Journal of Nematology 20(4):609–619. 1988.

Characterization of Carbohydrates on the Surface of Second-stage Juveniles of *Meloidogyne* spp.[1]

E. L. DAVIS,[2] D. T. KAPLAN,[2] T. A. PERMAR,[2]
D. W. DICKSON,[3] AND D. J. MITCHELL[4]

Abstract: Fluorescent conjugates of the lectins soybean agglutinin (SBA), Concanavalin A (Con A), wheat germ agglutinin (WGA), *Lotus tetragonolobus* agglutinin (LOT), and *Limulus polyphemus* agglutinin (LPA) bound primarily to amphidial openings and amphidial secretions of viable, preinfective second-stage juveniles (J2) of *Meloidogyne incognita* races 1 and 3 (Mi1, Mi3) and *M. javanica* (Mj). No substantial difference in fluorescent lectin binding was observed among the populations examined. Binding of only LOT and LPA were inhibited in the presence of 0.1 M competitive sugar. Structural differences in amphidial carbohydrate complexes among populations of Mi1, Mi3, and Mj were revealed by glycohydrolase treatment of preinfective J2 and subsequent labeling with fluorescent lectins.

A quantitative microfiltration enzyme-linked lectin assay revealed previously undetected differences in lectin binding to nonglycohydrolase-treated J2. Preinfective J2 of Mj bound the greatest amount of SBA, LOT, and WGA, whereas J2 of Mi1 bound the most LPA.

Key words: enzyme-linked lectin, fluorescent lectin, glycohydrolase, lectin, *Meloidogyne incognita*, *Meloidogyne javanica*, microfilter plate, recognition, root-knot nematode, specificity.

The importance of surface carbohydrate biochemistry in recognition and specificity between plants and micro-organisms has been the subject of many recent investigations and several discussions (2,5,7). Although few investigations concerning this phenomenon have been conducted between nematodes and plants, surface carbohydrates of nematodes have been implicated in recognition between nematodes and nematophagous fungi (12,35). Surface carbohydrates of some animal-parasitic helminths have been characterized and related to antigenicity and chemoresponse (4,25). The involvement of surface carbohydrate recognition in the specificity of interaction between nematodes and *Pasteuria penetrans* has also been investigated (34).

Carbohydrates on biological surfaces exist primarily as glycoconjugates such as glycolipids, polysaccharides, and especially as glycoproteins (23). The carbohydrate residues often consist of monosaccharide molecules covalently linked in various sequences and spatial arrangements (17). Surface carbohydrate accessibility to potential receptors on other surfaces or as receptors of chemostimuli may be obscured by attached carbohydrate molecules, as sometimes occurs with sialic acids in animal systems (30). Enzymatic or inorganic chemical degradation may reveal these "masked" carbohydrates. Conversely, enzymes (glycohydrolases) that cleave specific carbohydrate residues from glycoconjugates can remove carbohydrates from biological surfaces and potentially alter biological interactions. An example of this latter phenomenon is the apparent loss of chemosensory perception of *Escherichia coli* culture filtrates by the nematodes *Caenorhabditis elegans* and *Panagrellus redivivus* after treatment of these nematodes with mannosidase or sialidase (13).

Lectins, proteins that bind to specific carbohydrate residues, are excellent probes for surface carbohydrates (7,18). Several methods, including lectin probes, have been used to characterize surface carbohydrates of a number of free-living and plant-parasitic nematodes (12,35). Application of lec-

Received for publication 4 April 1988.

[1] Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

[2] U.S. Department of Agriculture, Agricultural Research Service, 2120 Camden Road, Orlando, FL 32803.

[3] Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

[4] Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

We thank Drs. A. M. Golden, J. G. Baldwin, and J. D. Eisenback, for their assistance in identification of *Meloidogyne* spp.

tins to soil infested with *Meloidogyne incognita* (Kofoid and White) Chitwood reduced the number of nematode-induced galls on tomato roots, but the function of lectins was unclear (19).

Several studies have attempted to relate nematode surface carbohydrates to specificity in plant pathogenicity (6,8,21,28). Differences in binding of fluorescent lectins to pathotypes of *Globodera* spp. and *Meloidogyne* spp. were reported (6,8,21). Lectins labeled with hemocyanin and with tritium have been used to quantify relative amounts of carbohydrates on the surface of nematodes and bacteria, respectively (20,26). The difficulty in production and handling of radiolabeled lectins and the sophisticated equipment required to observe hemocyanin conjugates on the nematode surface limit the practicality of these methods. We have adapted a microfiltration enzyme-linked lectin assay (22,24) to quantify the amount of lectin that binds to second-stage juveniles (J2) of *Meloidogyne* spp. The objective of this investigation was to characterize the surface carbohydrates of preinfective J2 of three Florida populations of *Meloidogyne* using selected lectins and glycohydrolases.

Materials and Methods

Populations of *Meloidogyne incognita* races 1 and 3 (Mi1 and Mi3) and *M. javanica* (Treub) Chitwood (Mj) were maintained in greenhouse culture on roots of tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) and eggplant (*Solanum melongena* L. cv. Black Beauty). *Meloidogyne* spp. populations were identified by female perineal patterns, J2 lengths, and differential plant hosts (29). Species identifications were also confirmed by three independent nematode taxonomists. Eggs were collected from host roots with 0.53% NaOCl for 30 seconds (11) and hatched at room temperature on a Baermann funnel. Preinfective J2 that had hatched within 48 hours were used as test organisms in each experiment. A few eggs were present in each suspension of J2.

Surface carbohydrates of preinfective J2: Fluorescent lectin probes were used to identify and locate carbohydrates on the surface of preinfective J2 of Mi1, Mi3, and Mj. Tetramethylrhodamine isothiocyanate (TRITC) conjugates of soybean agglutinin (SBA), wheat germ agglutinin (WGA), Concanavalin A (Con A), *Lotus tetragonolobus* agglutinin (LOT), and *Limulus polyphemus* agglutinin (LPA) were used (E-Y Labs, San Mateo, CA). The ratios of absorbance at 550 to 280 nm were 0.44 for SBA, 0.55 for WGA, 0.41 for Con A, 0.57 for LOT, and 0.20 for LPA. The specific (competitive) sugars to which each lectin binds are listed in Table 1.

Approximately 1,000 J2 of each population were suspended in distilled water for a control treatment. The remaining J2 were concentrated into 2.0 ml of the appropriate buffer by centrifugation at 1,000 *g* for 3 minutes. Buffer solutions included 0.01 M phosphate-buffered saline (PBS) at pH 7.2 for SBA, WGA, and LOT; 0.05 M Tris-saline plus 0.01 M $CaCl_2$ at pH 7.5 for Con A; and 0.05 M Tris-saline plus 0.01 M $CaCl_2$ at pH 8.0 for LPA. Approximately 5,000 J2 of each population were incubated in lectin–TRITC conjugate (200 μg/ml) for 2 hours at 4 C. Additional treatments included ca. 5,000 J2 incubated in lectin-TRITC plus 0.1 M corresponding competitive sugar (Table 1) to inhibit lectin binding, J2 incubated in 0.1 M sugar plus buffer, J2 incubated in buffer minus sugar, and J2 incubated in unconjugated TRITC solution. Treated J2 were transferred three times to microcentrifuge tubes containing fresh buffer or water and allowed to settle to the bottom of the tube. About 500 J2 in final wash solution were placed on a glass microscope slide and covered with a cover glass. The edges of the cover glass were sealed with clear fingernail polish. Approximately 50 specimens from each treatment were immediately observed at 1,000× under a Zeiss epifluorescence microscope equipped with a TRITC filter. When nematode movement ceased, ca. 1–3 hours after J2 were mounted on slides, photographs of selected nematodes were taken. Each test was repeated twice.

Surface carbohydrates of glycohydrolase-

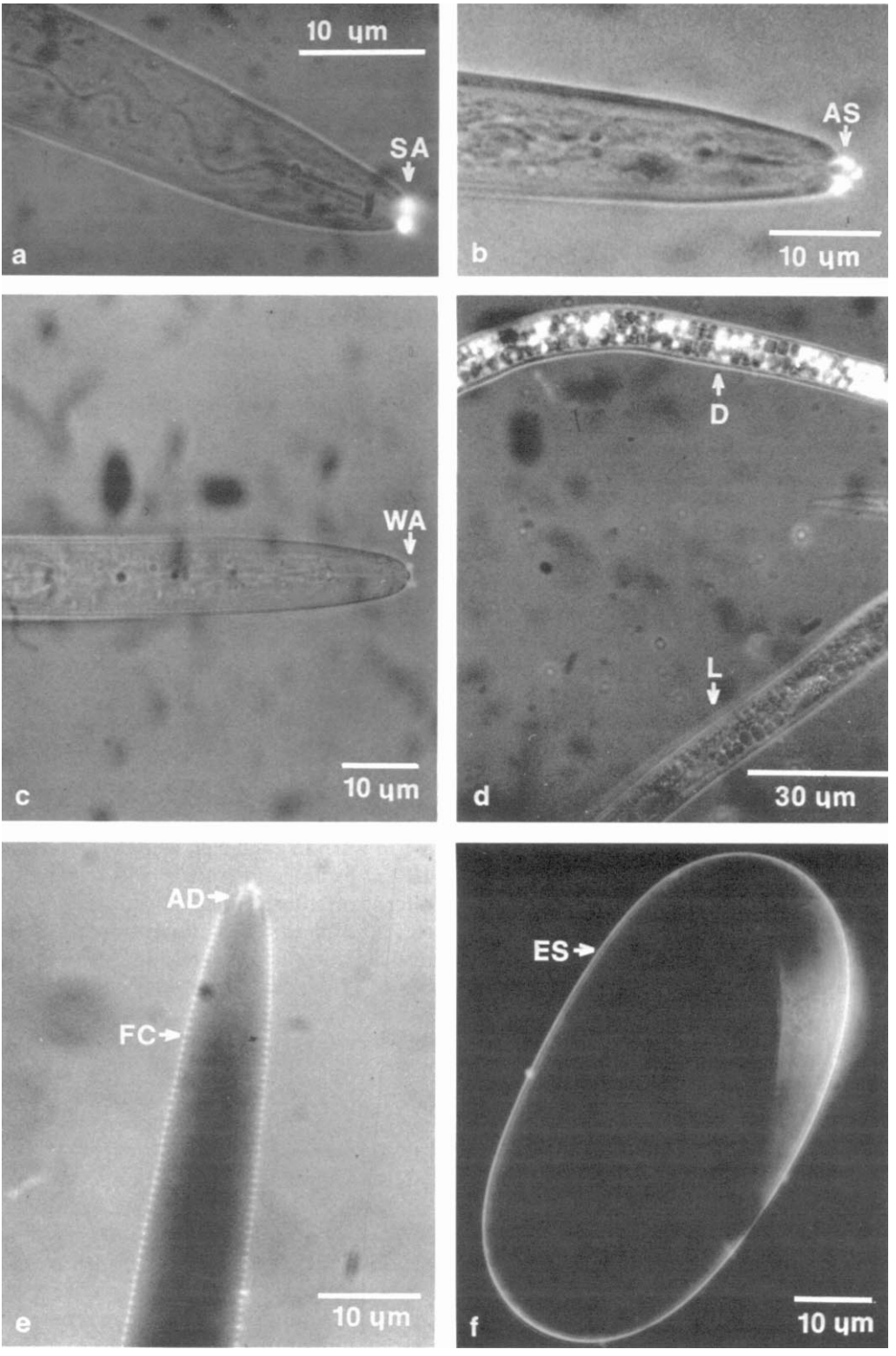
treated preinfective J2: Glycohydrolases were assayed for their effect on the surface carbohydrates of Mi1, Mi3, and Mj. The glycohydrolases tested consisted of the following: α-galactosidase (α-gal) EC 3.2.1.22 from recombinant *E. coli,* α-L-fucosidase (α-fuc) EC 3.2.1.51 and β-N-acetyl-glucosaminidase (β-glu) EC 3.2.1.30 from beef kidney, α-mannosidase (α-man) EC 3.2.1.24 from *Canavalia ensiformis,* and neuraminidase (sialidase) EC 3.2.1.18 from *Clostridium perfringens*; each was obtained from, and assayed by, Boehringer Mannheim Biochemicals, Indianapolis, Indiana.

Preinfective J2 were concentrated into 2.0 ml of the appropriate buffer by centrifugation at 1,000 *g* for 3 minutes. Buffer solutions included 0.01 M phosphate buffer (pH 7.2) for α-gal, 0.05 M sodium citrate buffer (pH 5.0) for α-fuc, 0.05 M sodium citrate buffer (pH 4.5) for β-glu, 0.05 M sodium citrate buffer plus 1.0 mM $ZnSO_4$ (pH 4.5) for α-man, and 5.0 mM sodium acetate buffer plus 72.0 mM NaCl and 7.0 mM $CaCl_2$ (pH 5.0) for neuraminidase. Enzyme buffers were formulated at the pH for optimum enzyme activity indicated by the manufacturer. Approximately 5,000 J2 of each population were incubated in either α-gal (1.0 U/ml), α-fuc (0.25 U/ml), β-glu (1.0 U/ml), α-man (1.0 U/ml), or neuraminidase (0.25 U/ml) solution for 18 hours at 37 C. Nematodes were also incubated in enzyme plus 0.1 M corresponding competitive sugar (Table 1) to inhibit enzyme activity. Control treatments consisted of J2 in buffer alone and J2 in buffer plus 0.1 M sugar at 37 C for 18 hours. Nematode viability after treatment with enzyme buffers under experimental conditions was confirmed by bioassay (6). Glycohydrolase-treated nematodes were washed three times with the appropriate lectin buffer, treated with separate lectin–TRITC conjugates, and observed under epifluorescence microscopy as described for nonglycohydrolase-treated J2.

Quantification of lectin binding to preinfective J2: The quantity of lectin binding to preinfective J2 of Mi1, Mi3, and Mj was determined using a modified enzyme-linked lectin assay (22). The modification was the incorporation of microfiltration procedures (24) to rinse excess lectin from nematode surfaces and reduce background readings. Lectins conjugated with horseradish peroxidase (HRP) were purchased from E-Y Labs; they included SBA, Con A, WGA, LOT, and LPA. The sugar specificity and corresponding buffer solutions were identical to those for lectin–TRITC assays.

Ninety-six well microfilter plates (SV-96, Millipore Corp., Bedford, MA) with a 5-μm pore size were incubated with 200 μl 1.0% bovine serum albumin (BSA) in PBS per well at 37 C for 2 hours. These plates were washed three times with PBS on a microfiltration apparatus (Millipore Corp.) before use in the following assay.

Preinfective J2 were incubated in 500 μl lectin–HRP solution (200 μg/ml) for 2 hours at 4 C. Control treatments included untreated J2 in buffer and 500 μl lectin solution (200 μg/ml) minus J2 (lectin wash). Five 100-μl aliquots from each treatment (ca. 10,000 J2 suspended in lectin solution) were placed in separate wells on a microfilter plate. Each well was washed five times with the appropriate lectin buffer on a microfiltration apparatus. The treated J2 or lectin wash in each well were suspended in 100 μl buffer and transferred to separate microcentrifuge tubes. Buffer was added to 250 μl, and 50 μl suspension was withdrawn from each tube to quantify the number of J2 per 50-μl sample. Four 50-μl suspensions of treated J2, untreated J2, and lectin wash were transferred from each tube to separate wells on a fresh 96-well microfilter plate. One hundred microliters of peroxidase substrate ([2,2′-azino-di-(3-ethylbenzthiazoline)sulfonic acid] ABTS) (Sigma Chemical Co., St. Louis, MO) was added to each well (31). After 30 minutes at room temperature in the dark, the solution from each well was transferred to corresponding wells on a 96-well EIA (enzyme immunoassay) plate using a microfiltration apparatus to remove J2. Absorbance (414 nm) of solution in each well was determined on an automated microplate


10 µm
SA
a
AS
b
10 µm
WA
c
10 µm
D
L
d
30 µm
AD
FC
e
10 µm
ES
f
10 µm

reader (Model EL309, Bio-Tek Instruments, Winooski, VT). Twenty separate absorbance values were determined for each treatment combination.

Absorbance values were compared with the linear portion of a standard curve prepared for each lectin–HRP conjugate. Standard curves were prepared by diluting (1:1, v/v) 50-μl volumes of lectin–HRP solution across a 96-well EIA plate and adding 100 μl ABTS solution per well. The quantity of lectin that adsorbed to a single J2 was determined by dividing the observed lectin–HRP value by the number of J2 estimated for that sample (ca. 500–2,000 J2). Each test was repeated once.

Hemagglutination assays: The relative binding capacity of each lectin–TRITC and lectin–HRP conjugate was determined through hemagglutination assay (26). Twenty-five-microliter volumes of lectin were serially diluted (1:1) with the appropriate buffer across a 96-well microtiter plate. Twenty-five microliters of a 4% suspension of trypsinized, gluteraldehyde-stabilized human type O red blood cells (Sigma Chemical Co.) was added to each well, except for wells containing LPA. A 4% suspension of gluteraldehyde-stabilized horse red blood cells (Sigma Chemical Co.) was used for LPA assays. The greatest dilution of lectin which exhibited visible hemagglutination (titer) was determined after 3 hours' incubation at room temperature. The titer divided by the lectin concentration of each sample was a measure of the specific hemagglutination activity of each lectin conjugate. Similar tests were conducted in the presence of 0.1 M competitive sugar to assess inhibition of lectin binding activity.

Results

Hemagglutination tests indicated that the binding capacity of all lectin–TRITC conjugates except LPA was relatively strong. Specific hemagglutination activities of 1,024 (SBA), 512 (Con A), 4,096 (WGA), 2,048 (LOT), and 16 (LPA) units/mg lectin were determined. Hemagglutination activity of all lectin–TRITC conjugates was completely inhibited in the presence of 0.1 M corresponding competitive sugar.

Viable, preinfective J2 were labeled with lectin–TRITC almost exclusively in the vicinity of the amphidial openings (Fig. 1a–c). At times fluorescent lectin labeling extended outward from these openings, suggesting that carbohydrates occur within amphidial secretions. Binding of fluorescent lectins to any other portions of the nematode surface was rarely observed, except as indicated for several glycohydrolase treatments. Nematodes that were apparently nonviable often exhibited strong labeling of the stylet, esophageal lumen, and especially the gut region after exposure to lectin–TRITC conjugates (Fig. 1d). No labeling of viable J2 with unconjugated TRITC was observed.

Few differences in fluorescent lectin labeling were observed among untreated J2 of the *Meloidogyne* spp. tested (Table 1). Amphids of Mi1, Mi3, and Mj labeled weakly with SBA, Con A, and LPA and strongly with WGA and LOT. No binding of TRITC-conjugated *Limax flavus* agglutinin (LFA, sialic acid-specific) to J2 was

←

FIG. 1. Binding of fluorescent (rhodamine) lectin conjugates to *Meloidogyne* spp. second-stage juveniles (J2) and egg. a) Strong amphidial (SA) fluorescence of *M. incognita* race 3 (Mi3) labeled with *Lotus tetragonolobus* agglutinin. b) Binding of wheat germ agglutinin (WGA) to amphidial secretions (AS) of *M. javanica* (Mj) after α-galactosidase treatment. c) Weak amphidial fluorescence (WA) of *M. incognita* race 1 (Mi1) labeled with *Limulus polyphemus* agglutinin. d) Fluorescence of dead (D) vs. living (L) Mi1 J2 after soybean agglutinin treatment. e) Fluorescent cuticle (FC; note annulation) and amphidial ducts (AD) of Mj labeled with Concanavalin A after J2 exposure to α-galactosidase. f) Binding of WGA to egg shell (ES) of Mi3. (Note: Incandescent light provided to enhance J2 image in a–d results in artifactual cuticular glow. True labeling of J2 cuticle by fluorescent lectin is presented in e.)

TABLE 1. Binding of fluorescent lectins to preinfective second-stage juveniles of *Meloidogyne incognita* races 1 and 3 (Mi1, Mi3), and *M. javanica* (Mj) incubated in lectin solution ± competitive sugar.

Lectin†	Competitive sugar	Mi1		Mi3		Mj	
		− Sug	+ Sug	− Sug	+ Sug	− Sug	+ Sug
SBA	D-galactose	++	++	+	+	++	++
WGA	N-acetyl-D-glucosamine	+++	+++	+++	+++	++++	++++
Con A	D-mannose	++	++	++	++	++	++
LOT	L-fucose	+++	NF	+++	NF	+++	NF
LPA	N-acetylneuraminic (sialic) acid	++	NF	++	NF	++	NF

Epifluorescent microscope observations: + = very weak amphidial fluorescence; ++ = weak amphidial fluorescence; +++ = strong amphidial fluorescence; ++++ = very strong amphidial fluorescence; NF = no fluorescence.

† Soybean agglutinin (SBA), wheat germ agglutinin (WGA), Concanavalin A (Con A), *Lotus tetragonolobus* agglutinin (LOT), and *Limulus polyphemus* agglutinin (LPA).

observed in preliminary tests (unpubl.), and binding of LPA–TRITC to J2 was not observed until viewed with an improved fluorescence microscope light source (50-watt mercury lamp; Carl Zeiss, West Germany). Inhibition of lectin binding in the presence of the appropriate competitive sugar was observed only for LOT and LPA. All lectins tested bound to egg shells of Mi1, Mi3, and Mj (Fig. 1f), and binding was not inhibited in the presence of corresponding competitive sugar.

Differences in lectin labeling among the populations of *Meloidogyne* tested were observed after J2 were treated with various glycohydrolases (Table 2). Lectin labeling of J2 treated with glycohydrolases was compared to labeling of J2 incubated in enzyme buffer minus glycohydrolase. In most cases, enzyme activity was inhibited in the presence of the appropriate competitive sugar, except where indicated.

Treatment of J2 with α-gal eliminated binding of SBA–TRITC to the amphids of Mj and Mi1, but not to amphids of Mi3. Binding of LOT–TRITC to the amphids of Mj was reduced by treatment of J2 with α-gal. The cuticle on the anterior half of the body of Mj and Mi1 labeled weakly with Con A–TRITC, with fluorescence of body annulation growing weaker from head to midbody (Fig. 1e). In Mj, enzyme activity was not inhibited in the presence of 0.1 M D-galactose. Similar cuticular labeling was not observed for J2 of Mi3 treated with α-gal; however, binding of Con A–TRITC to the amphids of Mi3 was eliminated. Binding of LPA–TRITC to the amphids of J2 was unchanged on Mj, increased on Mi1, and eliminated on Mi3 after treatment with α-gal.

Treatment of J2 of Mi1 with β-glu reduced binding of WGA–TRITC to amphids and promoted binding of WGA–TRITC to the anterior cuticle of Mi1. Binding of LPA–TRITC to the amphids of J2 of Mj and Mi1 was eliminated by β-glu treatment; however, binding of LPA–TRITC to the amphids of Mi3 increased after β-glu treatment.

Binding of SBA–TRITC to the amphids of Mi1 and LPA–TRITC to the amphids of Mj, Mi1, and Mi3 was eliminated by α-man. Binding of LOT–TRITC to the amphids of Mj was eliminated by treatment of J2 with α-man, but α-man activity was not inhibited by 0.1 M mannose. Amphids of Mj and Mi3 did not label with Con A–TRITC after α-man treatment, but Con A–TRITC did bind to the anterior cuticle of Mi1 after treatment with α-man buffer plus or minus enzyme.

After treatment of J2 with α-fuc, binding of LOT–TRITC to the amphids of Mj and Mi1 was reduced and binding of LPA–TRITC to amphids of Mj, Mi1, and Mi3 was eliminated. Weak labeling of the anterior cuticle of Mi1 with Con A–TRITC and LPA–TRITC occurred after incubation of J2 in α-fuc buffer with or without the enzyme.

Treatment of J2 with neuraminidase

TABLE 2. Binding of fluorescent lectins to preinfective second-stage juveniles of *Meloidogyne incognita* races 1 and 3 (Mi1, Mi3) and *M. javanica* (Mj) after incubation in glycohydrolase buffer ± glycohydrolase.

Enzyme and Lectin‡	Mi1		Mi3		Mj	
	−Enz	+Enz	−Enz	+ Enz	−Enz	+Enz
α-galactosidase						
SBA	++	NF	+	+	++	NF
WGA	+++	+++	+++	+++	+++	+++
Con A	++	++, WC	++	NF	++	++, WC
LOT	+++	+++	+++	+++	+++	++
LPA	+	+++	++	NF	+	+
β-N-acetyl-glucosaminidase						
SBA	++	++	+	+	+	+
WGA	+++	++, WC	+++	+++	+++	+++
Con A	++	++	+	+	++	++
LOT	+++	+++	+++	+++	+++	+++
LPA	++	NF	NF	++	++	NF
α-mannosidase						
SBA	++	NF	++	++	++	++
WGA	+++	+++	+++	+++	+++	+++
Con A	++, WC	++, WC	++	NF	++	NF
LOT	+++	+++	++	++	+++	NF
LPA	++	NF	++	NF	++	NF
α-L-fucosidase						
SBA	++	++	+++	+++	++	++
WGA	+++	+++	+++	+++	+++	+++
Con A	++, WC	++, WC	++	++	+++	+++
LOT	+++	++	+++	+++	+++	+
LPA	+++, WC	WC	++	NF	++	NF
Neuraminidase						
SBA	NF	NF	+	+	+	+
WGA	+++	+++	+++	+++	+++	+++
Con A	++, WC	++, WC	+++	++	++, WC	+++, WC
LOT	+++	+++	+++	++	+++	+
LPA	++	NF	++	++	++	NF

Epifluorescent microscope observations: + = very weak amphidial fluorescence; ++ = weak amphidial fluorescence; +++ = strong amphidial fluorescence; ++++ = very strong amphidial fluorescence; NF = no fluorescence; WC = weak fluorescence of J2 cuticle along anterior half of body.

† See Table 1 for key to abbreviations.

partially inhibited binding of LOT–TRITC to amphids of Mj and Mi3 and completely inhibited binding of LPA–TRITC to amphids of Mj and Mi1. Neuraminidase treatment increased binding of Con A–TRITC to amphids of Mj but reduced binding of Con A–TRITC to amphids of Mi3. The anterior cuticle of Mj and Mi1 labeled weakly with Con A–TRITC after incubation in neuraminidase buffer with or without enzyme.

Hemagglutination tests indicated that the binding capacity of all lectin–HRP conjugates except LPA was relatively strong. Specific hemagglutination activities of 512 (SBA), 512 (Con A), 4,096 (WGA), 2,048 (LOT), and 8 (LPA) units/mg lectin were determined. Hemagglutination activity of all lectin–HRP conjugates was completely inhibited in the presence of 0.1 M corresponding competitive sugar.

To reduce background to negligible levels, we found that it was critical to incubate the first plate in BSA and transfer the treated J2 to a clean microfilter plate before addition of peroxidase substrate. The reduction in background levels allowed the detection of differential amounts

TABLE 3. Binding of peroxidase-labeled lectins to second-stage juveniles (J2) of *Meloidogyne incognita* races 1 and 3 (Mi1, Mi3) and *M. javanica* (Mj), as determined by microfiltration assay in two separate experiments.

Lectin‡	Picograms lectin per J2†		
	Mj	Mi1	Mi3
SBA			
Exp. 1	4.20 ± 0.35	1.36 ± 0.07	0.63 ± 0.03
Exp. 2	3.63 ± 0.21	1.30 ± 0.09	0.55 ± 0.03
Con A			
Exp. 1	0.66 ± 0.04	0.81 ± 0.03	0.62 ± 0.04
Exp. 2	1.04 ± 0.07	0.86 ± 0.06	0.70 ± 0.04
LOT			
Exp. 1	9.07 ± 1.01	4.79 ± 0.35	4.40 ± 0.49
Exp. 2	6.18 ± 0.43	4.17 ± 0.15	4.33 ± 0.13
WGA			
Exp. 1	3.42 ± 0.25	0.39 ± 0.11	0.21 ± 0.03
Exp. 2	1.92 ± 0.10	0.78 ± 0.07	0.54 ± 0.07
LPA			
Exp. 1	1.18 ± 0.05	2.31 ± 0.07	0.65 ± 0.05
Exp. 2	1.25 ± 0.07	3.13 ± 0.13	0.83 ± 0.05

Mean of 20 observations ± standard error.
† Picograms lectin divided by the number of J2 (ca. 500–2,000 J2) estimated for each sample.
‡ See Table 1 for key to abbreviations.

of lectin in microplate wells that contained lectin-treated nematodes. No peroxidase activity above background levels was detected among untreated J2 and lectin wash treatments.

About 10,000 J2 per microplate well were required at the initiation of each experiment because as many as 75% of the J2 per well remained in the first microfilter plate after transfer of washed nematodes to a fresh microfilter plate for peroxidase substrate reaction. No J2 were observed in the wash solution that had passed through the first microfilter plate. Thus, the number of nematodes in each sample was determined when washed nematodes were transferred to the second microfilter plate.

Approximately 500–2,000 J2 per well were used to quantify the amount of lectin bound to nematodes after addition of peroxidase substrate. Lectins were most likely bound to the surface of the J2 examined because microscopic observation of lectin-treated J2 from samples transferred to peroxidase substrate reaction plates indicated that J2 were intact and viable. Different amounts of lectin bound to J2 of Mi1, Mi3, and Mj (Table 3). Preinfective J2 of Mj bound more SBA, LOT, and WGA than did J2 of Mi1 or Mi3, and preinfective J2 of Mi1 bound more LPA than did J2 of Mj and Mi3 in two experiments. Populations of Mi3 bound less lectin than J2 of Mi1 and Mj in all tests except the LOT experiments. Preinfective J2 of Mi1 bound the most Con A in experiment 1, and J2 of Mj bound the most Con A in experiment 2. Considerable differences in the relative amount of lectin that bound to J2 within a single lectin-nematode combination were detected between experiment 1 and experiment 2.

DISCUSSION

Fluorescein isothiocyanate (FITC)–lectin conjugates were not used in fluorescence assays because untreated J2 of the *Meloidogyne* populations strongly autofluoresced at the excitation wavelength of FITC. Difficulty with autofluorescence of *C. elegans* and *P. redivivus* at the excitation wavelength of FITC has also been reported (14). Lectins conjugated with rhodamine (TRITC) fluorophors were more appropriate for our study of lectin binding to nematodes because untreated J2 did not autofluoresce when viewed through the TRITC microscope filter.

It is apparent from our observations that nematode viability is critical for true labeling of nematode surfaces with fluorescent lectins. Living (motile) J2 bound fluorescent lectin almost exclusively in the vicinity of the amphidial openings. The entire body of nonviable nematodes fluoresced after TRITC–lectin treatment, especially within the gut region. The fluorescence of dead J2 was similar to the enzymatically induced fluorescence of dead nematodes reported by Bird (3). This phenomenon may have influenced fluorescent observation of sialyl residues over the entire body of J2 of *M. javanica* as reported by Spiegel et al. (32). In our studies, labeling of sialyl residues with fluorescent LPA was relatively weak and confined to

the amphidial region of viable J2 of *Meloidogyne*. Because the specific hemagglutination activity and absorbance ratio (550 nm : 280 nm) of LPA–TRITC was relatively low compared with the other lectins tested, it may be possible that more sialic acid exists on the J2 surface than can be detected with fluorescent lectin probes. Lectin binding to the tail region of *M. incognita* has also been reported (20), but we did not observe this. The binding of fluorescent lectins to egg shells of *Meloidogyne* spp. in our investigations has been reported for eggs of *M. javanica* (33).

Only the binding of LOT and LPA to specific sugars on J2 were substantiated by competitive sugar inhibition. Soybean agglutinin, Con A, and WGA bind to other molecular forms of galactose, mannose, and N-acetylglucosamine, respectively, and WGA has multiple carbohydrate binding sites (9). It is possible that the affinity of SBA, Con A, and WGA for carbohydrate-specific sites near amphidial openings was too strong to be inhibited by the competitive sugar solutions used in our assays. However, the binding of SBA, Con A, and WGA to amphidial secretions of J2 of *Meloidogyne* spp. may result from binding of these lectins to hydrophobic ligands (possibly lipids), as reported elsewhere (27). Incubation of J2 in fluorescent SBA, Con A, or WGA in the presence of 1,8-anilinonaphthalenesulfonic acid plus or minus competitive sugar may confirm the presence of hydrophobic binding because the hydrophobic and carbohydrate binding sites are independent of each other (27). The direct binding of unconjugated TRITC to surface lipids of J2 was not apparent in our assays because J2 did not fluoresce after incubation in unconjugated TRITC.

The greater intensity of fluorescent labeling by LOT and WGA conjugates may be due to their relatively higher binding capacities. Lack of differential lectin labeling among nonglycohydrolase-treated, preinfective J2 of Mi1, Mi3, and Mj makes it difficult to extrapolate a potential role of constitutive surface carbohydrates in the specificity of pathogenicity (16). Lectin binding substances were concentrated, however, and sometimes emanated from the amphidial region of J2 of *Meloidogyne*; this binding has also been reported for invasive juveniles of pathotypes of potato-cyst nematode and other populations of *Meloidogyne* (8,21). Because the head region is the portion of the nematode body around which some postinfectional, incompatible plant responses occur (10,15), it may be possible that carbohydrates in amphidial secretions of postinfectional J2 affect plant–nematode interactions.

Differences in lectin binding among populations of *Meloidogyne* that were not detected in our assays with fluorescent lectins were detected by microfiltration enzyme-linked lectin assay. Variability in estimation of the number of J2 per sample most likely influenced the quantitative differences in lectin binding to J2 determined among Mi1, Mi3, and Mj and between experiments 1 and 2. If this were a major influence, however, standard errors should have been greater than those calculated for each experiment. The quantitative differences in lectin binding detected in these experiments could have been due to the production of carbohydrates in amphidial secretions of J2 of *Meloidogyne* as reported here and elsewhere (21). Differences between experiments 1 and 2 may be due to the handling and relative age of the groups of J2 used in separate experiments. The rate of production of amphidial secretions and the amount of amphidial secretion (if any) lost through the initial centrifugation or microfiltration wash remains unknown. Loss of excess amphidial secretion was noted in preliminary lectin–TRITC assays when centrifugation was used for all nematode washes, but this occurrence was inconsistent (unpubl.).

Results of experiments involving glycohydrolases suggested that carbohydrates located in the amphidial region of J2 may occur in complexes and that these complexes may be structurally different among *Meloidogyne* populations. The inability of enzymes to alter carbohydrate residues on

some nematode surfaces may be a reflection of the substrate specificity exhibited by glycohydrolases (1). Enzyme treatment did reveal cuticular carbohydrates (especially mannose, glucose, or both) on the anterior half of some J2, and sialyl residues were often removed from J2 amphids by a number of different glycohydrolases. This may indicate that sialic acids are some of the outermost residues present in amphidial carbohydrate complexes of J2 of *Meloidogyne*. Whether surface carbohydrate changes similar to those reported for glycohydrolase treatments occur once *Meloidogyne* J2 enter plant roots is unknown. Alterations in the structure of carbohydrate complexes or relative quantity of specific carbohydrates in amphidial secretions of *Meloidogyne* J2 may influence nematode host-finding and recognition of invasive J2 by receptors in specific plant genotypes.

Literature Cited

1. Agrawal, K. M. L., and O. P. Bahl. 1972. α-galactosidase, β-galactosidase, β-glucosidase, β-N-acetylglucosaminidase, and α-mannosidase from pinto beans (*Phaseolus vulgaris*). Methods in Enzymology B 28:720–728.

2. Albersheim, P., and A. J. Anderson-Prouty. 1975. Carbohydrates, proteins, cell surfaces and the biochemistry of pathogenesis. Annual Review of Plant Physiology 26:31–52.

3. Bird, A. F. 1979. A method of distinguishing between living and dead nematodes by enzymatically induced fluorescence. Journal of Nematology 11:103–105.

4. Bone, L. W., and K. P. Bottjer. 1985. Cuticular carbohydrates of three nematode species and chemoreception by *Trichostrongylus colubriformis*. Journal of Parasitology 71:235–238.

5. Daly, J. M. 1984. The role of recognition in plant diseases. Annual Review of Phytopathology 26: 31–52.

6. Davis, E. L. 1988. The interactions of surface carbohydrates of *Meloidogyne* spp. with soybean roots. Ph.D. dissertation, University of Florida, Gainesville.

7. Etzler, M. E. 1985. Plant lectins: Molecular and biological aspects. Annual Review of Plant Physiology 36:209–234.

8. Forrest, J. M. S., and W. M. Robertson. 1986. Characterization and localization of saccharides on the head region of four populations of the potato cyst nematode *Globodera rostochiensis* and *G. pallida*. Journal of Nematology 18:23–26.

9. Goldstein, I. J., and R. D. Poretz. 1986. Isolation, physicochemical characterization, and carbohydrate-binding specificity of lectins. Pp. 33–244 *in* I. E. Liener, N. Sharon, and I. J. Goldstein, eds. The lectins: Properties, functions, and applications in biology and medicine. New York: Academic Press.

10. Huang, J. S., and K. R. Barker. 1986. Radioimmunoassay of glyceollin I in soybean roots infected with Race 1 of *Heterodera glycines*. Phytopathology 76:1139 (Abstr.).

11. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025–1028.

12. Jansson, H.-B. 1987. Receptors and recognition in nematodes. Pp. 153–158 *in* J. A. Veech and D. W. Dickson, eds. Vistas on nematology. Society of Nematologists.

13. Jansson, H.-B., A. Jeyaprakash, R. A. Damon, Jr., and B. M. Zuckerman. 1984. *Caenorhabditis elegans* and *Panagrellus redivivus*: Enzyme-mediated modification of chemotaxis. Experimental Parasitology 58:270–277.

14. Jansson, H.-B., A. Jeyaprakash, G. C. Coles, N. Marban-Mendoza, and B. M. Zuckerman. 1986. Fluorescent and ferritin labelling of cuticle surface carbohydrates of *Caenorhabditis elegans* and *Panagrellus redivivus*. Journal of Nematology 18:570–574.

15. Kaplan, D. T., I. J. Thomason, and S. D. Van Gundy. 1979. Histological studies of the compatible and incompatible interaction of soybeans and *Meloidogyne incognita*. Journal of Nematology 11:338–343.

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

17. Kornfeld, R., and S. Kornfeld. 1970. The structure of a phytohemagglutinin receptor site from human erythrocytes. Journal of Biological Chemistry 245:2536–2545.

18. Lis, H., and N. Sharon. 1986. Applications of lectins. Pp. 293–370 *in* I. E. Liener, N. Sharon, and I. J. Goldstein, eds. The lectins: Properties, functions, and applications in biology and medicine. New York: Academic Press.

19. Marban-Mendoza, N., A. Jeyaprakash, H.-B. Jansson, R. A. Damon, Jr., and B. M. Zuckerman. 1987. Control of root-knot nematodes on tomato by lectins. Journal of Nematology 19:331–335.

20. McClure, M. A., and B. M. Zuckerman. 1982. Localization of cuticular binding sites of Concanavalin A on *Caenorhabditis elegans* and *Meloidogyne incognita*. Journal of Nematology 14:39–44.

21. McClure, M. A., and B. A. Stynes. 1988. Lectin binding sites on the amphidial exudates of *Meloidogyne*. Journal of Nematology 20:321–326.

22. McCoy, J. P., Jr., J. Varani, and I. J. Goldstein. 1983. Enzyme-linked lectin assay (ELLA): Use of alkaline phosphatase-conjugated *Griffonia simplicifolia* B_4 isolectin for the detection of α-galactopyranosyl end groups. Analytical Biochemistry 130:437–444.

23. Monsigny, M., C. Kieda, and A.-C. Roche. 1983. Membrane glycoproteins, glycolipids, and membrane lectins as recognition signals in normal and malignant cells. Biology of the Cell 47:95–110.

24. Permar, T. A., and T. R. Gottwald. 1988. Rec-

ognition of *Xanthomonas campestris* pv. *citri* Florida nursery isolate using a monoclonal antibody probe in a microfiltration EIA. Phytopathology, in press.

25. Philipp, M., and F. D. Rumjaneck. 1984. Antigenic and dynamic properties of helminth surface structures. Molecular and Biochemical Parasitology 10:245–268.

26. Pueppke, S. G., T. G. Freund, B. C. Schulz, and H. P. Friedman. 1980. Interaction of lectins from soybean and peanut with rhizobia that nodulate soybean, peanut, or both plants. Canadian Journal of Microbiology 26:1489–1497.

27. Roberts, D. D., and I. J. Goldstein. 1983. Binding of hydrophobic ligands to plant lectins: Titration with arylaminonaphthalenesulfonates. Archives of Biochemistry and Biophysics 224:479–484.

28. Robertson, W. M., Y. Spiegel, H.-B. Jansson, N. Marban-Mendoza, and B. M. Zuckerman. 1988. Surface carbohydrates of plant parasitic nematodes. Nematologica, in press.

29. Sasser, J. N., and C. C. Carter. 1985. Overview of the International *Meloidogyne* Project 1975–1984. Pp. 19–24 *in* J. N. Sasser and C. C. Carter, eds. An advanced treatise on *Meloidogyne*, vol. I. Biology and control. Raleigh: North Carolina State University Graphics.

30. Schauer, R. 1982. Chemistry, metabolism, and biological functions of sialic acids. Advances in Carbohydrate Chemistry and Biochemistry 40:131–234.

31. Snyder, M. L., and G. A. Erickson. 1981. Recommended minimum standards for an enzyme-linked immunosorbent assay (ELISA) in pseudorabies serodiagnosis. Publication of the National Veterinary Services Laboratories, USDA APHIS, Ames, Iowa.

32. Spiegel, Y., E. Cohn, and S. Spiegel. 1982. Characterization of sialyl and galactosyl residues on the body wall of different plant parasitic nematodes. Journal of Nematology 14:33–39.

33. Spiegel, Y., and E. Cohn. 1982. Lectin binding to *Meloidogyne javanica* eggs. Journal of Nematology 14:406–407.

34. Stirling, G. R., A. F. Bird, and A. B. Cakurs. 1986. Attachment of *Pasteuria penetrans* spores to the cuticles of root-knot nematodes. Revue de Nematologie 9:251–260.

35. Zuckerman, B. M., and H. B. Jansson. 1984. Nematode chemotaxis and possible mechanisms of host/prey recognition. Annual Review of Phytopathology 22:95–113.