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Root Tissue Response of Two Related Soybean Cultivars to Infection by Lectin-treated *Meloidogyne* spp.

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Abstract: Treatment of second-stage juveniles (J2) of *Meloidogyne incognita* race 1 and *M. javanica* with soybean agglutinin, Concanavalin A, wheat germ agglutinin, *Lotus tetragonolobus* agglutinin, or *Limax flavus* agglutinin or the corresponding competitive sugars for each of these lectins did not alter normal root tissue response of soybean cultivars Centennial and Pickett 71 to infection by *M. incognita* race 1 or *M. javanica.* Giant cells were frequently induced in Centennial and Pickett 71 roots 5 and 20 days after inoculation of roots with untreated J2 of a population of *M. incognita* race 3. Treatment of J2 of *M. incognita* race 3 with the lectins or carbohydrates listed above caused Centennial, but not Pickett 71, root tissue to respond in a hypersensitive manner to infection by *M. incognita* race 3. Penetration of soybean roots by J2 of *Meloidogyne* spp. was strongly inhibited in the presence of 0.1 M sialic acid. Treatment of J2 with sialic acid was not lethal to nematodes, and the inhibitory activity of sialic acid was apparently not caused by low pH. These results suggest that carbohydrates may influence plant–nematode interactions.

Key words: carbohydrate, giant cell, *Glycine max,* hypersensitive reaction, lectin, *Meloidogyne incognita, Meloidogyne javanica,* resistance, sialic acid, soybean, specificity.

Plant incompatibility with nematodes often results from active plant defense reactions to infection by phytoparasitic nematodes (8–10,16,18,24,32). Although several mechanisms of active incompatibility have been proposed, little is known about nematode characteristics that may elicit plant responses incompatible with nematode development and their relation to specificity of plant–nematode interactions (18). The occurrence of physiological races of phytoparasitic nematodes (29,31) suggests that specific interactions occur between nematodes and plant cultivars and that populations of phytoparasitic nematodes evolve to overcome incompatibility.

Evidence that surface biochemistry, especially glycoconjugates of cells and organisms, promotes specificity in plant–microbe interactions has been the subject of several reviews (1,3,4,19,28). Keen (19) suggested that biochemical surface interactions were important in the specificity of incompatibility in gene-for-gene systems between plant cultivars and microbial pathogens. Whether surface interactions are important in plant–nematode incompatibility is unclear. The existence of carbohydrates on the surface of nematodes and evidence that surface carbohydrates may be important in interactions between nematodes and microbes have been reported and summarized (33). Zuckerman and Jansson (33) proposed that interaction between nematodes and other organisms may be altered by obliteration or blocking of carbohydrates on the nematode surface.

To evaluate this concept with respect to specificity in plant–nematode interactions, we chose to study a model system consisting of two related soybean (*Glycine max* (L.) Merr.) cultivars, Centennial and Pickett 71, that differed in compatibility with three *Meloidogyne* spp. populations. The incompatibility of Centennial soybean with *Meloidogyne incognita* has been associated with a hypersensitive reaction (HR) of soybean root tissue in the region of penetration by second-stage juveniles (J2) (15). The HR

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was strongly correlated with the accumulation of glyceollin, but elicitors of the HR were not identified (17).

The surface carbohydrates of J2 of populations of *M. incognita* races 1 and 3 and *M. javanica* have been characterized with fluorescent lectin probes (5,22). Fluorescent lectins bound primarily to the amphidial exudate of infective J2 of *Meloidogyne* spp. Soybean root tissue responses to *Meloidogyne* spp. J2 that were treated with various lectins and carbohydrates are reported here.

Materials and Methods

Populations of *Meloidogyne incognita* (Kofoid and White) Chitwood races 1 and 3 and *M. javanica* (Treub) Chitwood 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 using female perineal patterns, juvenile lengths, and differential host plant responses (25). Species identifications were confirmed independently by three nematode taxonomists. Eggs of each nematode population were extracted 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.

Purified soybean agglutinin (SBA), wheat germ agglutinin (WGA), *Lotus tetragonolobus* agglutinin (LOT), Concanavalin A (Con A), or *Limax flavus* agglutinin (LFA) (E-Y Labs, San Mateo, CA) were bound to surface carbohydrates of *Meloidogyne* spp. J2 by incubating nematodes in the separate lectin solutions. The sugar specificity, appropriate lectin buffers, corresponding competitive sugars, and procedure used to determine the specific hemagglutination activity for each lectin were described elsewhere (5). The sugar specificity of LFA is N-acetyl-neuraminic (sialic) acid; LFA assays were conducted in buffer containing 0.05 M Tris-saline plus 0.01 M $CaCl_2$ at pH 7.5.

Preinfective J2 of *M. incognita* races 1 and 3 and *M. javanica* were concentrated in the appropriate buffer or in distilled water by centrifugation at 1,000 *g* for 3 minutes. Approximately 8,400 J2 of each nematode population were incubated in 1) lectin solution (200 μg/ml), 2) lectin (200 μg/ml) plus 0.1 M competitive sugar, or 3) 0.1 M sugar solution minus lectin for 2 hours at 4 C. Seventy-microliter suspensions of juveniles in these solutions were used as direct (unwashed treatment) inoculum for subsequent soybean root challenge. In addition, J2 exposed to each lectin and sugar treatment were washed three times in buffer and similarly used as inoculum for soybean root challenge. Control treatments included J2 in buffer and J2 in distilled water.

Two related soybean cultivars, Pickett 71 and Centennial, were used for root tissue challenge by treated J2 of *Meloidogyne* spp. Pickett 71 was reported to be compatible and Centennial incompatible with *M. incognita* and both were compatible with *M. javanica* (15). Seeds of each variety were dusted with Thiram 75WP (Kerr–McGee Chemical Corp., Jacksonville, FL), rolled in moist germination paper (ragdolls), and incubated in the dark at 27 C until they germinated. Newly germinated seedlings with roots 3–5 cm long were placed on trays containing autoclaved Astatula fine sand (hyperthermic, uncoated typic quartzipsamments), and the root tips were covered with a small amount of sand. A suspension of approximately 600 J2 from each treatment was placed on the sand covering the root tips of separate soybean plants (15,21). Trays containing inoculated soybeans in sand were incubated in the dark at 27 C. Dark conditions more closely simulated the soil environment experienced by roots and nematodes. There were seven replicates of each treatment combination.

Approximately 40 hours after inoculation, soybeans were removed from trays, and sand and nematodes that had not penetrated were washed from the roots. The seedlings were then placed on moist germination paper and the positions of the

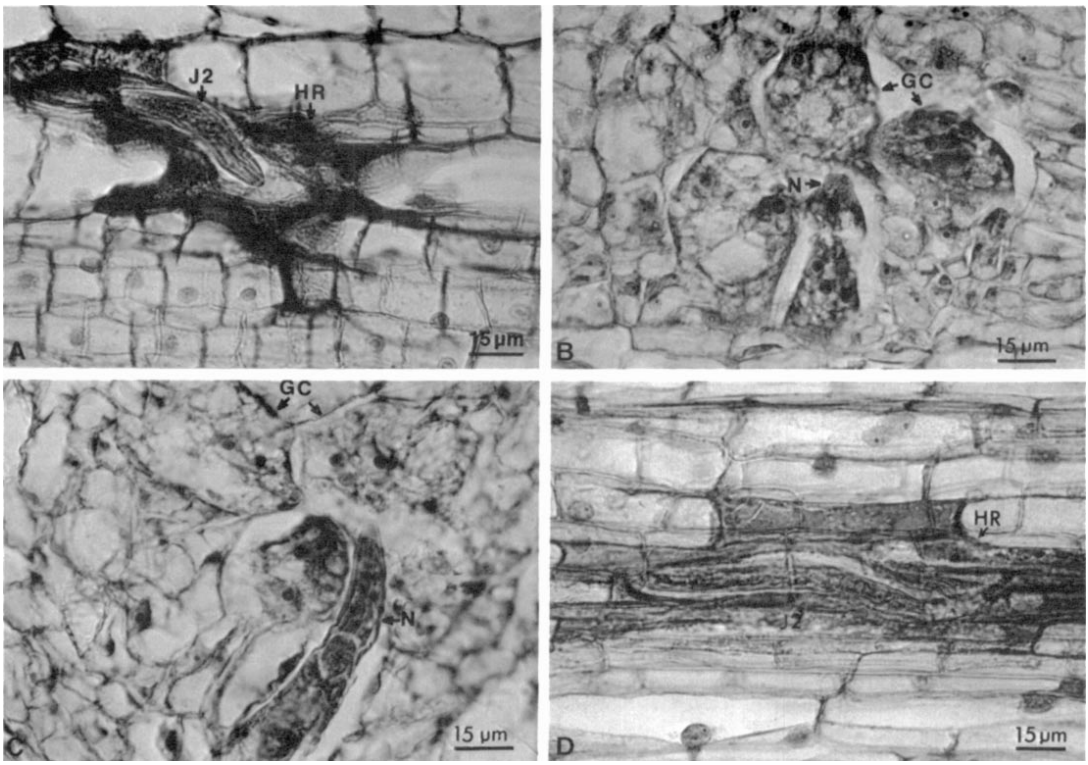

FIG. 1. Response of Centennial soybean root tissue within 5 days of infection by lectin-treated second-stage juveniles (J2) of *Meloidogyne* spp. A) Hypersensitive reaction (HR) to *M. incognita* race 1 treated with soybean agglutinin. B) Giant cells (GC) induced by the untreated nematode (N), *M. incognita* race 3. C) Giant cells induced by *M. javanica* treated with soybean agglutinin plus galactose. D) Hypersensitive reaction induced by *M. incognita* race 3 treated with N-acetylglucosamine.

inoculated portions of the roots were marked on the paper. The germination paper that contained inoculated seedlings was covered with an additional piece of moist germination paper, carefully rolled into ragdolls, and incubated in the dark at 27 C for 80 additional hours.

Inoculated soybean root segments were excised 5 days after inoculation and immediately fixed in 10% alcoholic formalin (1:9, formalin; 95% ETOH, v:v). Additional treatments of J2 of *M. incognita* race 3 in water that were applied to Centennial and Pickett 71 soybean roots were excised 20 days after inoculation and processed in the same manner. Fixed root segments were dehydrated through a tert-butyl alcohol series and embedded in paraffin. Serial sections (12-µm thick) were mounted on glass slides, stained with safranin and fast green, and observed with a light microscope. Sections from seven replicates of each combination were examined for tissue responses to individual nematode infection. Root tissue responses that occurred in $< 5\%$ of the total observations within a given treatment were not recorded. This experiment was repeated.

Since a 0.1-M solution of sialic acid in buffer is acidic (pH ~ 3.0), a soybean root penetration bioassay was conducted to determine the effect of low pH on the activity of J2 of *Meloidogyne* spp. Second-stage juveniles of *M. incognita* races 1 and 3 and *M. javanica* were incubated for 2 hours at 4 C in a solution of 0.1 M sialic acid (pH ~ 3.0), 0.1 M sialic acid neutralized with NaOH (pH 7.0), 0.05 M Tris-buffer saline adjusted with HCl (pH 3.0), 0.05 M Tris-buffer saline (pH 8.0), or in distilled water. Juveniles suspended in 100 µl incubation solution (ca. 200 J2) were applied to Pickett

TABLE 1. Tissue responses† of Pickett 71 (P) and Centennial (C) soybean roots 5 days after exposure to second-stage juveniles of *Meloidogyne incognita* races 1 and 3, and *M. javanica* incubated in soybean agglutinin (SBA) solution (200 μg/ml) and (or) 0.1 M galactose (gal) solution prior to inoculation.

Treatment	*M. incognita* race 1		*M. incognita* race 3		*M. javanica*	
	P	C	P	C	P	C
Washed‡						
SBA	GC, GRCY	HR, NR	GC, NR	HR, EGC	GC, S	GC, S
SBA + gal	GC	HR	GC, GRCY	HR, NR	GC, GRCY	GC, S
Gal	GC, GRCY	HR	GC, GRCY	HR, NR	GC, GRCY	GC
Unwashed						
SBA	GC, GRCY	HR, EGC	GC, S	HR, EGC	GC, S	GC, S
SBA + gal	GC, GRCY	HR, EGC	EGC, GRCY	HR, EGC	GC, EGC	GC, S
Gal	GC, S	HR, GRCY	GC, S	HR, NR	GC, S	GC, S
Buffer	GC, GRCY	HR, GRCY	GC, S	GC, GRCY	GC, S	GC, S
Distilled H_2O	GC, S	HR	GC, S	GC, GRCY	GC, S	GC, S

GC = giant cells; GRCY = granular cytoplasm within giant cells; HR = hypersensitive response; NR = no response; EGC = early giant cells; S = swollen juvenile.

† The first and second (if observed) most frequent tissue responses to individual J2 of *Meloidogyne* spp. are listed consecutively.

‡ Second-stage juveniles were incubated in treatment solution and washed three times with buffer before inoculation.

71 soybean root tips growing on trays of autoclaved sand. An identical set of sialic acid and buffer solutions containing J2 were diluted 1:16 as described in a similar test (6) and used as inoculum for Pickett 71 root tip inoculation. The inoculated segment of each soybean root was excised after 40 hours and stained with acid fuchsin (2), and the numbers of J2 within each root segment were counted. There were six replicates of each treatment and this test was repeated.

RESULTS

The binding capacity of pure lectins, except LFA, was relatively strong. Specific hemagglutination activities of 4,096 (SBA), 4,096 (Con A), 8,192 (WGA), 8,192 (LOT), and 4 (LFA) units/mg lectin were determined. The ability of lectins to agglutinate red blood cells by multivalent binding to cell surface carbohydrates was completely inhibited in the presence of a 0.1 M solution of the specific sugar to which each lectin binds.

The most frequent response of Pickett 71 soybean root tissue to infection by *Meloidogyne* spp. J2 was giant cell production and of Centennial root tissue was HR (Tables 1–5). Infective juveniles incubated in buffer or water became enlarged and induced giant cell formation in compatible interactions, but they remained vermiform and were associated with a hypersensitive reaction in incompatible combinations (Fig. 1). Giant cells of normal appearance were very frequently observed in roots of Pickett 71 and Centennial soybean 5 and 20 days after roots were exposed to *M. incognita* race 3 incubated in buffer or water. Giant cells associated with *M. incognita* race 3 controls in Centennial soybean often contained granular cytoplasm. No gall formation, evidence of hyperplasia of pericycle cells adjacent to feeding sites, or development of *M. incognita* race 3 past third-stage juvenile was observed in Centennial root tissue 20 days after inoculation with J2 of *M. incognita* race 3.

The primary tissue response of both soybean cultivars to J2 of *M. incognita* race 1 or *M. javanica* exposed to any lectin or sugar treatment was essentially unchanged from that of tissues infected by J2 incubated in buffer or water. Lectin or sugar treatment of J2 of *M. incognita* race 3 did not influence host response in Pickett 71, but in Centennial soybean roots, treatment of J2 of *M. incognita* race 3 with any lectin or sugar stimulated a hypersensitive response rather than giant cells.

Soybean tissue responses to infection by

TABLE 2. Tissue responses† of Pickett 71 (P) and Centennial (C) soybean roots 5 days after exposure to second-stage juveniles of *Meloidogyne incognita* races 1 and 3, and *M. javanica* incubated in Concanavalin A (Con A) solution (200 μg/ml) and (or) 0.1 M mannose (man) solution prior to inoculation.

	M. incognita race 1		*M. incognita* race 3		*M. javanica*	
Treatment	P	C	P	C	P	C
Washed‡						
Con A	GC, NR	NR, HR	GC, S	HR	GC	GC, S
Con A + man	GC, GRCY	HR, NR	GC, S	HR, NR	GC, S	GC, S
Man	GC, S	HR	GC	NR, GC	GC, GRCY	GC, GRCY
Unwashed						
Con A	GC, S	HR	GC, EGC	HR, NR	GC, S	GC, S
Con A + man	GC, S	HR	GC, S	HR, EGC	GC, S	GC, S
Man	GC, S	NR, EGC	GC, S	GC, GRCY	GC, S	GC, S
Buffer	GC, S	HR, NR	GC, S	GC, GRCY	GC, S	GC, S
Distilled H_2O	GC, S	HR, GRCY	GC, S	GC, EGC	GC, S	GC, S

GC = giant cells; GRCY = granular cytoplasm within giant cells; HR = hypersensitive response; NR = no response; EGC = early giant cells; S = swollen juvenile.
† The first and second (if observed) most frequent tissue responses to individual J2 of *Meloidogyne* spp. are listed consecutively.
‡ Second-stage juveniles were incubated in treatment solution and washed three times with buffer before inoculation.

individual J2 that were atypical of the most frequent tissue response occurred frequently enough (> 10% of observations) to warrant report. These included occurrence of early giant cells in Centennial tissue challenged by *M. incognita* race 1 and *M. incognita* race 3 treated with SBA or SBA plus galactose, unwashed, and *M. incognita* race 3 treated with SBA, washed, was in contrast to the primary response of hypersensitivity (Table 1). Early giant cell formation was also observed in Centennial challenged by *M. incognita* race 1 treated with mannose, unwashed (Table 2), or with LOT, washed (Table 3).

Second-stage juveniles of *Meloidogyne* spp. were observed in soybean roots with no apparent plant tissue reaction to their presence. This was the primary observation in Centennial root tissue inoculated with *M. incognita* race 1 treated with Con A, washed; *M. incognita* race 1 treated with mannose,

TABLE 3. Tissue responses† of Pickett 71 (P) and Centennial (C) soybean roots 5 days after exposure to second-stage juveniles of *Meloidogyne incognita* races 1 and 3, and *M. javanica* incubated in *Lotus tetragonolobus* agglutinin (LOT) solution (200 μg/ml) and (or) 0.1 M fucose (fuc) solution prior to inoculation.

	M. incognita race 1		*M. incognita* race 3		*M. javanica*	
Treatment	P	C	P	C	P	C
Washed‡						
LOT	GC, S	HR, EGC	GC, S	HR, EGC	GC, S	GC, GRCY
LOT + fuc	GC, S	HR, NR	GC, NR	HR, GRCY	GC, S	GC, S
Fuc	GC, GRCY	HR	GC, EGC	HR, NR	GC, S	GC, S
Unwashed						
LOT	GC, GRCY	HR	GC, GRCY	HR, EGC	GC, S	GC, GRCY
LOT + fuc	GC, GRCY	HR, GRCY	GC, S	HR, EGC	GC, EGC	GC, NR
Fuc	GC, GRCY	HR, GRCY	GC, S	HR, EGC	GC, S	GC, S
Buffer	GC, S	HR	GC, S	GC, EGC	GC, S	GC, S
Distilled H_2O	GC, S	HR	GC, S	GC, S	GC, S	GC, S

GC = giant cells; GRCY = granular cytoplasm within giant cells; HR = hypersensitive response; NR = no response; EGC = early giant cells; S = swollen juvenile.
† The first and second (if observed) most frequent tissue responses to individual J2 of *Meloidogyne* spp. are listed consecutively.
‡ Second-stage juveniles were incubated in treatment solution and washed three times with buffer before inoculation.

TABLE 4. Tissue responses† of Pickett 71 (P) and Centennial (C) soybean roots 5 days after exposure to second-stage juveniles of *Meloidogyne incognita* races 1 and 3, and *M. javanica* incubated in wheat germ agglutinin (WGA) solution (200 μg/ml) and (or) 0.1 M N-acetylglucosamine (GlcNAc) solution prior to inoculation.

Treatment	*M. incognita* race 1		*M. incognita* race 3		*M. javanica*	
	P	C	P	C	P	C
Washed‡						
WGA	GC, EGC	HR, NR	GC, NR	HR, NR	GC, NR	GC
WGA + GlcNAc	GC, NR	HR, NR	GC, EGC	HR, NR	GC, S	GC, S
GlcNAc	GC, HR	HR, GRCY	GC, S	NR, HR	GC, S	GC, S
Unwashed						
WGA	GC, NR	HR, NR	GC, S	HR, GRCY	GC, S	GC, GRCY
WGA + GlcNAc	GC, HR	NR, HR	GC, S	HR	GC, S	GC, S
GlcNAc	GC, HR	HR	GC, S	HR, EGC	GC, S	GC, S
Buffer	GC, S	HR, NR	GC, S	GC, NR	GC, S	GC, S
Distilled H_2O	GC, S	HR, NR	GC, S	GC, GRCY	GC, S	GC, S

GC = giant cells; GRCY = granular cytoplasm within giant cells; HR = hypersensitive response; NR = no response; EGC = early giant cells; S = swollen juvenile.

† The first and second (if observed) most frequent tissue responses to individual J2 of *Meloidogyne* spp. are listed consecutively.

‡ Second-stage juveniles were incubated in treatment solution and washed three times with buffer before inoculation.

unwashed; *M. incognita* race 3 treated with mannose, washed; *M. incognita* race 1 treated with WGA plus N-acetyl-D-glucosamine (GlcNAc), unwashed; *M. incognita* race 3 treated with GlcNAc, washed; and *M. incognita* race 1 treated with LFA, washed (Tables 4, 5). No tissue response was observed in Pickett 71 to *M. incognita* race 3 treated with LFA plus sialic acid, washed. A hypersensitive response was sometimes observed in Pickett 71 challenged by *M. incognita* race 1 treated with GlcNac, washed; *M. incognita* race 1 treated with WGA plus GlcNAc, unwashed; and *M. incognita* race 1 treated with GlcNAc, unwashed. Very few J2 were located in soybean roots challenged by any population of *Meloidogyne* spp. that received unwashed LFA and sialic acid treatments. In these instances, the tissue response was similar to comparable treatments with SBA.

Both 100 mM sialic acid at pH 3.0 and 100 mM sialic acid at pH 3.0 diluted to 6.25 mM before inoculation caused a significantly greater reduction in penetration of soybean roots by J2 of all three nematode populations than any other treatment (Table 6). In experiment 1 100 mM sialic acid neutralized to pH 7.0 with NaOH significantly reduced root penetration by J2 of *Meloidogyne* spp., compared with controls, but this effect was apparently lost when neutralized sialic acid was diluted to 6.25 mM. Undiluted 50 mM Tris-saline at pH 3.0 reduced penetration of soybean roots by J2 of all three nematode populations, compared with controls, but neither this treatment nor diluted Tris-saline at pH 3.0 reduced J2 penetration of roots as much as nonneutralized sialic acid.

DISCUSSION

Incubation of J2 of *M. incognita* race 1 in the lectins or sugars tested appeared to have little effect on soybean root tissue response in the incompatible pathosystem. If the concept that preformed sites (carbohydrate moieties) on the surface of J2 of *M. incognita* race 1 are responsible for recognition by plant cell surface receptors and subsequent plant defense reaction were valid, blockage of these sites with lectins should have prevented the HR observed in Centennial root tissue. In a few instances, early giant cell formation in Centennial by *M. incognita* race 1 was noted, but the HR was much more common. The more frequent occurrence of HR in Pickett 71 infected by *M. incognita* race 1 treated with N-acetyl-D-glucosamine may indicate an alteration of nematode surface carbohydrates which promoted incompatibility.

TABLE 5. Tissue responses† of Pickett 71 (P) and Centennial (C) soybean roots 5 days after exposure to second-stage juveniles of *Meloidogyne incognita* races 1 and 3, and *M. javanica* incubated in *Limax flavus* agglutinin (LFA) solution (200 μg/ml) and (or) 0.1 M N-acetylneuraminic (sialic) acid solution prior to inoculation.

Treatment	*M. incognita* race 1		*M. incognita* race 3		*M. javanica*	
	P	C	P	C	P	C
Washed‡						
LFA	GC, S	NR, HR	GC, EGC	HR, NR	GC, S	GC, S
LFA + sialic	GC, S	HR	NR, EGC	HR, EGC	GC, S	GC, S
Sialic	GC, GRCY	HR, GRCY	GC, EGC	HR	GC, S	GC, S
Unwashed						
LFA	—§	—	—	—	—	—
LFA + sialic	—	—	—	—	—	—
Sialic	—	—	—	—	—	—
Buffer	GC, S	HR, GRCY	GC, S	GC, EGC	GC, S	GC, S
Distilled H_2O	GC, S	HR, NR	GC, S	GC, GRCY	GC, S	GC, S

GC = giant cells; GRCY = granular cytoplasm within giant cells; HR = hypersensitive response; NR = no response; EGC = early giant cells; S = swollen juvenile.

† The first and second (if observed) most frequent tissue responses to individual J2 of *Meloidogyne* spp. are listed consecutively.

‡ Second-stage juveniles were incubated in treatment solution and washed three times with buffer before inoculation.

§ In the unwashed treatments, few or no nematodes were observed within soybean root tissue.

The fate of lectin bound to J2 of *Meloidogyne* spp., once the nematode entered soybean root tissue, is questionable. Unwashed treatments were included in this study to ensure that J2 were present in an environment of lectin and (or) sugar until they penetrated roots. No treatments had an effect on the compatible interaction between *M. javanica* and root tissue of either soybean cultivar. Differences in the quantity, balance, or accessibility of secretory carbohydrates to potential plant receptors,

TABLE 6. Effect of 100 mM sialic acid at pH 3.0, 100 mM sialic acid neutralized to pH 7.0 with NaOH, and 50 mM Tris-saline at pH 3.0 on penetration of Pickett 71 soybean roots by second-stage juveniles (J2) *Meloidogyne* spp.

Treatment†	Experiment	J2/root segment (no.)		
		M. javanica	*M. incognita* race 1	*M. incognita* race 3
1. Sialic acid (100 mM, pH 3.0)	1	1.67 ± 0.96	0.17 ± 0.15	1.67 ± 0.69
	2	0.67 ± 0.31	0.50 ± 0.31	1.33 ± 0.45
2. Sialic acid (100 mM, pH 7.0)	1	3.67 ± 1.22	5.50 ± 1.78	7.00 ± 1.03
	2	10.50 ± 1.12	10.17 ± 1.92	9.17 ± 0.93
3. Tris-saline (50 mM, pH 3.0)	1	9.00 ± 1.76	9.33 ± 2.09	13.50 ± 2.14
	2	7.67 ± 1.15	10.50 ± 1.71	8.33 ± 1.58
4. Sialic acid (6.25 mM, pH, 3.0)	1	1.00 ± 0.47	1.17 ± 0.60	0.67 ± 0.45
	2	0.67 ± 0.45	1.50 ± 0.70	0.83 ± 0.37
5. Sialic acid (6.25 mM, pH 7.0)	1	12.83 ± 1.83	13.67 ± 2.87	14.33 ± 1.80
	2	14.50 ± 1.76	15.33 ± 2.09	20.17 ± 1.23
6. Tris-saline (3.13 mM, pH 3.0)	1	12.33 ± 1.74	7.17 ± 1.56	11.00 ± 1.65
	2	16.17 ± 1.42	16.17 ± 2.23	19.00 ± 1.96
7. Tris-saline (50 mM, pH 8.0)	1	10.83 ± 1.92	6.67 ± 1.73	14.00 ± 2.40
	2	25.00 ± 1.53	20.83 ± 1.87	22.50 ± 1.35
8. Distilled water	1	12.17 ± 1.72	12.33 ± 1.49	18.67 ± 3.30
	2	25.17 ± 1.88	22.17 ± 2.00	24.17 ± 1.36

Data are means of six observations ± standard error.

† Treatments 4, 5, and 6 were identical to treatments 1, 2, and 3, respectively, except that suspensions of J2 in incubation solutions were diluted from 1.0 to 16.0 ml to provide ca. 200 J2/100 μl for inoculation of soybean roots.

however, may promote incompatibility or compatibility. Previous reports indicated that lectins bind to amphidial secretions of J2 of *Meloidogyne* spp. (5,22), but the rates of production of amphidial secretions by J2 of *Meloidogyne* spp. and the quantities of this material that are sloughed off in plant tissue or the soil environment are unknown.

The frequent occurrence of normal giant cells in Centennial 5 and 20 days after inoculation of root tips with J2 of *M. incognita* race 3 incubated in water was unexpected. This host–parasite relationship was apparently incomplete compared with *M. incognita* race 3 in Pickett 71, however, because pericyclic hyperplasia and nematode development were strongly inhibited 20 days after inoculation. Variability in the degree of soybean resistance to different populations of *M. incognita* has been reported (27). Treatment of J2 of *M. incognita* race 3 with any lectin or sugar promoted active incompatibility (HR) in Centennial soybean and may actually have facilitated recognition of invasive *M. incognita* race 3 and subsequent defense response by the plant. The lack of specificity of lectin or sugar effects in the *M. incognita* race 3–Centennial interaction makes it unlikely that alteration of surface carbohydrate composition of preinfective J2 was responsible for promoting incompatibility. If the interaction of nematode surface carbohydrates with potential plant cell surface receptors is a "lock and key" phenomenon, a slight alteration in surface carbohydrate composition may be sufficient to enhance binding to host cell surface receptors and promote incompatibility to *M. incognita* race 3 in Centennial soybean roots. Possibly a greater alteration of the carbohydrates examined here on *M. incognita* race 1 and *M. javanica*, or alteration of surface carbohydrates not examined in these studies, would influence their host–parasite interactions.

Treatment of J2 of *M. incognita* race 3 with lectin or sugar may have stimulated the production of a substance by the nematode that induces HR in Centennial soybean roots. Juveniles of *M. incognita* race 1 may have an inherent capacity to induce HR, whereas J2 of *M. javanica* cannot promote incompatibility in soybean no matter what the treatment. For some populations, such as in the case of *M. incognita* race 3, incompatibility may be a process that can be stimulated. Conversely, substances produced by J2 of *Meloidogyne* spp. (i.e., amphidial or stylet secretions) may be essential to induce compatibility between host and parasite, and these substances were altered sufficiently in *M. incognita* race 3 to inhibit compatibility in Centennial soybean roots.

A single soil application of Con A, and LFA at relatively higher concentrations, was reported to significantly reduce galling of tomato roots induced by *M. incognita* (20). Little effect of Con A on soybean root penetration by J2 of any *Meloidogyne* spp. population was observed in our investigations, even though J2 were incubated in Con A solution (200 μg/ml) before their application to roots in soil.

The ability of J2 of the three nematode populations tested to penetrate roots of both soybean varieties was apparently strongly impaired when J2 were introduced to roots in a solution that contained sialic acid. Whether J2 emerged from roots within the 40-hour "pulse inoculation" was not determined. The inhibition of soybean root penetration by J2 of *Meloidogyne* spp. treated with sialic acid appeared to be more than an effect of the low pH of a 0.1-M sialic acid solution. The activity of sialic acid was apparently reduced when the solution pH was neutralized.

Sialic acids appear to be important in the adhesion of conidia of *Meria coniospora* to nematode surfaces, especially at the sites of chemosensory organs (12–14). Reports concerning the occurrence of sialic acids in protostome invertebrates are controversial (26), and conflicting, indirect evidence for the existence of sialic acids on the surface of phytoparasitic nematodes has been presented (5,7,22,23,30). The occurrence of sialic acids in plants has not been unequivocally established (26). In the studies reported here, nematode viability after

LFA and sialic acid treatment was confirmed by infectivity of washed J2. Viable J2 of *Meloidogyne* spp. have been observed microscopically after similar treatment (5). Removal of sialic acids from amphidial secretions by a number of selective glycohydrolases suggests that sialic acids may be some of the outermost carbohydrate residues of amphidial glycoconjugates (5). Incubation of J2 of *Meloidogyne* spp. in solutions of sialic acid may promote binding of excess sialic acid to amphidial carbohydrate complexes that acts as a barrier between some external chemical stimuli and nematode chemosensory receptors. Sialic acid residues present in amphidial carbohydrate complexes may have masking or regulatory effects similar to those observed in other animal systems (26) and therefore merit further investigation.

Literature Cited

1. 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.

2. Byrd, D. W., Jr., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15:142–143.

3. Callow, J. A. 1977. Recognition, resistance and the role of plant lectins in host–parasite interactions. Pp. 1–49 *in* R. D. Preston and H. W. Woolhouse, eds. Advances in botanical research, vol. 4. New York and London: Academic Press.

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

5. Davis, E. L., D. T. Kaplan, T. A. Permar, D. W. Dickson, and D. J. Mitchell. 1988. Characterization of carbohydrates on the surface of second-stage juveniles of *Meloidogyne* spp. Journal of Nematology 20: 609–619.

6. Davis, E. L., D. T. Kaplan, D. W. Dickson, and D. J. Mitchell. 1988. Reproduction of lectin-treated *Meloidogyne* spp. in two related soybean cultivars. Revue de Nématologie, in press.

7. 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. pallia*. Journal of Nematology 18:23–26.

8. Giebel, J. 1974. Biochemical mechanisms of plant resistance to nematodes: A review. Journal of Nematology 6:175–184.

9. Gommers, F. J. 1981. Biochemical interactions between nematodes and plants and their relevance to control. Helminthological Abstracts, Series B 50:9–24.

10. Huang, J. S. 1985. Mechanisms of resistance to root-knot nematodes. Pp. 165–174 *in* J. N. Sasser and C. C. Carter, eds. An advanced treatise on *Meloidogyne*, vol. 1. Biology and control. Raleigh: North Carolina State University Graphics.

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., and B. Nordbring-Hertz. 1983. The endoparasitic nematophagous fungus *Meria coniospora* infects nematodes specifically at the chemosensory organs. Journal of General Microbiology 129: 1121–1126.

13. Jansson, H.-B., and B. Nordbring-Hertz. 1984. Involvement of sialic acid in nematode chemotaxis and infection by an endoparasitic nematophagous fungus. Journal of General Microbiology 130:39–43.

14. Jansson, H.-B., A. Jeyaprakash, and B. M. Zuckerman. 1985. Differential adhesion and infection of nematodes by the endoparasitic fungus *Meria coniospora* (Deuteromycetes). Applied and Environmental Microbiology 49:552–555.

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 N. T. Keen. 1980. Mechanisms conferring plant incompatibility to nematodes. Revue de Nématologie 3:123–134.

17. Kaplan, D. T., N. T. Keen, and I. J. Thomason. 1980. Association of glyceollin with the incompatible response of soybean roots to *Meloidogyne incognita*. Physiological Plant Pathology 16:309–318.

18. 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.

19. Keen, N. T. 1982. Specific recognition in gene-for-gene host–parasite systems. Pp. 35–81 *in* D. S. Ingram and P. H. Williams, eds. Advances in plant pathology, vol. 1. New York: Academic Press.

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

21. McClure, M. A., and J. Robertson. 1973. Infection of cotton seedlings by *Meloidogyne incognita* and a method of producing uniformly infected root segments. Nematologica 19:428–434.

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

23. Robertson, W. M., Y. Spiegel, H.-B. Jansson, N. Marban-Mendoza, and B. M. Zuckerman. 1989. Surface carbohydrates of plant parasitic nematodes. Nematologica, in press.

24. Roy, T. K. 1981. Biochemical aspects of host–parasite relationships in plant parasitic nematodes. Proceedings of Indian National Science Academy B 47:919–936.

25. 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. 1. Biology and control. Raleigh: North Carolina State University Graphics.

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

27. Schmitt, D. P., and G. R. Noel. 1984. Nematode parasites of soybeans. Pp. 13–59 *in* W. R. Nickle, ed. Plant and insect nematodes. New York: Marcel Dekker.

28. Sequeira, L. 1978. Lectins and their role in host–pathogen specificity. Annual Review of Phytopathology 16:453–481.

29. Sidhu, G. S., and J. M. Webster. 1981. The genetics of plant–nematode parasitic systems. Botanical Review 47:387–419.

30. 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.

31. Sturhan, D. 1971. Biological races. Pp. 51–71 *in* B. M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Plant parasitic nematodes, vol. 2. New York: Academic Press.

32. Veech, J. A. 1981. Plant resistance to nematodes. Pp. 377–403 *in* B. M. Zuckerman and R. A. Rohde, eds. Plant parasitic nematodes, vol. 3. New York: Academic Press.

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