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# Stage-specific Differences in Lectin Binding to the Surface of *Anguina tritici* and *Meloidogyne incognita*[1]

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*Abstract:* The occurrence and distribution of several lectin binding sites on the outer surfaces of eggs, preparasitic second-stage juveniles (J2), parasitic second-stage juveniles (PJ2), females, and males of two tylenchid nematodes, *Anguina tritici* and *Meloidogyne incognita* race 3, were compared. In both species, a greater variety of lectins bound to the eggs than to other life stages; lectin binding to eggs was also more intense than it was to other life stages. Species-specific differences also occurred. More lectins bound to the amphids or amphidial secretions of *M. incognita* J2 than to the amphids or amphidial secretions of *A. tritici* J2. Lectins also bound to the amphids or amphidial secretions of adult male and female *A. tritici*, but binding to the cuticle occurred only at the head and tail and was not consistent in all specimens. *Canavalia ensiformis* and *Ulex europaeus* lectins bound specifically to the outer cuticle of *M. incognita*. Several other lectins bound nonspecifically. Oxidation of the cuticle with periodate under mild conditions, as well as pretreatment of the nematodes with lipase, markedly increased the binding of lectins to the cuticle of *A. tritici* J2 but not, in most cases, to *M. incognita* J2 or eggs of either species.

*Key words: Anguina tritici*, carbohydrate, cuticle, glycocalyx, lectin, lipase, *Meloidogyne incognita*, nematode, periodate.

In the last decade, the presence and biological significance of carbohydrates on the cuticular surface of plant-parasitic nematodes has been documented extensively (4,7,13,19). Most of these studies focused on a single stage in the life cycle of the nematode, usually the infective stage (5). In one investigation, however, sialyl and galactosyl residues were detected on preparasitic juveniles (J2), females, and males of *Meloidogyne javanica* and *Tylenchulus semipenetrans* (16). Differences in the structure and distribution of body wall glycans between life stages of a single species indicated that development of nematodes may be accompanied by changes in surface saccharides (18). Age-related differences in binding of wheat germ agglutinin (WGA) to the cuticle of *Caenorhabditis elegans* (16) supported previous findings of a decrease in net negative charge during the aging process (6).

The specific association between some microorganisms and the cuticle surface of plant-parasitic nematodes has attracted the attention of several investigators. Adhesion of endospores of *Pasteuria penetrans* to *Meloidogyne* spp., *Heterodera* spp., or *Pratylenchus brachyurus* (14,17), as well as adhesion of the fungus *Dilophospora alopecuri* and the bacterium *Clavibacter* sp. (syn. *Corynebacterium rathayi*) to *Anguina agrostis* (2), appears to be both species and stage specific. The target is the infective juvenile rather than adults or other juvenile stages.

The possibility that these stage-specific interactions may, in part, be mediated by lectin-hapten binding led us to investigate the occurrence and distribution of several lectin binding sites on the outer surfaces of eggs, juveniles, females, and males of two plant-parasitic nematodes that differ in their parasitic habit. *Anguina tritici* is a seed gall nematode with a restricted host range and a life cycle closely linked to that of its host. *Meloidogyne incognita* is a polyphagous sedentary parasite of roots.

## MATERIALS AND METHODS

Preparasitic second-stage juveniles (J2) of *A. tritici* were extracted from brown seed

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galls of wheat, harvested the previous season, by dissection in 0.06 M phosphate buffered saline, pH 7.2 (PBS). Liberated nematodes were allowed to migrate through a 10-μm-pore nylon cloth to remove extraneous plant material and were washed thoroughly with distilled water and PBS. Eggs and adults were obtained from green galls on infected plants maintained in the greenhouse. Wheat kernels (*Triticum aestivum* cv. Tonichi) were sown in pasteurized sandy loam soil inoculated with seed galls containing J2 (10). When green galls developed on the inflorescence, they were harvested and the nematodes within were removed by dissection. Eggs and adults were recovered by hand-picking or pipetting and washing in PBS.

*Meloidogyne incognita* race 3 was propagated on eggplant, *Solanum melongena* cv. Black Beauty, in the greenhouse. Eggs were collected from infected plants (9) and hatched to obtain infective J2. Parasitic second-stage juveniles (PJ2) and adult females were dissected from infected plants 2 and 4 weeks after inoculation. Hand-picked or pipetted nematodes were washed with PBS.

*Lectin labeling:* Fluorescein isothiocyanate (FITC) conjugates of lectins were obtained from E-Y laboratories (San Mateo, CA). They included Con A (*Canavalia ensiformis* agglutinin), DBA (*Dolichos biflorus* agglutinin), GSII (*Griffonia* [*Bandeiraea*] *simplicifolia* agglutinin), MPA (*Maclura pomifera* agglutinin), PNA (*Arachis hypogaea* agglutinin), SBA (*Glycine max* agglutinin), UEA (*Ulex europaeus* agglutinin), and WGA (*Triticum vulgaris* agglutinin). Con A was diluted to a concentration of 100 μg/ml with 0.015 M Tris, pH 7.0, containing 0.015 M NaCl, 0.01 M $CaCl_2$, and 0.001 M $MnCl_2$. Other lectins were diluted to 100 μg/ml in PBS. From 1,000 to 2,000 eggs or J2 or 10–15 PJ2, males, or females were incubated in the buffered lectin solutions for 45 minutes at room temperature. Controls consisted of untreated nematodes and parallel blocking experiments similar to the lectin treatments except that the buffer used for diluting the lectin contained 0.2 M lectin-specific hapten: α-methyl mannopyranoside for Con A, α-D-galactosamine for DBA, N-acetylglucosamine for GSII, N-acetylgalactosamine for MPA and SBA, D-galactose for PNA, L-fucose for UEA and chitobiose for WGA. Nonspecific staining of nematodes by FITC was determined by incubating nematodes in FITC (21 μg/ml in PBS) for 60 minutes at room temperature. All treatments were followed by three washes with the appropriate buffer. Live nematodes treated with FITC-conjugated lectins were then 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.

*Periodate pretreatment:* Nematodes (J2) were incubated for 60 minutes in the dark at 0 C (ice water bath) with freshly made 10 mM sodium metaperiodate ($NaIO_4$) in 100 mM sodium acetate buffer, pH 4.5. The J2 were then washed with cold PBS, and both nematode species were incubated with FITC-conjugated lectins as described in the previous paragraph. Nematodes stored in the dark at 0 C but not treated with $NaIO_4$ were the controls.

*Lipase pretreatment:* PBS-washed J2 were incubated at 37 C overnight with crystalline lipase from wheat germ (Sigma, St. Louis, MO) (2 mg/ml, 17 units/mg) in PBS. Controls consisted of nematodes incubated overnight at 37 C in PBS. All experiments and results were repeated two or more times.

## Results

Binding of FITC-conjugated lectins to the eggs of *A. tritici* and *M. incognita* was quantitatively and qualitatively greater than to other life stages of these two nematodes (Table 1). All lectins tested bound to the eggs of *M. incognita* and all but Con A and UEA bound to eggs of *A. tritici.* Conversely, a greater variety and amount of lectins bound to the J2 of *A. tritici* than to the J2 of *M. incognita.* Only Con A bound to the cuticle of the latter species, whereas GSII, PNA, UEA, and WGA bound to the cuticle of *A. tritici.* Binding of lectins to amphids

TABLE 1. Fluorescent lectin binding to cuticular surface of *Anguina tritici* and *Meloidogyne incognita* eggs, preparasitic juveniles (J2), parasitic juveniles (PJ2), females, and males.

| Lectin | Eggs | J2 | PJ2 | ♀ | ♂ |
|---|---|---|---|---|---|
| | | *A. tritici* | | | |
| Con A | — | — | | a, v | — |
| DBA | +++b | — | | — | — |
| GSII | +b | ++b, a | | a, h, t | a, h, t |
| MPA | ++++b | a | | a | a |
| PNA | +++, ++++b | ++b | | a | a |
| SBA | +++, ++++b | — | | a | — |
| UEA | — | ++++b | | a, h, t | a, h, t |
| WGA | ++++b | ++++b | | a, h, t, v | a, h, t |
| | | *M. incognita* | | | |
| Con A | ++++b | ++b, a | — | ++b, eh | a |
| DBA | +b | — | — | ++b, ns, eh | — |
| GSII | ++++b | — | — | +++b, ns, eh | a |
| MPA | ++++b | a | a | ++b, ns, eh, a | a |
| PNA | ++, +++b | — | — | — | — |
| SBA | ++, +++b | a | — | +++b, eh | ++b, a |
| UEA | ++++b | a | a, h | ++++b, a, v | |
| WGA | ++++b | a | a, h | a | +b, a |

— = no labeling.
+b, ++b, +++b, ++++b = increased intensities of labeling of entire cuticle.
a, h, t, v = labeling limited to amphids, head, tail, or vulva areas.
ns = nonspecific labeling.
eh = labeling of entire cuticle, except for the head region.

or amphidial secretions (A-AS) was greater for *M. incognita* J2 than for *A. tritici* J2. In contrast, lectins bound primarily to A-AS of *A. tritici* males and females. Only some lectins bound to the cuticle of *A. tritici* adults, and this generally was at the head or tail and not to cuticle of the midbody region. Exceptions to this pattern of labeling were DBA, which did not bind to any parts of males or females, and Con A and SBA, which did not bind to males. Several lectins nonspecifically bound to the body wall cuticle of *M. incognita* females, but only SBA, Con A, and UEA bound specifically (i.e., were displaced by their competitive haptens). Whether specific or nonspecific, binding of most lectins to females of *M. incognita* occurred over the entire body wall except for the head and neck. UEA bound uniformly over the outer cuticle and amphids, whereas WGA bound only to the amphids and PNA did not bind at all.

Binding of lectins to the cuticle of *A. tritici* J2, shown by an increase in fluorescence, was markedly increased by pretreatment of the J2 with periodate or lipase (Fig. 1). Binding to A-AS was not affected. Neither treatment had any effect on the binding of lectins to *M. incognita*, except for periodate oxidation of *M. incognita* eggs, where GSII and SBA binding was inhibited. FITC did not label nematodes.

## DISCUSSION

Binding of lectins differed between *Meloidogyne incognita* race 3 and *A. tritici* and among life stages of each species. *Anguina tritici* juveniles had a greater diversity of cuticular binding sites for the lectins tested than did adults, whereas the opposite was true for *M. incognita*. Binding of lectins to A-AS of *M. incognita* was greater for the J2 than for postinfective stages and greater than binding to A-AS of *A. tritici*. The significance of these differences may relate to the functions that have been suggested for A-AS glycoproteins: chemical mediators in chemotaxis and host recognition (7) or elicitors of phytoalexins in defense responses of hosts (8). If, indeed, the differences we observed are function related, then they could reflect differences in host preference and parasitic habit.

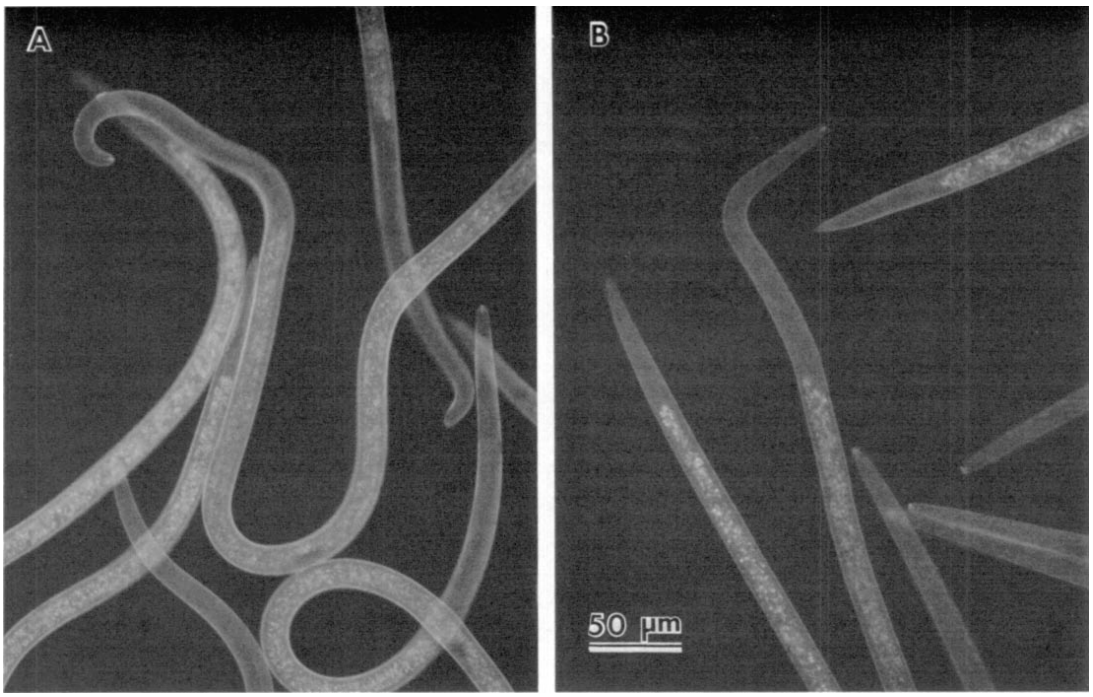


FIG. 1. *Anguina tritici* labeled with fluorescein-conjugated *Arachis hypogaea* agglutinin. A) Pretreated with 10 mM sodium metaperiodate. B) Not pretreated.

Reduction of surface carbohydrates specific for GSII, PNA, UEA or WGA during maturation of *A. tritici* supports the concept that there may be age-related attrition in specific cuticular binding sites as nematodes develop to maturity (3,6). That the opposite seems to be the case for *M. incognita* could result from chemical changes accompanying morphological modifications of the cuticle during maturation. Cuticular morphology changes dramatically as the nematode develops from a preparasitic juvenile to a parasitic juvenile, and the cuticle of the adult female bears little resemblance to that of the J2 (1,12).

Because of the strong FITC-conjugated lectin binding to *M. incognita* females, it is difficult to explain the apparent lack of glycoproteins in cuticular preparations of *M. incognita* adult females purified with SDS and extracted with β-mercaptoethanol (12). Detergents strip surface antigens from nematodes (11), and glycans on the surface of *M. incognita* may have been lost during purification procedures utilizing SDS. Alternatively, extractable surface glycoproteins could occur in quantities below the limits of detection on electrophoretic gels treated with Schiff's reagent or other stains of glycoproteins and amplification procedures may be required for visualization (4).

Surface glycans may also exist as glycolipids, but pretreatment with lipase had no observable effect on the fluorescence of *M. incognita* treated with FITC-conjugated lectins. That lipase enhanced the binding (fluorescence) of lectins to *A. tritici* but not to *M. incognita* suggests at least two possibilities for the nature of the surface glycans: The sugar residues in *A. tritici* are masked by a lipid layer that is susceptible to lipase, or there is a lack of digestible lipids on the surface of *M. incognita* J2.

Periodic acid can cleave selectively by oxidation of molecules that bear adjacent hydroxyl groups (i.e., glycols). According to Schrevel et al. (15), optimum cleavage occurs under the conditions used during this investigation. Periodate treatment of *A. tritici* J2 probably exposes glycosyl residues of glycans whose conformation otherwise masks lectin binding sites. Presum-

ably these sites are subsurface, because periodate oxidation under more rigorous conditions (room temperature, exposure to light) stripped the surface coat but did not eliminate the binding of WGA (unpubl.).

Another concern that must be addressed when considering surface coats of plant-parasitic nematodes is the likely contribution of host materials. Preparasitic juveniles of *M. incognita* have no direct contact with the host and, therefore, should be relatively free of host saccharides, especially when the eggs, prior to hatching, are isolated by treatment with sodium hypochlorite (9). On the other hand, the female of *M. incognita* and all stages of *A. tritici* are in intimate contact with the host. The exception may be *A. tritici* J2 emerging from eggs dissected from green galls. But, although these J2 may be free from host contaminants within the egg, recent evidence suggests that lectins adhering to the egg shell are capable of binding to emerging J2 (Spiegel, McClure, Kahane, Robertson, and Salomon, unpubl.). The possibility that the host may produce enzymes or other substances that expose nematode surface glycans, or that stimulate the nematode to produce surface glycans, must also be considered.

While the physiological role of surface glycans on the cuticle of plant-parasitic nematodes has yet to be determined, changes that occur in glycan diversity and distribution as nematodes mature point to significant functions of these components in nematode biology and pathology.

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