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Lectin Binding to Some Developmental Stages of *Meloidogyne incognita*

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Abstract. Lectin binding to the external cuticle and the amphids of the second stage juveniles, eggs and females of *Meloidogyne incognita* was studied. Fluorophore-conjugated lectins Concanvalin A (Con A), *Phytolacca americana* (PAA), *Ulex europaeus* (UEA), and *Helix pomatia* (HPA) all bound to the cuticle; fluorescence was uniform throughout the cuticle. Con A-associated fluorescence was strong, PAA was moderate, UEA and HPA were weak, but wheat germ agglutinin (WGA) did not bind to the cuticle. Con A, WGA, PAA and UEA showed limited binding to the anterior face, but HPA was negative. All lectins tested bound to the cuticle of *Meloidogyne incognita* females showing strong fluorescence. WGA, UEA, PAA and Con A bound to nematode eggs.

Key words: cuticle lectins, amphids, root-knot nematodes, female, second stage, egg

Introduction

Surface carbohydrates of animal-parasitic nematodes are believed to be implicated in antigenicity and in the response of the host to infection [1]. Among plant-parasitic nematodes, surface carbohydrates may be involved in recognition and in specificity of nematodes with host plants and fungal pathogens [2,3]. In cells, carbohydrates exist in large and complex molecules, glycoconjugates, containing lipid (glycolipids) or protein (glycoproteins or proteoglycans) and also as polysaccharides [4]. Most cell surface carbohydrates exist as glycoprotein with the remainder being glycolipids [5].

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Cell surface glycoconjugates may be characterised readily using lectins. Lectins are proteins or glycoproteins of non-immune origin that selectively bind to carbohydrates. Lectins can be isolated from a wide variety of plant and animal sources including seeds, plant roots and bark, fungi, bacteria, seaweed, sponges, molluscs, fish eggs, body fluids of invertebrates and lower vertebrates, and mammalian cell membranes [6]. Lectins make useful probes because they vary in specificity for carbohydrates, and generally bind to either particular monosaccharides or to very short oligosaccharide sequences. Widespread-use of lectins has been made to define the carbohydrates present in cells and tissues and on the surface of whole organisms as well [7,8].

Lectin binding studies of plant parasitic nematodes have usually focused on free-living juveniles. They have shown, for example, differences in the surface carbohydrates of *Meloidogyne* races [9]. High levels of carbohydrates were reported around the amphidia [9,10,11] with lesser amount being detected elsewhere on the nematode surface.

The present study compares lectin binding to eggs, juveniles and females of *Meloidogyne incognita.*

Materials and Methods

Preparation of second stage- *Meloidogyne incognita*

A population of *M. incognita* [12,13] provided by Imperial College, Ascot, Berkshire, has been maintained at Leeds University, U.K. on tomatoes, since 1975. Infective juveniles were extracted from plants in this colony. A galled plant was selected and washed to remove soil from the roots. The roots were cut into short lengths and shaken in 0.5% sodium hypochlorite (NaOCl) for 5 min. Then preparation was poured immediately through a 250 μm sieve, and the filtrate was diluted into several litres of water. This material was allowed to settle for 30 min before the filtrate was decanted from the sediment and mixed with a sucrose solution of specific gravity (SG) 1.2. This was centrifuged for two min. at 1000g in a bench centerifuge. The eggs, which float on the sucrose, were transferred into 1 L of water. Egg number was estimated in 100 μl aliquots before the liquid was poured through a 30 μl nylon sieve. Eggs were washed off from the sieve with water and set to hatch on 30 μm nylon sieve. Eggs were washed off from the sieve with water and set to hatch on 30 μm nylon sieves in 5-10 ml of water within glass containers at 25°C.

Collection of females from roots

Root-tips of dwarf bean, previously infected with *M. incognita,* were incubated in 10 mg/ml pectolyase (Sigma Biochemical Co.) in 50 mM M2-N-Morpholino ethanesulfonic acid (MES) buffer at pH 6.0 for 1-2 hours at room temperature. The cortex and vascular tissues of the roots were then carefully separated using fine needles under a dissecting microscope and nematodes were released from the plant tissues. Nematodes were collected, and examined under a dissecting microscope. Live young egg laying females were used.

Preparation of lectins

Concanvalin A (Con A), wheat germ agglutinin (WGA), *Phytolacca americana* lectin (PAA), *Ulex europaeus* (UEA), and *Helix pomatia* lectin (HPA) were obtained as tetra methyl rhodamine isothiocyanate (TRITC) conjugates from one source (Sigma). They were made up immediately before use at 200 μg protein/ml buffer (50 mM 3-morpholino) propanesulfonic acid (MOPS) adjusted to pH 7.2 with potassium hydroxide). A preliminary experiment using Con A, suggested that 200 μg/ml was the optimum lectin concentration for experiments with both J2 and adult females.

Lectin binding to nematodes

Freshly hatched *M. incognita* second-stage juveniles and females were added to the TRITC-conjugated lectin. Control treatments consisted of nematodes in buffer (MOPS) alone and J2 in buffer plus 0.2 M of an appropriate inhibitor sugar (see the Table). After one hour at room temperature in dark, the nematodes were washed carefully with MOPS and transferred to microscope slides. Second-stage nematodes were killed using a freezer spray and viewed under the fluorescence microscope. They were examined under a 40 X oil immersion lens (Olympus) fitted to a microscope with epi-illumination (BH 2-RFL Olympus) using a green excitation filter with a 530nm additional filter and a secondary filter with a long bandpass of wave length 590 nm.

Results and Discussion

Two potential lectin binding sites on second state juveniles of *M. incognita* were studied: (i) the external cuticle and (ii) the anterior face. Con A, PAA, UEA, and HPA all bound to the cuticle and fluorescence was uniform throughout the cuticle. Con A fluorescence was strong, PAA was moderate, but UEA and HPA were weak (Fig. 1, a-b). WGA did not bind to the cuticle. Con A, WGA, PAA and UEA all showed moderate reactivity with the anterior face (Fig. 1, a-b) but HPA was negative as the autofluorescence control and inhibitory sugar control (Table).

Table 1. Lectin binding to *M. incognita*

Lectin	*M. incognita* J2			
	Egg	Cuticle	Anterior head region	Female cuticle
Con A	+++	+++	++	+++
WGA	+++	0	++	+++
PAA	+++	++	++	+++
UEA 1	+++	+	++	+++
HPA	ND	+	0	+++
Autofluorescence control		0	0	0
HPA in N-acetyl glucosamine		0	0	
PAA in glucose		0	0	

Con A = Concanavalin A; WGA = Wheatgerm agglutinin; PAA = *Phytolacca americana* lectin; UEA = *Ulex europaeus* lectin; HPA = *Helix pomatia* lectin, 0 = Fluorescence not detected, + = weak; ++ = moderate; +++ = strong fluorescence; ND = not determined. *All lectins and sugars were made up in MOPS buffer.

The cuticle was the principal lectin binding site on females of *M. incognita*. All lectins tested bound to cuticle and all showed strong fluorescence (Fig. 1,c). Several lectins (WGA, UEA, PAA & Con A) recognised the egg shell. (Fig. 2a).

The M. incognita Race 2 population used in the present study and the lectin binding reported in this work is consistent with the findings for this race by [9].

Major differences between second stage, juveniles and females can be seen. Much WGA bound to female cuticle but none to second stage cuticle (Fig. 2b). UEA and HPA showed little binding to second stage cuticle but strong fluorescence was seen on females incubated with these lectins. It is possible that binding of lectins to the anterior face of *M. incognita* female was masked by the strong binding to the cuticle. It is clear, however, that considerable changes in surface carbohydrates take place between the free living J2 and parasitic adult. These may be adaptations to life within the host plant.

PAA bound strongly to the cuticle of *M. incognita* juvenile, but wheat germ agglutinin did not. Although both of these lectins reacted with N-acetyl glucosamine residues, this result may be explained by the fact that PAA is thought to bind to oligosaccharides consisting of three N-acetylglucosamine residues (N,N′,N″ chitotriose) [14], while WGA binds to two such residues (N,N′ chitobiose) [11,15,16]

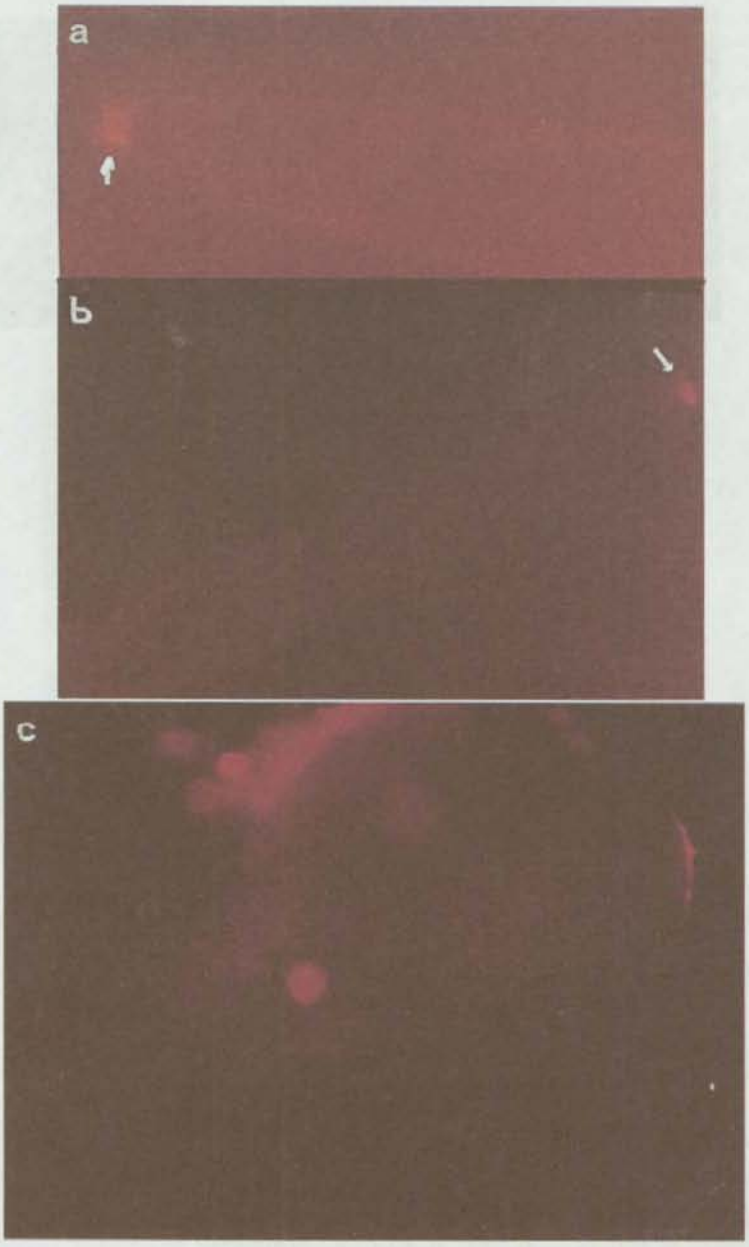

Fig. 1. Binding of fluorescent lectins to nematodes. a) Binding of Con A to *M. incognita* J2. b) Binding of UEA 1 to *M. incognita*. c) Binding of WGA to female *M. incognita*. Arrows show regions of binding to anterior face of nematodes.

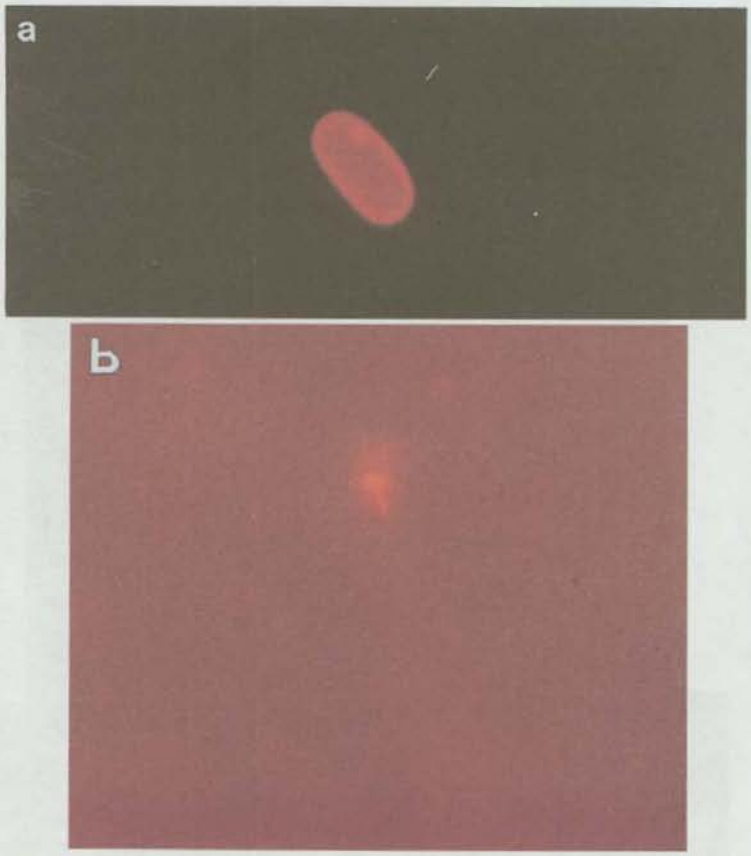

Fig. 2. Binding of fluorescent lectins to different stages of nematodes. a) WGA binding to *M. incognita* egg. b) WGA binding to *M. incognita* J2, head region.

found that a wide range of lectins exhibited strong binding to the surface of *M. javanica* female. The results of that study were broadly similar to this except that labelled PAA gave only slight fluorescence on *M. javanica* whereas strong fluorescence was seen in *M. incognita* .

Lectin binding sites at the anterior end of the nematode are generally considered to be associated with the amphidia [9,10,11,17] although binding sites elsewhere on the head have been reported by [18]. Amphidial secretions have been suggested to be the source of cuticle surface carbohydrates [19]. In support of this, [11] detected an increase in binding of Con A and WGA to J2 of *M. javanica* during aging. Freshly hatched juveniles had few binding sites for these lectins, but these increased by 24 hrs post- hatching.

The results of this study suggest that the cuticle of *M. incognita* second stage juvenile contains a restricted complement of exposed carbohydrates. The principal carbohydrates are mannose and/or glucose and N-acetylglucosamine, and there are lesser amount of L-fucose and D-galactose; while the cuticle of the adult female has a wider range of sugars (mannose/glucose, L-fucose, galactose, N-acetylglucosamine and/or N-acetyl neuraminic acid) and larger amount of these residues [11]. These results could form the basis for future studies into interactions between nematodes and their host plants.

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التصاق مجموعة من البروتينات النباتية Lectin بمراحل مختلفة من تطور نيماتودا تعقد الجذور *Meloidogyne incognita*

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ملخص البحث. تم استخدام أنواع من البروتينات النباتية (ليكتن)، فكان لها مقدرة للالتصاق بمراحل مختلفة من تطور نيماتودا تعقد الجذور *Meloidogyne incognite* ، وتم الاستعانة باستخدام تقنية المركبات المميزة وميضًا للتعرف على درجة التصاق هذه البروتينات بالبيض والطور اليرقي الثاني والإناث وكانت النتائج كالتالي:

أولًا التصاق البروتينات HPA-UEA-PAA-ConA على الكيوتيكل مع ظهور اختلاف في شدة الوميض من بروتين لآخر. أما البروتين WAG فلم يلتصق بالكيوتيكل.

ثانيًا: التصاق البروتينات السابقة بمقدمة النيماتودا كان متماثلاً مع أربعة بروتينات. أما بروتين HPA فلم يلتصق بالمقدمة.

ثالثًا: التصاق البروتينات المستخدمة في هذه الدراسة بإناث وبيض نيماتودا تعقد الجذور كان قويًا.