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Wheat Germ Agglutinin Binding to the Outer Cuticle of the Plant-parasitic Nematode *Anguina tritici*[1]

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Several carbohydrates have been identified on the surface of free-living, plant-parasitic, and animal-parasitic nematodes (1–3,5,7,8). The function of these carbohydrates is uncertain, but they could be involved in the nematode–host recognition process (9). This note reports on the occurrence and identity of carbohydrates or saccharides on the cuticle of *Anguina tritici*, a host-specific parasite that has a life cycle closely linked with that of its cereal hosts (4).

Infective, second-stage *A. tritici* juveniles were extracted from infected, seedlike wheat galls. The J2 were washed thoroughly with distilled water and phosphate-buffered saline (PBS, pH 7.4) and incubated with different lectins conjugated with fluorescein or rhodamine isothiocyanate (FITC, TRITC) as described elsewhere (2,6). The lectins used were Concanavalin A (Con A), *Dolichos biflorus* agglutinin (DBA), *Limax flavus* agglutinin (LFA), *Limulus polyphemus* agglutinin (LPA), soybean agglutinin (SBA), *Ulex europaeus* agglutinin (UEA), and wheat germ agglutinin (WGA). The conjugated lectins were obtained from Bio-Yeda (Rehovot, Israel) or Sigma (Poole, Dorset, U.K.). The specificity of the observed lectin adsorption and the fluorescence microscopy observations was determined as described elsewhere (2,6). Ferritin conjugated to WGA was obtained from Sigma; nematode treatments and preparations for transmission electron microscopy were done as described by Forrest and Robertson (2). Proteolytic digestion of PBS-washed nematodes and lipase pretreatment were accomplished as described previously (6).

Conjugated WGA was the only lectin that produced a strong fluorescence on the outer surface of *A. tritici.* The entire body surface was strongly labeled (Fig. 1), except for the head where the binding was marked only at the tip region. The specificity of this binding was confirmed by testing all the lectins conjugated with FITC, TRITC, and ferritin (Figs. 1, 3–5). Pre-incubation of WGA with its specific sugars (N-acetylglucosamine or a crude mixture of oligomers of this sugar) blocked its adsorption by the nematode cuticle. Moreover, post-incubation of the lectin-bound nematode with those specific sugars significantly decreased the fluorescence (Fig. 2). These last two findings support the view that the lectin binding pattern did not result from nonspecific labeling or uptake by the nematode outer surface.

The other lectins tested did not label the nematode outer surface, indicating that specific binding sites for these lectins are not present, or if present they are not exposed or accessible to these lectins.

Juveniles of *A. tritici* pretreated with pronase or protease did not subsequently retain WGA labeling at the tip of the head or on the body wall, indicating that the

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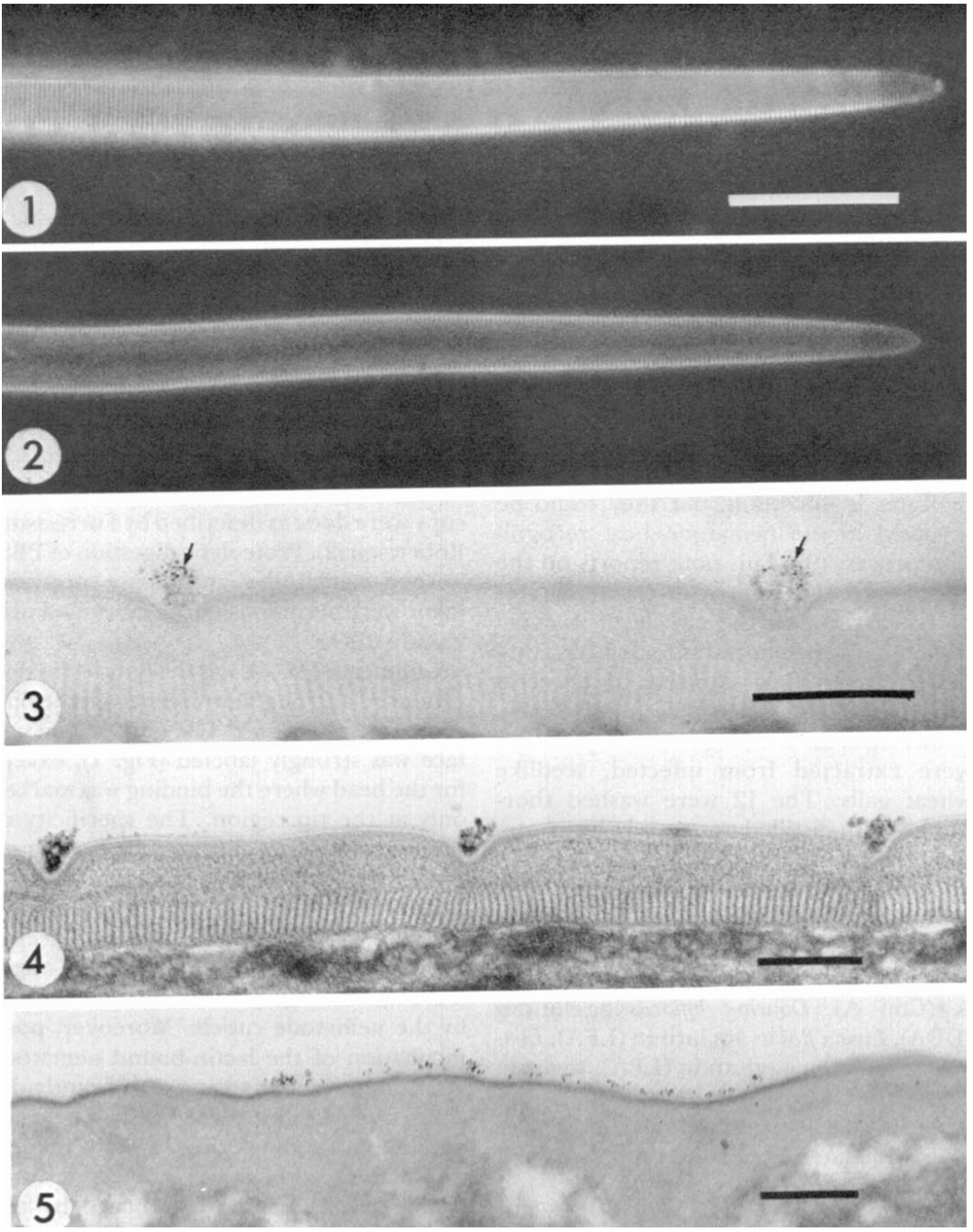

FIGS. 1, 2. Anterior end of *Anguina tritici.* 1) Juvenile labeled with WGA–TRITC. Bar represents 50 μm. 2) Reduced labeling of WGA–TRITC-labeled juvenile following incubation in N-acetyl glucosamine oligomers. Same scale as Figure 1.

FIGS. 3–5. Electron micrographs of sections through the cuticle of *A. tritici.* Bars represent 500 nm. 3) Labeling in annular grooves with WGA-ferritin (arrows). Section unstained. 4) As Figure 3 but section stained with uranyl acetate and lead citrate to show structure of cuticle. 5) Tip of head showing labeling with WGA-ferritin.

TABLE 1. Binding of wheat germ agglutinin–fluorescein isothiocyanate (WGA–FITC) conjugate to nontreated or pretreated *Anguina tritici* with different enzymes.

Treatment	Labeling intensity	
	Body wall	Tip of head
WGA-FITC (control)	+++	+++
Pronase	++	−
Protease	+	−
Lipase	++++	++++

− = no labeling; +, ++, +++, ++++ = increased intensities of labeling.

sugar residues are probably part of the surface glycoprotein, as in *Tylenchulus semipenetrans* (7). Pretreatment with lipase increased labeling by WGA (Table 1), probably because removal of lipid residues on the outer cuticle provided better access for the conjugated lectin.

Interestingly, a parasite that is specific to certain cereals, including wheat, labeled only with WGA. Whether this apparent specificity between the carbohydrate on the body surface of *A. tritici* and the lectin in the host is related to recognition of the host by the nematode or to the nematode seeking to avoid recognition by the host is uncertain.

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