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Influence of Lectins on Constricting Ring Formation by *Arthrobotrys dactyloides*

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Abstract: Incubation of *Arthrobotrys dactyloides* conidia in the presence of *Radopholus citrophilus* in lectin solutions with their corresponding sugars did not alter the stimulation of trap formation in solutions containing lectins alone. The lack of inhibition of lectin-stimulated trap formation by sugars or by lectin denaturation and the lack of lectin specificity indicate that the carbohydrate-binding regions of the particular lectins studied are not the stimulatory moieties of these macromolecules.

Key words: *Arthrobotrys*, biological control, carbohydrate, fungus, lectin, *Radopholus citrophilus*, recognition.

Glycoconjugates function in molecular recognition in many biological systems (4,15,17). Lectin probes have been used to indirectly identify carbohydrate-containing components of nematode body walls and secretions (5,16). A fungal lectin specific for N-acetylgalactosamine, which may be involved in prey recognition, occurs in the walls of traps of *Arthrobotrys oligospora* Dreschler (2,10,12,13). In contrast, Boag et al. (1) determined that attachment of adhesive knobs of *A. dasguptae* (Shome & Shome) to nematodes was not influenced by lectins or carbohydrates. The relative influence of lectin-carbohydrate binding for *A. oligospora* was less for trapping than for penetration of nematode prey (16).

Some fungi spontaneously produce rings that trap nematodes. In contrast, spores of *A. dactyloides* Dreschler germinated under flooded conditions do not produce traps spontaneously (3). Trap formation is stimulated in vitro by a thermostable factor in sterile human and guinea pig blood serum (14); by nemin, which is the liquid from mass rearing of nematodes (6,11); and possibly by contact with fungistatic soils (3,8). It is not known if lectin-carbohydrate binding is a component of the molecular stimulus for trap formation.

We investigated the influence of aqueous lectin and carbohydrate solutions on conidial germination and trap formation by *A. dactyloides* in the presence and absence of nematodes. The lectins and their competitive sugars included Concanavalin A (Con A) and methyl α-mannopyranoside or mannose, *Lotus tetragonolobus* agglutinin (LOT) and fucose, soybean agglutinin (SBA) and N-acetylgalactosamine, and wheat germ agglutinin (WGA) and N-acetylglucosamine.

Materials and Methods

Newly formed conidia were collected from cultures of *A. dactyloides* previously isolated from a citrus grove in Apopka, Florida. Approximately 30 conidia were placed in wells of flat-bottom, 96-well, microtiter plates containing 50-μl test solutions that contained no nematodes or 300 adult *Radopholus citrophilus* Huettel, Dickson & Kaplan obtained from carrot disk cultures (7). Test solutions consisted of distilled water, 0.01 M MOPS buffer (3-[N-Morpholino]propanesulfonic acid in 0.01 M $CaCl_2$, pH 6.5) with or without 100 μg/ml lectin, and 50-mM sugar solutions. An inverted light microscope was used to monitor eight replications of each treatment for germination and trap formation of 25 randomly selected conidia from each well at 16 and 40 hours after test initiation. Fluorescent microscopy (5) was used to determine if lectins bound to *A. dactyloides* conidia.

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[1] Mention of a trademark, warranty, proprietary product, or vendor does not constitute a guarantee by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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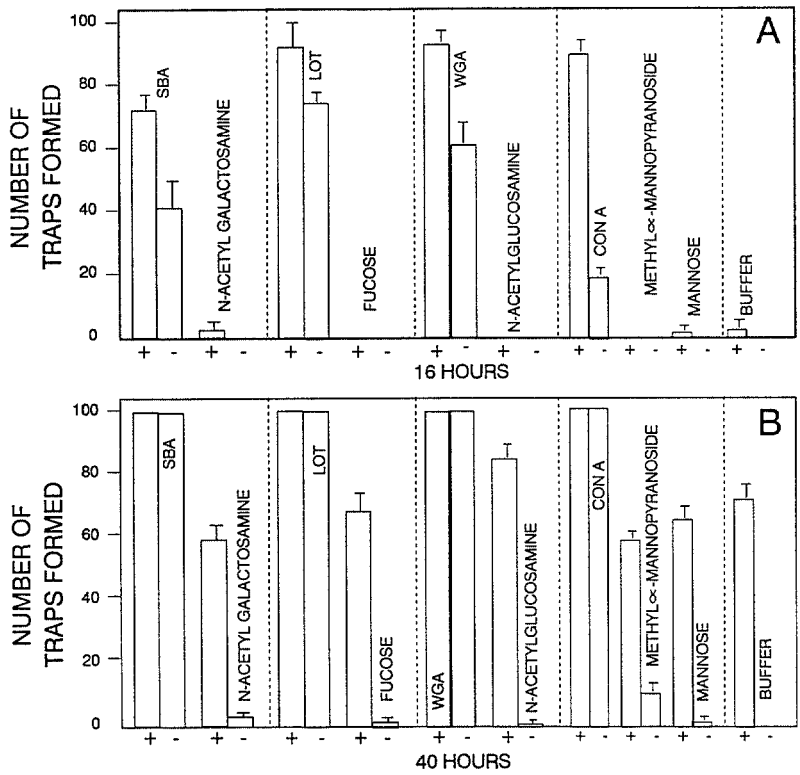

FIG. 1. Mean numbers of traps produced by the nematode-trapping fungus *Arthrobotrys dactyloides* in vitro. A) Traps formed after 16 hours of incubation in 100 μg/ml soybean agglutinin (SBA), 50 mM N-acetylgalactosamine, 100 μg/ml *Lotus tetragonolobus* agglutinin (LOT), 50 mM fucose, 100 μg/ml wheat germ agglutinin (WGA), 50 mM N-acetylglucosamine, 100 μg/ml Concanavalin A (Con A), 50 mM methyl α-mannopyranoside, 50 mM mannose, or in 0.01 M MOPS buffer (3-[N-morpholino]propanesulfonic acid) containing 0.01 M $CaCl_2$, pH 6.5 (+ = 300 adult *Radopholus citrophilus* present in microtiter well with conidia; − = no nematodes added to well). B) Traps formed in same series after 40 hours of incubation.

RESULTS AND DISCUSSION

Conidia of *A. dactyloides* germinated readily when placed in all test solutions. In the absence of nematodes, traps were rarely formed by germinated conidia incubated in distilled water, in the 0.01 M MOPS buffer, or in any 50-mM sugar solution (Fig. 1). Addition of nematodes to these control solutions resulted in a slight increase in trap formation after 16 hours but considerably increased trap formation after 40 hours. All lectins markedly increased trap formation at 16 hours of incubation, and this effect was enhanced in the presence of nematodes. At 16 hours of incubation, stimulation of trap formation in the absence of nematodes was greatest in the LOT treatments and least in the Con A treatments, but trap formation in the presence of nematodes was comparable in all lectins. At 40 hours of incubation, maximum trap formation occurred in lectin solution regardless of the presence of nematodes. Lectin and sugar solutions had little influence on nematode activity or appearance.

Incubation of conidia in lectin solutions containing 50 mM of their corresponding competitive sugars did not alter the extent of trap formation from that for lectins alone at 16 and 40 hours. Denaturation of lectins in boiling water for 30 minutes had little influence on stimulation of trap formation by lectins.

Conidia in most lectin-only and nematode-only treatments germinated to produce an initial length of mycelium prior to production of traps. However, conidia incubated in LOT often germinated directly

to traps. Heat treatment of LOT was associated with loss of this trap formation pattern.

The binding of fluorescent lectin conjugates to conidia of *A. dactyloides* was also observed. Fluorescence patterns of *A. dactyloides* conidia incubated in TRITC-conjugated lectins differed from one another. Con A–TRITC labeled both cells of the two-celled conidia, whereas SBA-TRITC labeled only the cell in each conidium that had been proximal to the conidiophore. Conidia incubated in LOT-TRITC and WGA-TRITC fluoresced from within the spore wall.

The lectins studied stimulated trap formation by *A. dactyloides,* especially in the presence of nematodes. However, the lack of lectin specificity and the limited inhibition of trap formation by heat denaturation of lectins or by incubation of lectins with their competitive sugars suggest that the carbohydrate-binding regions of the lectins studied are not the stimulatory moieties of these macromolecules. Possibly, only very small amounts of free active lectin are required for stimulation to occur or lectin peptides may stimulate trap formation. An earlier report indicates that peptides may stimulate trap formation (14) but not as effectively as intact nematodes (9).

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