department of Plant Pathology, North Carolina State University, and propagated on eggplant, *Solanum melongena* cv. Black Beauty, in the greenhouse. Eggs were collected from infected plants (8) and hatched (1) to obtain infective, second-stage juveniles (J2). Prior to testing, J2 were rinsed with distilled water and concentrated, after settling, by decanting excess water.

Nematodes were centrifuged at 200 *g* in 1.5-ml microcentrifuge tubes. Supernatant was aspirated and the pellet of nematodes,

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containing 1,000–2,000 specimens, was resuspended in 0.2 ml of the appropriate lectin preparation. Controls consisted of untreated nematodes and parallel blocking treatments similar to those described above except that the buffer used for diluting the lectin contained 200 μM lectin-specific saccharide. Because blocking of lectin-binding activity is seldom complete and partial blocking is difficult to measure by fluorescence microscopy, only the following lectins and their specific saccharides were selected for blocking experiments: WGA (chitobiose), Con A (α-methyl mannose), UEA (fucose), MPA (D-galactose-*N*-acetylgalactosamine), and GS II (*N*-acetylglucosamine).

After incubation for 90 minutes in blocked and unblocked lectins, treated specimens were rinsed three times with the buffer used for dilution of the lectin and prepared for light and electron microscopy. Live nematodes treated with FITC-lectins were mounted under coverslips on glass microscope slides and examined by incident fluorescence microscopy with an excitation wavelength of 450–490 nm and a barrier filter of 520 nm. Nematodes for scanning electron microscopy (SEM) were fixed, dehydrated, and observed by methods described previously (10). Specimens for transmission electron microscopy were fixed for 90 minutes at 21–24 C in 0.06 M phosphate buffer, pH 7.3, containing 3% glutaraldehyde, and then decapitated in the same solution. Severed heads were transferred to processing chambers (9) where they were rinsed three times with PBS, postfixed for 1 hour at 21–24 C with 1% osmium tetroxide, dehydrated in acetone (10% increments), and infiltrated with low viscosity resin (13) in acetone (10% increments). Heads in undiluted resin were cast between two glass microscope slides (12) and cured at 60 C for 18 hours; those whose amphids were labeled with colloidal gold were selected for sectioning by examination at 400× with transmitted-light, brightfield microscopy. Silver sections of selected heads were mounted on uncoated 200-mesh copper grids, stained for 20 minutes with 2% uranyl acetate, poststained with Reynold's lead citrate, and photographed on a Hitachi H-500 TEM at 75 kV.

Carbohydrate constituents of amphidial exudate were demonstrated by the periodic acid-Schiff's (PAS) reaction (5). Infective J2 of *M. incognita* Race 3 in processing chambers (9) were fixed for 72 hours in 3% glutaraldehyde in 0.06 M phosphate buffer, pH 7.3, rinsed with 100 volumes of distilled water over 30 minutes, and incubated for 15 minutes in 0.5% periodic acid. They were then rinsed for 30 minutes in 100 volumes of distilled water, stained for 15 minutes in Schiff's reagent, rinsed for 10 minutes in 50 volumes of distilled water, and placed in 2% sodium bisulfite for 2 minutes. After a final rinse in distilled water, they were mounted on glass microscope slides for observation and photomicroscopy. Controls consisted of nematodes similarly treated but without exposure to periodic acid.

Results

Two principal lectin binding sites were observed when J2 of *Meloidogyne* spp. were treated with FITC-conjugated lectins: the external cuticle and the amphids and (or) their secretions (Table 1). All lectins except LPA bound to the amphidial exudate of at least one species, whereas detectable cuticular binding occurred only with Con-A (all populations except *M. incognita* Race 1 and *M. hapla* Race B), SBA (*M. hapla* Race B), and UEA (*M. incognita* Races 1 and 2, *M. arenaria* Races 1 and 2, and *M. javanica*).

At high magnification, the amphidial exudate appeared as a mass of small globular secretions (Fig. 1) which completely filled the amphidial pouch (Fig. 2D, E).

Lectins bound to amphidial exudate generally were confined to the external surface of the exudate (Fig. 2A, D, E), although the label occasionally was detected deep within the amphidial ducts of specimens treated with FITC-WGA (not shown). Densely packed material (Fig. 2E) would not be penetrated by gold-labeled lectin and perhaps FITC-labeled lectin. Lectins bound to the cuticle chiefly in the incisures.

Blocking experiments with single lectin-

TABLE 1. FITC-lectin labeling of *Meloidogyne* species.

Lectin, site	Inc1	Inc2	Inc3	Inc4	Aren1	Aren2	Jav	HapA	HapB
Con A									
Cuticle	–	+	++	+	+	+	+	+	–
Amphids	+	+	+	+	+	+	+	+	+
WGA									
Cuticle	–	–	–	–	–	–	–	–	–
Amphids	+++	+	+++	++	+++	+++	+++	+++	+++
PNA									
Cuticle	–	–	–	–	–	–	–	–	–
Amphids	–	–	–	–	–	+	–	+	–
SBA									
Cuticle	–	–	–	–	–	–	–	–	+
Amphids	–	–	+	–	+	+	+++	+	–
MPA									
Cuticle	–	–	–	–	–	–	–	–	–
Amphids	+++	+++	+++	+++	+++	+++	+++	+	+
UEA									
Cuticle	++	++	–	–	+	+	+	–	–
Amphids	+++	+++	+++	+++	+++	+++	+++	–	+++
GS I									
Cuticle	–	–	–	–	–	–	–	–	–
Amphids	–	–	+	+	–	–	++	+	+
GS II									
Cuticle	–	–	–	–	–	–	–	–	–
Amphids	–	–	–	–	–	–	–	+++	–
RCA									
Cuticle	–	–	–	–	–	–	–	–	–
Amphids	+++	+++	+++	+++	+++	+++	+++	+++	+++
DBA									
Cuticle	–	–	–	–	–	–	–	–	–
Amphids	–	–	–	–	–	–	+	+	–
LPA									
Cuticle	–	–	–	–	–	–	–	–	–
Amphids	–	–	–	–	–	–	–	–	–

Inc1 = *M. incognita* Race 1. Inc2 = *M. incognita* Race 2. Inc3 = *M. incognita* Race 3. Inc4 = *M. incognita* Race 4. Aren1 = *M. arenaria* Race 1. Aren2 = *M. arenaria* Race 2. Jav = *M. javanica*. HapA = *M. hapla* Race A. HapB = *M. hapla* Race B. + = detectable labeling (fluorescence). +++ = intense labeling. ++ = labeling intensity intermediate between + and +++. – = no detectable labeling.

specific saccharides were inconclusive and difficult to interpret. Reduction of amphidial labeling in *M. incognita* Race 3 was complete or nearly so (80–90% reduction in labeling frequency and reduced labeling intensity) on specimens treated with RCA, Con A, WGA, and the lectin-specific saccharide. Addition of specific saccharides had little noticeable effect on the frequency or intensity of UEA, GS II, and MPA labeling.

Schiff's reagent reacted positively with amphidial exudate (Fig. 2C) and the regions of the amphidial ducts (Fig. 2B) and glands (Fig. 2C). Faint staining of the rectal gland also occurred.

DISCUSSION

Evidence supporting the concept that surface carbohydrates play a crucial role in nematode chemotaxis and host–parasite interaction has been reviewed (16). Citing the occurrence of lectin binding sites on the heads of many nematodes and inhibi-

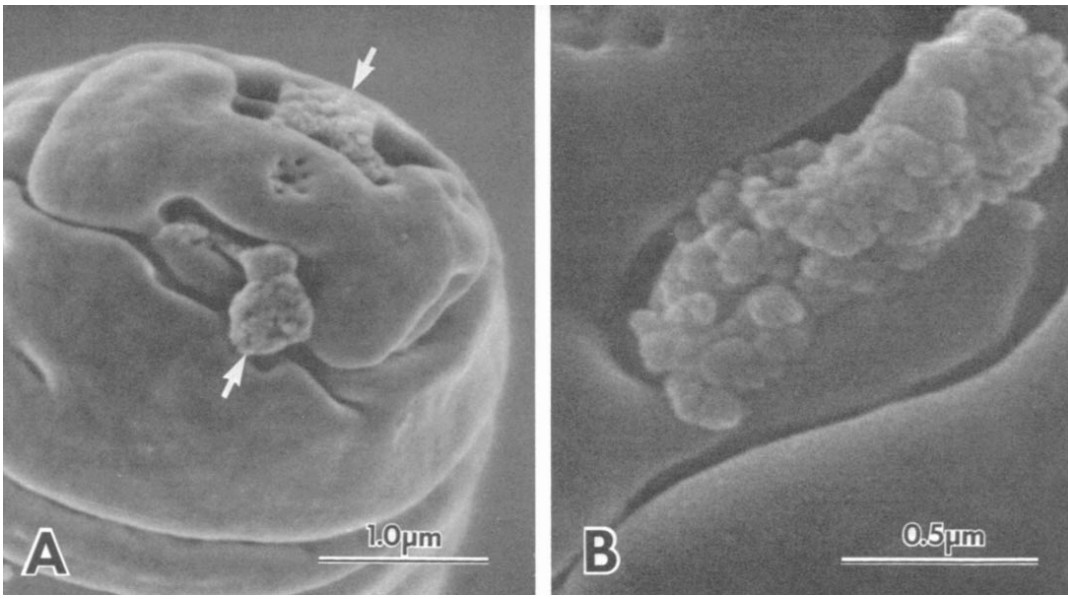

FIG. 1. Scanning electron micrographs of the heads of *Meloidogyne incognita* Race 3 infective juveniles. A) Amphidial exudate (arrows) emanating from the amphidial apertures. B) Details of the exudate showing aggregates of globules.

tion of host finding by Con A-treated *Caenorhabditis elegans,* Zuckerman (15) has proposed that the potential exists for highly specific control measures by intervention in nematode chemoresponses.

Our results demonstrate that binding of lectins to the head of *Meloidogyne* is concentrated in the region of the amphids or on the amphidial exudate. Species and races differed qualitatively in lectin binding ability. In some cases, races of a single species could be distinguished on the basis of their reaction to certain lectins. Since these taxa also differ in host preference, *Meloidogyne* species could provide a model system for future studies on host plant resistance and chemoresponses in nematodes.

It has been suggested, for example, that saccharide residues on the heads of nematodes may elicit phytoalexins (3) or inhibit elicitors of phytoalexins (14). If such is the case, production of phytoalexins should differ in response to infection by different species or races of *Meloidogyne.* The model could be broadened by including other members of the Heteroderidae such as *Globodera* species. Amphidial exudates of *G. rostochiensis* and *G. pallida* do not bind UEA (3), which, in our studies, was bound to the exudates of all species of *Meloidogyne* tested.

Binding sites of lectins have been localized with precision on *Globodera* with ferritin (3) and *Meloidogyne* with colloidal gold (this study). Penetration of these lectin-conjugated markers into the amphidial ducts is poor, however, and it is not known if, in addition to the amphidial exudate, binding sites occur within the amphid itself. We have observed FITC-lectin conjugates in the amphidial ducts up to 7 μm

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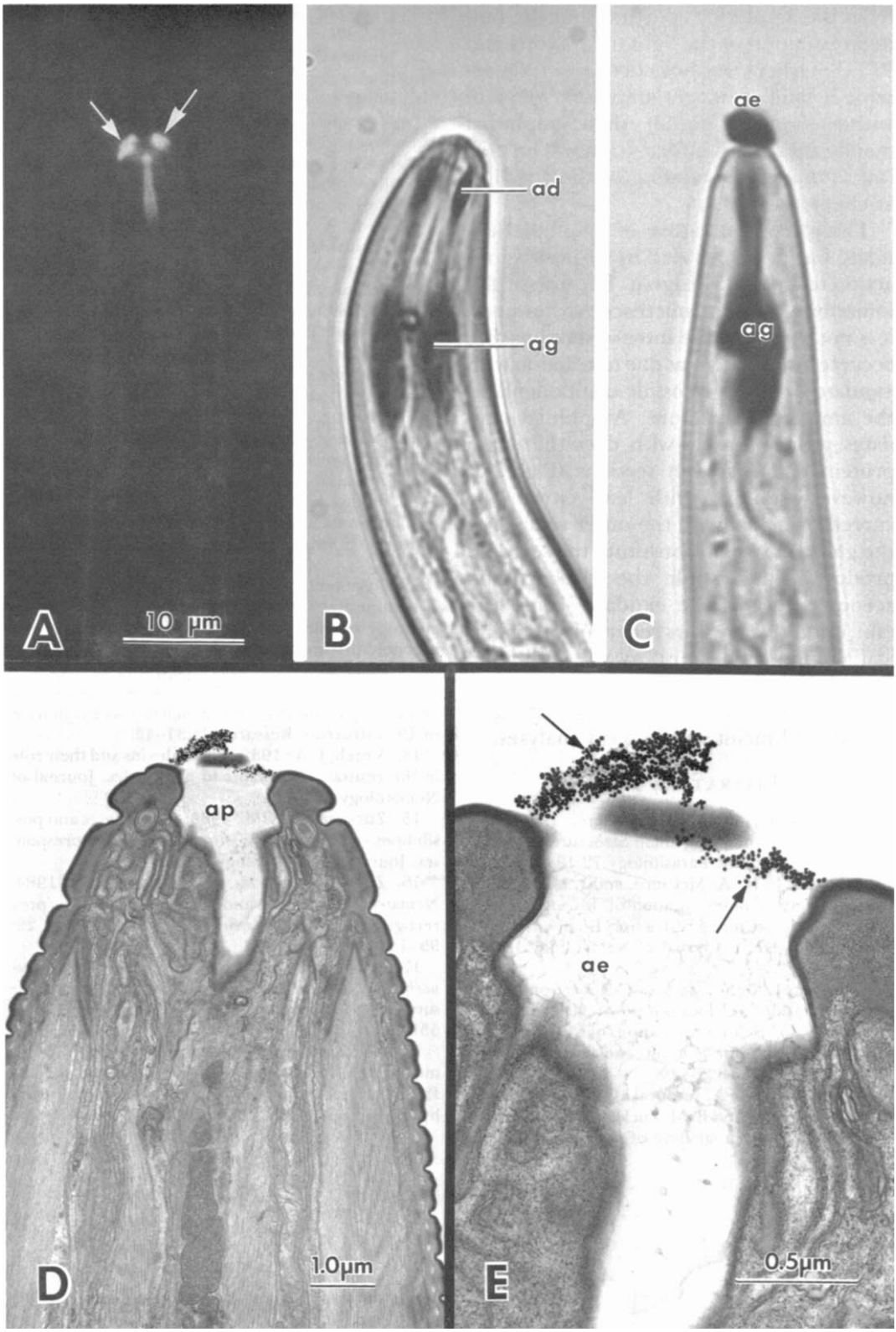
FIG. 2. Anterior ends of *Meloidogyne incognita* Race 3 infective juveniles. A) UV fluorescence of UEA-fluorescein isothiocyanate conjugate bound to amphidial exudate (arrows). Anterior end of stylet is autofluorescent. B, C) Juveniles treated with periodic acid-Schiff's reagent showing PAS-positive amphidial exudate (ae), amphidial ducts (ad), and amphidial glands (ag). B) Dorsal view. C) Lateral view. D, E) Transmission electron micrographs of parasagittal sections through the amphidial pouch (ap) of a specimen treated with UEA-colloidal gold, which is restricted to the extruded portions (arrows) of the amphidial exudate (ae).


A
10 µm
B
ad
ag
C
ae
ag
D
ap
1.0µm
E
ae
0.5µm

from the amphidial aperture, but the limited resolution of the light microscope and FITC markers was not adequate to determine if binding was to amphidial exudate within the duct rather than amphidial membranes. Such information will be critical to our understanding of the amphids as chemoreceptors.

The glycosidic nature of amphidial exudate was demonstrated by its positive reaction to the PAS reagent. Because of the limitations of light microscopy, however, it is not known if the intense staining that occurred internally was due to reaction with exudate or with glycosidic components of the amphidial structure. Amphidial exudates stain intensely with dyes that stain proteins (11). In thin sections (Fig. 2E), however, staining with lead citrate occurred principally on the outer surface of the globules, which constitute the secreted product. Thus, while the glycoproteinaceous nature of the exudate seems certain, definitive answers regarding its chemical composition must await traditional analytical approaches. Recently reported means for stimulating amphidial secretion (11) should facilitate chemical analyses.

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