Lectin Binding to Meloidogyne javanica Eggs

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The egg shell is one of the most important and least understood of the nematode's protective membranes. The egg shell of Meloidogyne incognita consists mostly of proteins (50%), chitin (30%), and traces of lipids and unidentified materials (1). In the present work we used fluorescein isothiocyanate (FITC)-labeled lectins (2) to identify the carbohydrate residues on the gelatinous matrix and the egg shells of the root-knot nematode, Meloidogyne javanica.

Egg masses and egg shells were dissected and separated from infected tomato roots as described by Bird and McClure (1). The fluorescein-labeled lectins—Concanavalin A (Con A), soybean agglutinin (SBA), and wheat germ agglutinin (WGA)—were reacted with the gelatinous matrix and the egg shells by the labeling technique described for neural crest cells (3).

The specificity of the observed lectin adsorption and the fluorescence microscopy observations were detected as described by Sieber-Blum and Cohen (3). Proteolytic digestion of phosphate-buffered saline (PBS, pH 7.4) washed nematodes and neuraminidase pretreatments were accomplished as described by us previously (4).

Con A and WGA caused a strong fluorescence intensity all over the outer surface of M. javanica gelatinous matrix (Fig. 1). SBA did not reveal any fluorescence in the gelatinous matrix, and pretreatment with neuraminidase or proteolytic enzymes did not change the results. Nevertheless, the presence of N-acetyl-galactosamine or galactose residues in the gelatinous matrix of M. javanica is not completely ruled out by the lectin-binding studies, since these sugars may be inaccessible to the lectin.

Egg shells of M. javanica were highly fluorescinated by FITC-labeled Con A, SBA, and WGA (Fig. 2). WGA binding provided additional proof that this outer membrane contains chitin as one of its components (1). Preincubation of lectins with their re-
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Spective haptenic sugars prevented their adsorption by the gelatinous matrix or the egg shells, whereas incubation with sugars other than the correct hapten inhibitor did not interfere with binding of the lectins. These last findings lend support to the idea that the described lectin-binding pattern was not a result of nonspecific adsorption or uptake by the gelatinous matrix or the egg shell.

Trypsin, chymotrypsin, or pronase did not abolish the fluorescence created both in the gelatinous matrix and in the egg shells. These results suggest at least two possibilities regarding the nature of the molecules bearing the receptors for these lectins on the gelatinous matrix and the egg shells. The sugar residues are included in either a polysaccharide or a glycolipid rather than a glycoprotein, or the peptides of the glycoprotein are inaccessible to the enzymes.

**LITERATURE CITED**


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Detection and Ultrastructural Description of a Larval Moul in the Egg of *Orrina phyllobia*

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Considerable work has now been done on the nematode *Orrina phyllobia* (Thorne, 1934) Brzeski, 1981, syn: *Notanguina phyllobia* (Thorne, 1934) Thorne, 1961, because of its potential as a biological control agent of the cosmopolitan weed, silver leaf nightshade (*Solanum elaengifolium*) Cav. (5,6). Detailed information on the nematode's pathological effects (7) and host range (5) is available. There are, however, some aspects of this nematode's life history which are not completely understood. One of these is if a larval moult occurs within the egg.

It is not always easy to determine if a moult has taken place within an egg, particularly if embryogenesis is rapid. Preliminary observations at Lubbock, Texas, (Orr, unpublished observations) have shown that embryogenesis in *O. phyllobia* takes about 36 h from single-celled eggs to hatching. This is particularly rapid when compared with some other plant parasitic nematodes. For instance, it is at least six times more rapid than *Meloidogyne javanica* (1) and *Anguina agrostis* (4) at their respective optima.

Occurrence of a larval moult within an egg is best detected when specimens, at the appropriate stage of development, are sectioned and viewed with the electron microscope. Therefore, eggs of *O. phyllobia* were collected and fixed in 4% buffered paraformaldehyde solution at Lubbock. The fixed eggs were shipped and examined in Adelaide, Australia. Eggs containing larvae were cracked, dehydrated, embedded, sectioned, and stained according to previously described techniques (3). Several eggs treated in this manner were sectioned and examined with a Philips EM 400 transmission electron microscope at 80 kV.

Two different stages of a moult within the egg are shown (Fig. 1). The cuticle of the first stage larva (L$_1$) of *O. phyllobia* appears to be thicker than that of *M. javanica* at this stage (2), although it is of similar

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