Hyphal Interactions Between *Trichoderma harzianum* and *Rhizoctonia solani*: Ultrastructure and Gold Cytochemistry of the Mycoparasitic Process

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ABSTRACT

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The interaction between *Trichoderma harzianum* and the soilborne plant pathogen *Rhizoctonia solani* was studied by both scanning (SEM) and transmission (TEM) electron microscopy. Macroscopic observations of fungal growth in dual cultures revealed that pathogen growth inhibition occurred soon after contact with the antagonist. SEM investigations demonstrated that coiling of the antagonist (*T. harzianum*) around its host (*R. solani*) was an early event preceding hyphal damage. Ultrastructural observations of hyphal interactions showed that contact between the two fungi was mediated by a fine, extracellular matrix originating from cells of *R. solani*. The use of gold-complexed *Ricinus communis* agglutinin provided evidence that this matrix was rich in galactose residues. Attachment of hyphae of *Trichoderma* to cells of *R. solani* was followed by a series of degradation events in the host. The cytochemical localization of *N*-acetylglucosamine residues with wheat germ agglutinin (WGA)/ovomucoid-gold complex revealed that chitin breakdown occurred gradually, suggesting a continuous production of chitinases by the antagonist. Disorganization of the cell-wall structure of *R. solani* appeared to be an early event that promoted internal osmotic imbalances that, in turn, triggered intracellular disorders, such as retraction of the plasma membrane and cytoplasm aggregation.

Rhizoctonia solani Kühn is a widespread, soilborne pathogen responsible for serious damage in many crops including canola, carrot, and soybean (16,19,21). Brown-girdling root rot, damping-off, and seedling blight are among the most important diseases that may lead to yield losses reaching 60% in some *R. solani*-infected growing areas (26). The wide host range of this pathogen as well as its ability to survive under adverse environmental conditions as sclerotia have markedly reduced the potential of crop rotation as a management strategy. In addition, a number of currently used fungicides, such as mercuric fungicides, have been withdrawn from the market (22), minimizing the use of fungicides as a means of managing *R. solani*. As a consequence, most efforts have been directed toward developing new alternatives for more effective disease management (7,8). The discovery of new biological control agents and the demonstration of their

value in reducing disease incidence and severity have opened new promising avenues for practical applications in agriculture and for promoting environmental safety (5). In this context, considerable attention has been paid to fungal antagonists (5), mainly because of their potential for reducing the inoculum density of the pathogen (13,18,31).

Among the fungal antagonists that have shown satisfactory degrees of control against soilborne pathogens, various species of *Trichoderma* have been reported to successfully inhibit pathogen growth and development (11,12). The ability of these antagonists to attack fungal pathogens at different stages of their development has led to the concept that they could be powerful biocontrol agents (7). However, one prerequisite for a rational utilization of the biological properties of a potential antagonist is an understanding of the mechanisms underlying the mycoparasitic process. The finding that some *Trichoderma* spp. are capable of producing either antibiotics (10) or extracellular, lytic enzymes (12) or both has provided key information on the nature of the degradation events associated with antagonism. Although hyphal interactions between *Trichoderma* spp. and *R. solani* have been investigated with scanning electron microscopy (SEM) and fluorescence microscopy (9,11), little is known about the cellular events occurring during the mycoparasitic process. The present study was undertaken to provide a detailed picture of the interaction between *Trichoderma harzianum* Rifai and *R. solani*, using lectin gold cytochemistry. We demonstrate that *T. harzianum* hyphae coil around and penetrate into cells of *R. solani* causing extensive damage, such as cell-wall alteration, retraction of the plasma membrane from the wall, and aggregation of the cytoplasm.

MATERIALS AND METHODS

Fungal isolates and growth conditions. The isolate of *T. harzianum* (T-203) used in this study was isolated by Elad et al (11) and was reported as an effective mycoparasite of *R. solani* (12). The strain of *R. solani* (AG-4) was recovered from peanut and inoculated into a medium consisting of 800 g of quartz sand, 25 g (dry weight) of Cream of Wheat, 30 g of corn meal, and 75 ml of water. The mixture was incubated at 25 C for 2 wk and dried at room temperature for 3 days. The inoculum was stored at 4 C until use. Samples of the *R. solani* inoculum were allowed to grow on Difco potato-dextrose agar (PDA; Difco Laboratories, Detroit, MI) for 5 days at 25 C, and the fungus was reinoculated on freshly prepared PDA to produce separate cultures. *T. harzianum* also was cultured separately on PDA at 25 C.

Dual culture tests. Hyphal interactions between *T. harzianum* and *R. solani* were studied according to the following procedure. Mycelial disks (5 mm in diameter), cut from actively growing colonies of both fungi, were placed 3 cm apart on the surface of the PDA. Petri dishes were incubated at 25 C under continuous light. The antagonist grew radially, and overgrowth of *R. solani* by *T. harzianum* began to occur by 2–3 days after inoculation. Mycelial samples from the interaction region were collected at 2, 4, and 6 days after inoculation and processed for electron microscopy.

Scanning electron microscopy (SEM). Mycelial samples from the interaction region were vapor-fixed with 2% (w/v) osmium tetroxide in distilled water for 20 h at room temperature, air-dried, and sputter-coated with gold palladium in a Polaron E 500 sputter coater. Samples were kept in a dessicator until examination with a Cambridge Stereoscan 5-150 scanning electron microscope (Cambridge Scientific Industries, Cambridge, MA) at 20 kV. Micrographs were taken on Polaroid type 52 positive film (Polaroid Corp., Cambridge, MA) with UV-haze and 02 orange filters. Two samples per sampling time were examined.

Transmission electron microscopy (TEM). Mycelial samples from the interaction region were fixed with 3% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 2 h at 4 C and post-fixed with 1% (w/v) osmium tetroxide in the same buffer for 1 h at 4 C. Samples were dehydrated in a graded ethanol series and embedded in Epon 812 (JBEM Chemical Co., Pointe-Claire, Québec, Canada). Ultrathin sections (0.07 μm) were collected on Formvar-coated nickel grids and either contrasted with uranyl acetate and lead citrate for direct examination with a JEOL 1200 EX transmission electron microscope (Tokyo) at 80 kV or processed for cytochemical labeling. Three samples per sampling time were examined with an average of 10 grid squares per sample.

Cytochemical labeling. Colloidal gold with an average particle diameter of 12 nm was prepared according to Frens (15). To study the distribution of chitin, a linear polysaccharide of β-1,4-linked *N*-acetylglucosamine residues, wheat germ agglutinin (WGA), a lectin with *N*-acetyglucosamine-binding specificity (3), was used in a two-step procedure. Ovomucoid, a high molecular-weight glycoprotein, was chosen as a second-step reagent because of its specific binding affinity for WGA (3). This glycoprotein was complexed to colloidal gold at pH 5.4. Sections were first incubated on a drop of PBS (phosphate-buffered saline), pH 7.2, for 5 min, then transferred on a drop of WGA (25 μg/ml of PBS, pH 7.2) for 30–60 min at room temperature in a moist chamber. After thorough washing with PBS, pH 7.2, sections were incubated on the ovomucoid-gold complex (1:30 in PBS-PEG [polyethylene glycol]). Sections were washed with PBS, rinsed with distilled water, and contrasted with uranyl acetate and lead citrate.

To study the distribution of galactose, *Ricinus communis* agglutinin (RcA), a lectin with galactose-binding specificity (3), was directly complexed to colloidal gold at pH 8.0, according to a previously described procedure (4). Sections were first incubated on a drop of PBS-PEG, pH 8.0, for 5 min and transferred to a drop of the RcA-gold complex for 30 min at room temperature in a moist chamber. After washing with PBS and rinsing with distilled water, sections were contrasted with uranyl acetate and lead citrate before examination by TEM. Two samples per sampling time were examined with an average of 10 grid squares per sample.

Specificity of the labeling obtained with both lectins was assessed through various control tests. For the indirect labeling with WGA, control tests included: 1) incubation with WGA to which was previously added *N*-*N'*-*N"*-triacetylchitotriose (1 mg/ml in PBS); 2) incubations with WGA, followed by uncomplexed ovomucoid and finally by ovomucoid-gold complex; and 3) direct incubation with gold-complexed ovomucoid with the lectin step omitted. For direct labeling with RcA, control tests included: 1) incubation with gold-complexed RcA, to which was previously added D-galactose (1 mg/ml in PBS); 2) incubation with an albumin-gold complex; and 3) incubation with the gold suspension alone.

Quantification of labeling. The density of labeling obtained with the WGA/ovomucoid-gold complex over cell walls was determined by counting the number of gold particles per square micrometer. Area determinations were carried out by the point-counting method established by Weibel (30), using negatives of electron micrographs projected on a lattice. Amount of labeling over specified wall areas (Sa) was estimated by counting the number of gold particles (Ni) on a photographic enlargement. Density of labeling (Ns) was calculated as follows: $Ns = Ni/Sa$, in which *Ns* represents the number of gold particles per unit surface.

Reagents. Lectins and glycoproteins were purchased from Sigma Chemical Co. (St. Louis, MO). PDA was obtained from Difco Laboratories, and tetrachloroauric acid was obtained from BDH Chemicals (Montreal). All other reagents and materials for electron microscopy were purchased from JBEM Chemical Co., (Pointe Claire, Québec, Canada).

RESULTS

Fungal growth in dual cultures. Examination of the extent of fungal growth in dual cultures revealed that the first apparent contact between hyphae of both fungi occurred within 2 days after inoculation. In the following days, *R. solani* mycelium was

totally encircled by *Trichoderma* hyphae, which grew very rapidly and colonized the agar. By 4 days after inoculation, overgrowth of the host colony was observed and *T. harzianum* began to sporulate abundantly.

SEM observations. Mycelial samples collected from the interaction region at different times after inoculation were observed with SEM (Fig. 1). The antagonist, *T. harzianum*, was most often distinguished from *R. solani* by hyphal diameter (Fig. 1A). Indeed, the average diameter of *T. harzianum* hyphae was estimated to be 2 μm, whereas those of *R. solani* hyphae ranged between 3 and 6 μm. At an early state of parasitism (2 days after inoculation), *T. harzianum* hyphae established close contact with the host by coiling around the hyphae. The coils were usually very dense (Fig. 1A) and appeared to tightly encircle the hyphae of *R. solani* (Fig. 1B). However, at this stage of the interaction, integrity of the cell surface of the host was well preserved (Fig. 1B).

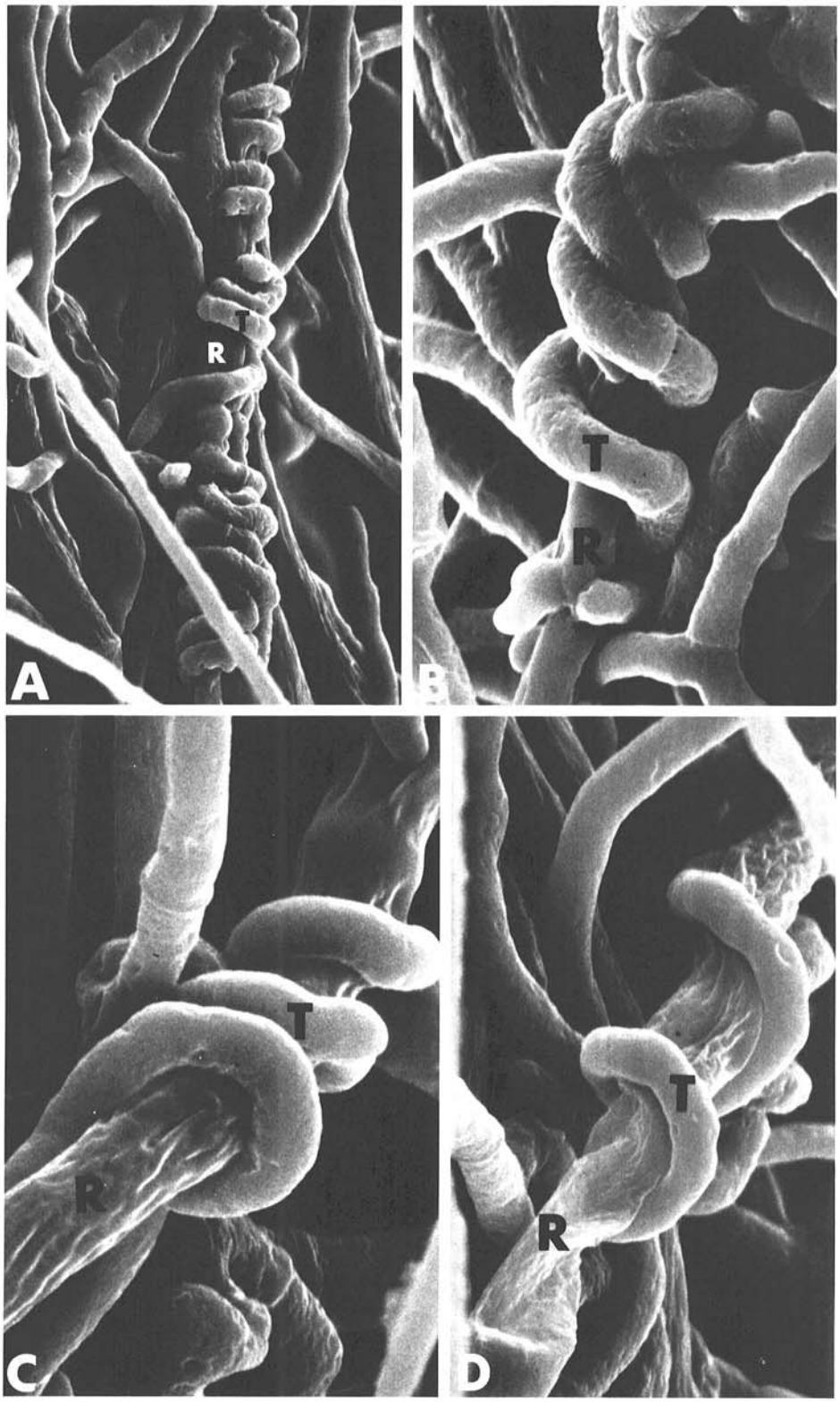

Fig. 1. Scanning electron micrographs of *Trichoderma harzianum* (T) hyphae interacting with cells of *Rhizoctonia solani* (R) in dual cultures. **A and B,** Two days after inoculation. *Trichoderma* hyphae form dense coils and tightly encircle hyphae of *R. solani*. **A,** 2,500×; **B,** 5,000×. **C,** Four days after inoculation. Early signs of collapse are visible in a *R. solani* hypha surrounded by *Trichoderma*; 6,000×. **D,** Six days after inoculation. There is loss of turgor and marked cell collapse in *R. solani*; 5,000×.

By 4 days after inoculation, the antagonist multiplied abundantly and coiling persisted (Fig. 1C). Early signs of collapse, as shown by the wrinkled appearance of the host cell surface, were often observed (Fig. 1C). Such features of cell alteration were not seen with unparasitized hyphae of *R. solani* (data not shown). Pronounced collapse and loss of turgor of *R. solani* hyphae were among the most typical features of alteration observed by 6 days after inoculation (Fig. 1D). In addition, cell-wall breakdown and hyphal disintegration occasionally were observed (data not shown). These SEM observations indicated that coiling of the antagonist around the pathogen was an early event preceding hyphal damage. A more precise investigation at the TEM level was essential to elucidate the nature of the antagonist–pathogen interaction.

TEM observations and cytochemical localization of *N*-acetyl-glucosamine residues (chitin). Ultrathin sections from individual colonies of either *T. harzianum* (Fig. 2A) or *R. Solani* (Fig. 2B and C) were incubated with the WGA/ovomucoid-gold complex. With both fungi, an intense labeling was specifically associated with the cell wall. In contrast, cytoplasm, organelles and vacuoles were nearly devoid of labeling. Gold particles also were distributed over septa (Fig. 2B and C) as well as over the triangular junctions

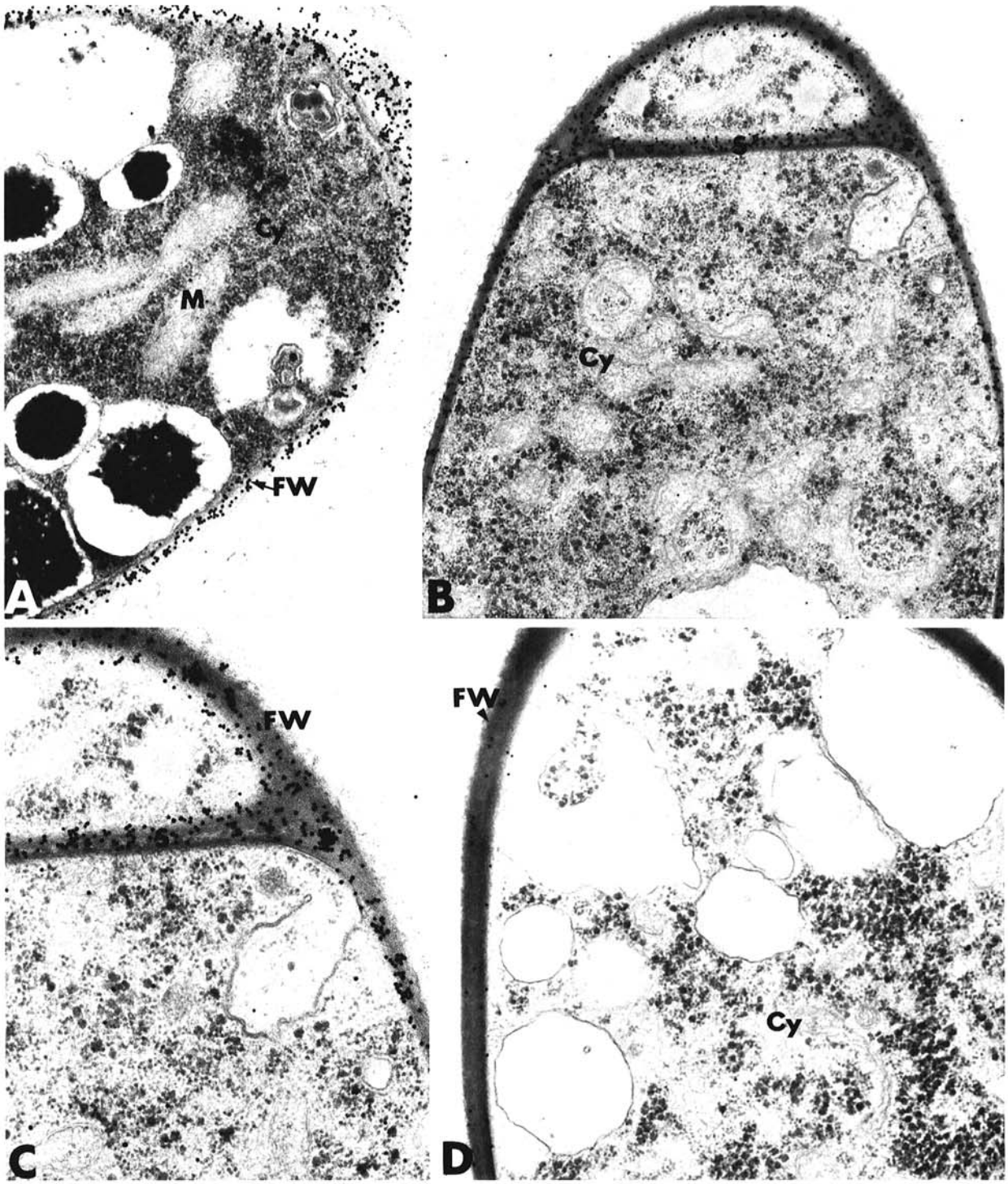

Fig. 2. Transmission electron micrographs of **A,** *Trichoderma harzianum* and **B–D,** *Rhizoctonia solani* in pure cultures. Labeling of chitin with the wheat germ agglutinin (WGA)/ovomucoid-gold complex. **A,** A regular deposition of gold particles occurs over the wall (FW) of a *T. harzianum* cell. Cytoplasm (Cy) and organelles such as mitochondria (M) are unlabeled; 27,000×. **B,** Labeling also is associated with the cell wall and septum (S) in *R. solani*, whereas the cytoplasm is unlabeled; 22,000×. **C,** Portion of **B** at a higher magnification showing the distribution of labeling over the cell wall; 36,000×. **D,** Control tests. Labeling is markedly reduced after adsorption of the WGA with *N*-*N*′-*N*″-triacetylchitotriose; 27,000×.

between septa and lateral walls (Fig. 2C). Control tests, including previous adsorption of WGA with *N-N'-N"*-triacetylchitotriose, resulted in a considerable reduction in cell-wall labeling (Fig. 2D).

Examination of ultrathin sections of the interaction region of 2-day-old dual cultures showed that cells of *T. harzianum* frequently encircled hyphae of *R. solani* (Fig. 3A). At low magnification, the structural integrity of the protoplasm of *R. solani* appeared intact (Fig. 3A). However, a close examination at a higher magnification revealed a slight retraction of the plasmalemma from the wall (Fig. 3B, arrows) and a decrease in the electron density of the outermost wall layers (Fig. 3B). Encircled cells of *R. solani* appeared delimited by shredded and loosened cell walls. Interestingly, cell walls of both the antagonist and the pathogen appeared diffuse when in close contact (Fig. 3C, double arrows). It was often difficult to clearly distinguish the two cell walls, although the innermost cell-wall layers of *R. solani* displayed a higher electron density than did the outer cell-wall layers (Fig. 3C). Incubation of these sections with the WGA/ovomucoid-gold complex resulted in the deposition of gold particles over cell walls of both fungi (Fig. 3B and C). However, labeling was irregularly distributed over *R. solani* cell walls (Table

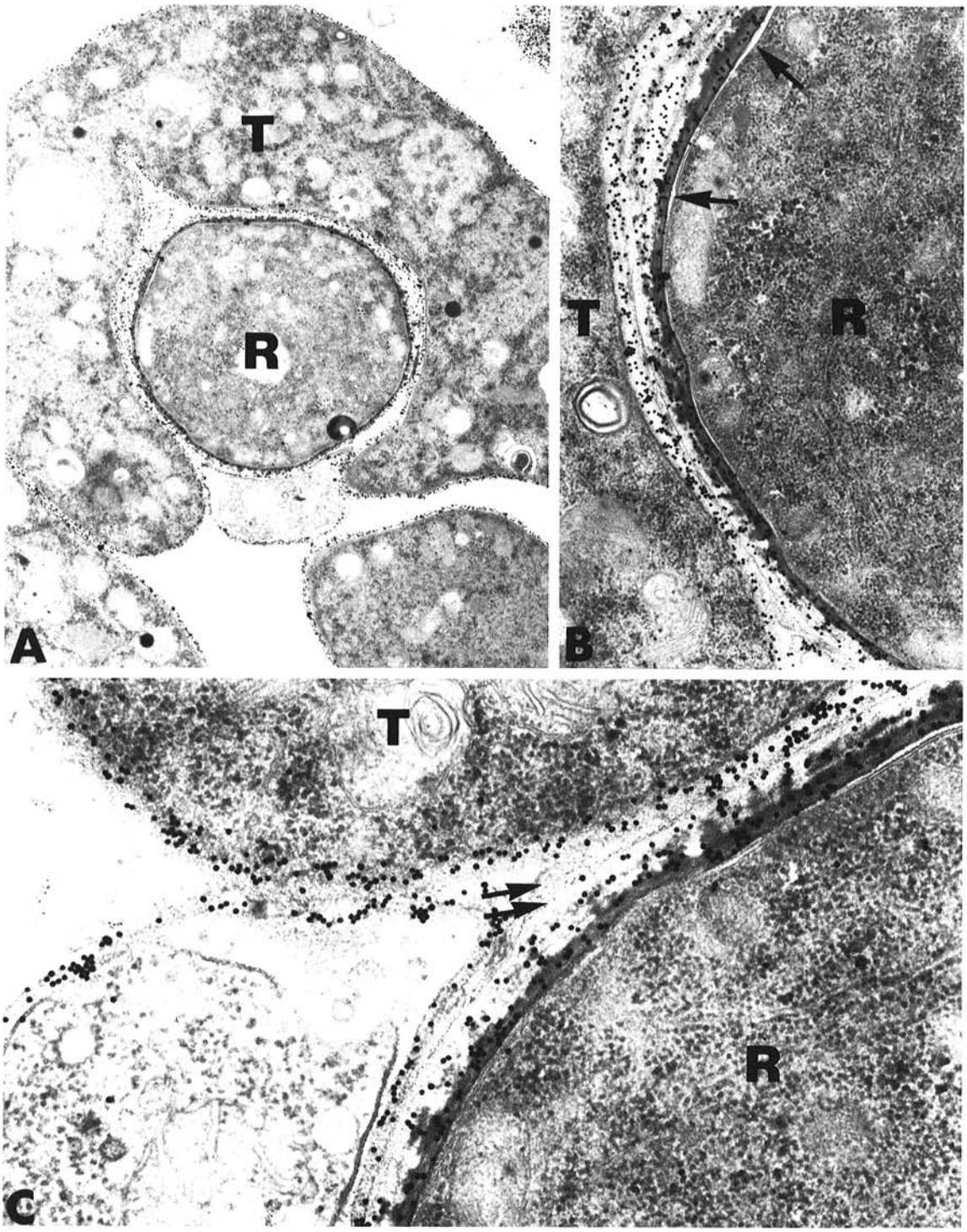

Fig. 3. Transmission electron micrographs of dual cultures between *Trichoderma harzianum* and *Rhizoctonia solani* 2 days after inoculation. Labeling of chitin with the wheat germ agglutinin (WGA)/ovomucoid-gold complex. **A,** A hypha of *T. harzianum* (T) is encircling a cell of *R. solani* (R); 9,000×. **B,** Portion of **A** at a higher magnification showing an early degradation of the outermost wall layers of the encircled *R. solani* hypha. Discrete signs of plasma-membrane retraction are visible (arrows); 27,000×. **C,** Labeling is significantly reduced over the outermost wall layers of *R. solani*, which are in apparent continuity with the antagonist cell wall (double arrows); 54,000×.

1). The electron-dense, innermost cell-wall layers were always more intensely labeled than were the outer, loosened wall layers over which only a few scattered gold particles could be seen (Fig. 3C; Table 1).

By 4 days after inoculation in dual cultures, pronounced alterations of *R. solani* hyphae were visible (Fig. 4). Observation of sections from the interaction region showed that the antagonist caused extensive damage to the host (Fig. 4B). Nearly all *R. solani* hyphae examined (~90%) exhibited obvious signs of disintegration. One of the most typical features of reaction was the retraction and often the rupture of the plasma membrane (Fig. 4A, arrow). This was associated with a marked cytoplasmic disorganization (Fig. 4A and B). In most cases, organelles were no longer discernible in the aggregated cytoplasm (Fig. 4B). Electron-dense inclusions as well as vesicular structures were deposited between the retracted plasma membrane and the cell wall (Fig. 4B, arrowhead). The host cell wall was reduced to its innermost layers. The only remnant of the outer cell-wall layers appeared to be a fine strand of fibrillar material that detached from the host cell wall and adhered closely to the antagonist cell wall (Fig. 4A arrowhead). The innermost cell-wall layers generally were more altered than at an earlier stage after inoculation and, in some cases, were reduced to sparse, electron-dense inclusions. After incubation with the WGA/ovomucoid-gold complex, scattered gold particles were associated with the altered innermost wall layers of *R. solani* (Fig. 4B). Quantification of labeling indicated a significant decrease in labeling intensity of these cell-wall layers as compared to that found in cells collected 2 days after inoculation (Table 1).

TABLE 1. Density of labeling obtained with the WGA/ovomucoid-gold complex over cell walls of *Rhizoctonia solani* during interaction with *Trichoderma harzianum*

Time after inoculation in dual cultures (days)	Gold particles/$\mu m^2 \pm$ SD[a]	
	Outer layer	Inner layer
Control[b]	61.1 ± 16.8	92.5 ± 18.2
2	18.7 ± 4.3	73.4 ± 10.1
4	9.4 ± 3.1	24.2 ± 8.3
6	6.8 ± 5.3	21.8 ± 9.6

[a]Densities were determined by counting the number of gold particles over specified areas of cell walls on 20 micrographs.
[b]Single culture.

At a more advanced state of parasitism (6 days after inoculation), the cell wall of *R. solani* appeared as a fine, convoluted strand that was labeled by the WGA/ovomucoid-gold complex (Fig. 5A). Adhesion of the thin outermost cell-wall remnant of *R. solani* to the *T. harzianum* cell wall was visible (Fig. 5A, arrow). Hyphae of the pathogen were highly distorted and very often penetrated by the antagonist as judged by the occurrence of *T. harzianum* hyphae within the altered *R. solani* cells (Fig. 5B). A large number of *R. solani* cells appeared completely disintegrated and were reduced to traces that could only be identified by the presence of some gold particles over remaining wall fibrils (Fig. 5C, arrow) and/or by the occurrence of cytoplasmic debris.

All control tests performed on sections of samples collected from the interaction region yielded negative results (data not shown).

Cytochemical localization of galactose residues. Incubation of sections from *R. solani* grown in pure culture with the gold-complexed RcA revealed that the cell wall was surrounded by

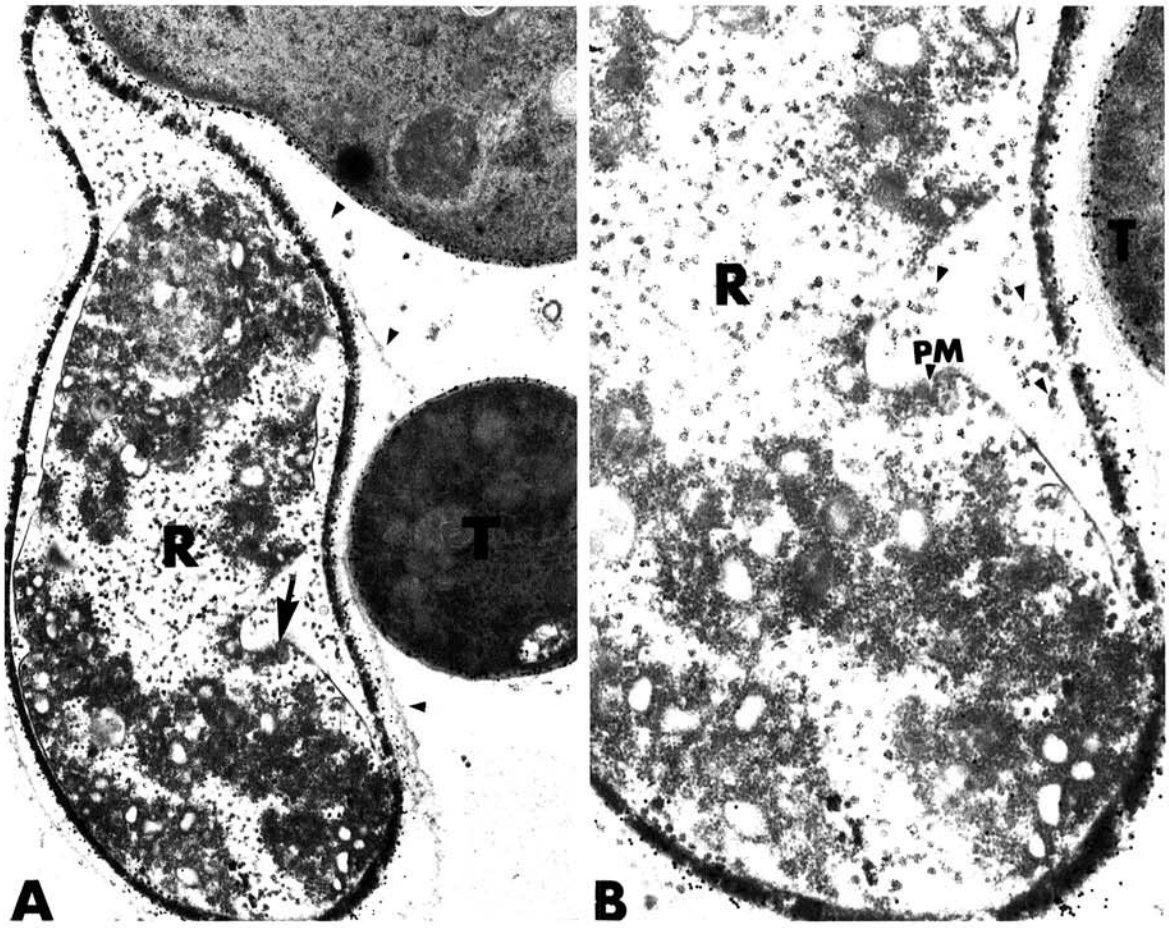

Fig. 4. Transmission electron micrographs of dual cultures between *Trichoderma harzianum* (T) and *Rhizoctonia solani* (R) 4 days after inoculation. Labeling of chitin with the wheat germ agglutinin (WGA)/ovomucoid-gold complex. **A,** A cell of *R. solani* shows signs of pronounced alteration characterized by plasma-membrane retraction (arrow) and cytoplasm disorganization; 14,500×. **B,** Portion of **A** at a higher magnification showing the formation of vesicules (arrowheads) between the altered cell wall and retracted plasma membrane (PM); 27,000×.

a fine matrix that was intensely labeled (Fig. 6A). Scattered gold particles also were associated with the electron-dense cell wall and occurred throughout the cytoplasm (Fig. 6A). Examination of *R. solani* cells in the interaction region 2 days after inoculation showed that the external matrix remained evenly labeled, although the cell wall was altered (Fig. 6B). As mentioned previously, this matrix detached from the wall of *R. solani* cells and stuck at the cell surface of *T. harzianum* hyphae (Fig. 6C).

Control tests, including previous adsorption of the RcA-gold complex with D-galactose prior to section labeling, yielded negative results as illustrated by the absence of labeling over the cell wall of *R. solani* (Fig. 6D).

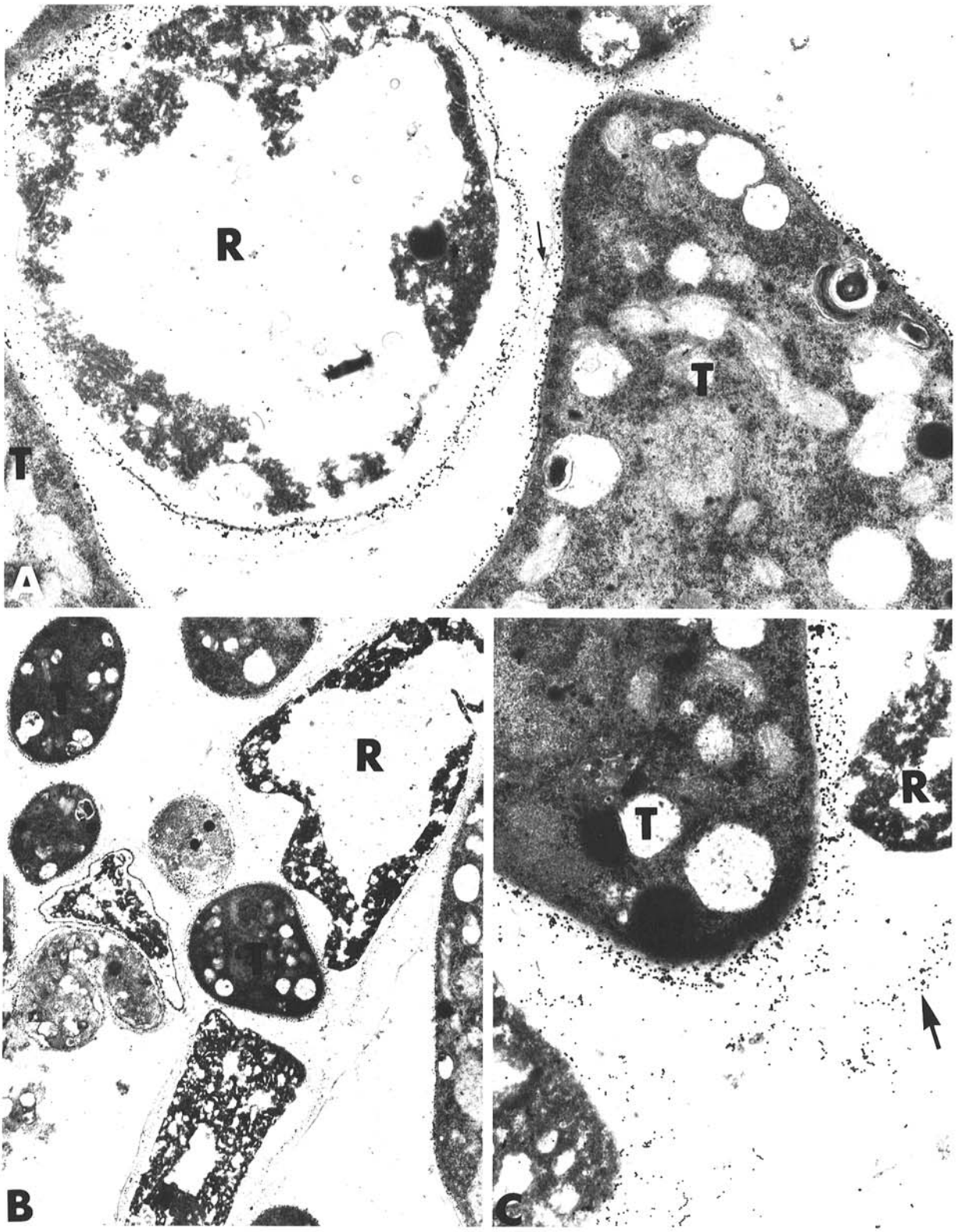

Fig. 5. Transmission electron micrographs of dual cultures between *Trichoderma harzianum* (T) and *Rhizoctonia solani* (R) 6 days after inoculation. Labeling of chitin with the wheat germ agglutinin (WGA)/ovomucoid-gold complex. **A,** The cell wall of a *R. solani* cell is reduced to a fine strand labeled by some gold particles. The fine external fibrillar matrix tends to stick on the *Trichoderma* cell wall (arrow); 18,000×. **B,** A hypha of *R. solani* is penetrated by a cell of *T. harzianum*; 5,500×. **C,** At an advanced stage of the interaction, cells of *R. solani* are disintegrated and are identified by a few labeled wall fragments (arrow); 22,000×.

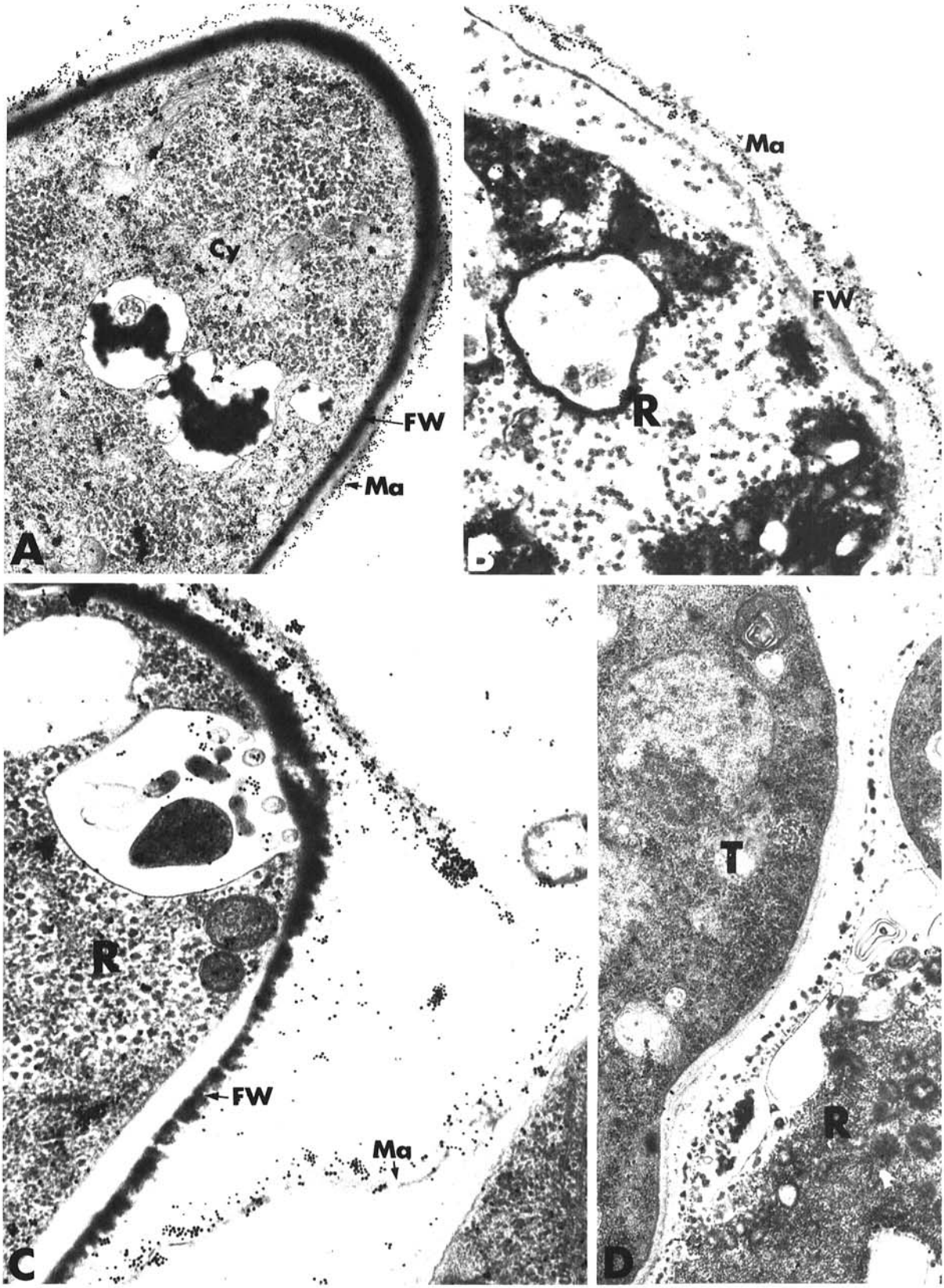

Fig. 6. Transmission electron micrographs of a single culture of **A,** *R. solani* and **B–D,** dual cultures between *Trichoderma harzianum* (T) and *Rhizoctonia solani* (R). Labeling of D-galactose with the *Ricinus communis* agglutinin (RcA)-gold complex. **A,** Gold particles (FW) are predominantly associated with an external layer surrounding the cell wall (Ma) of a *R. solani* hypha grown in single culture. Some gold particles also are scattered over the cytoplasm (Cy); 18,000×. **B,** The external layer is still regularly labeled in an altered *R. solani* hypha grown in dual cultures (4 days after inoculation). Labeling also is present over the aggregated cytoplasm; 22,000×. **C,** Detachment of the external layer of *R. solani* is accompanied by the release of fragments labeled by a few gold particles. The labeled external matrix adheres to the antagonist cell wall; 27,000×. **D,** Control test. Labeling is nearly abolished after adsorption of the RcA-gold complex with D-galactose; 22,000×.

DISCUSSION

Complementary to previous studies on *R. solani–Trichoderma* interactions (8,11–14,29), the present cytochemical investigation was undertaken to gain a better insight into the mechanisms underlying the process of mycoparasitism. Our results provide evidence that recognition events mediated by close contact between both fungi precede or coincide with major pathogen cell alterations, including cell-wall degradation, plasmalemma disorganization, and cytoplasm aggregation.

Visual estimation of fungal development in dual cultures showed that growth inhibition of the host mycelium occurred soon after contact with *Trichoderma* hyphae. This observation suggested that the mycoparasitic process between *T. harzianum* and *R. solani* did not result from the action of diffusible substances produced in advance of the antagonist itself, as has been reported for other fungus–fungus interactions (6,28). Instead, it seems likely that cell-surface interactions between both fungi are crucial in the subsequent steps leading to growth inhibition of the pathogen.

SEM investigations of the interaction region between both fungi demonstrated that damage to *R. solani* hyphae began to appear soon after coiling of *Trichoderma* around its host. This observation provided evidence supporting the assumption that the outcome of the interaction was likely determined by initial contact events that triggered firm binding of the antagonist to the host, which led to a series of events in pathogen degradation. The rapid collapse and loss of turgor of *R. solani* hyphae suggested that the antagonist produced antifungal substances in response to a signal generated by *Trichoderma* once contact between both partners was established.

TEM observations of sections from the interaction sites showed that cells of *Trichoderma* encircled and established close contact with the host mycelium. Interestingly, this contact appeared to be mediated by a fine, external matrix originating from *R. solani* hyphae. Whether a stimulus or a chemotropic response accounts for the attraction of this matrix toward cells of *Trichoderma* warrants investigation. The polysaccharidic nature of this fine layer was demonstrated with the gold-complexed *Ricinus* lectin. The occurrence of significant amounts of galactose residues in the external matrix of *R. solani* suggests that receptors with galactose-binding affinity are present at the cell surface of *Trichoderma*. Because lectins are sugar-binding proteins (20), a *Trichoderma* lectin may be responsible for the binding of the galactose-rich matrix of *R. solani*. In recent years, the key role of lectins in a number of fungus–fungus interactions has been well documented (1,2,20). A growing body of evidence from several studies indicates that lectin–sugar interactions probably correspond to early recognition events that trigger a subsequent program of specific responses, such as adhesion to, coiling around, and penetration into the host by the antagonist (17,20). A similar phenomenon is known to occur in several biological systems, including symbiotic associations in which *Rhizobia* attach to the root-hair surface of legumes provided that previous recognition between both partners is established through lectin-sugar binding (25). The present ultrastructural and cytochemical observations provide indirect evidence that a galactose-binding molecule is an important determinant in the mycoparasitic relationship between *T. harzianum* and *R. solani*. It would now be interesting to isolate and purify this molecule (lectin) from *T. harzianum* cell walls to determine its agglutinating properties toward spores and mycelium of *R. solani*.

After adhesion of *Trichoderma* hyphae to cells of *R. solani*, a sequence of degradation events including alteration of the host cell wall, retraction of the plasma membrane, and aggregation of the cytoplasm occurred. The observation that the amount of *N*-acetylglucosamine residues significantly decreased in the outer wall layers of *R. solani* correlates well with the idea that *Trichoderma* spp. display the ability to produce chitinase (6,7,12). Evidence for a gradual diffusion of the hydrolytic enzymes is that the alteration of *N*-acetylglucosamine residues occurred earlier and at a higher level in the outer than in the inner wall layers of the host. Thus, it is possible that a gradual production of chitinases by *Trichoderma* is required for penetration through a disrupted host cell wall. In that context, one may assume that chitin oligomers released from the outer wall layers of *R. solani* act as elicitors of further enzyme synthesis in a way similar to fungal elicitors of plant chitinases (24). This assumption is supported by the increase in chitinase production by *Trichoderma* when grown in the presence of chitin as the sole carbon source (12). It is also in line with previous studies indicating that the antagonistic activity of *Trichoderma* spp. is related mainly to the release of lytic enzymes, which are thought to be responsible for the cell-wall degradation of several pathogenic fungi, including *Sclerotium rolfsii* and *R. solani* (23,27). However, chitinase may not be the only enzyme responsible for the degradation of *R. solani* cell walls. It is likely that the coordinated action of several hydrolases (i.e., β-1,3-glucanases, lipases, and proteases) are required for a complete dissolution of the cell wall.

The observation that marked cellular changes such as retraction of the plasma membrane and cytoplasm aggregation occurred in cells of *R. solani* during the mycoparasitic process raises a question as to the involvement of antibiosis, in addition to enzymatic action, in host-cell degradation. *T. harzianum* may excrete toxic metabolites that interfere with the plasma membrane, causing alterations in permeability and fluidity properties. However, a disorganization of the cell-wall structure due to the hydrolysis of wall-bound compounds also may promote internal osmotic imbalances that lead to the observed intracellular disorders. This explanation is supported by our ultrastructural observations, which clearly showed that, at an early stage of the interaction, features of cell-wall degradation occurred in advance of intracellular disorders. Hence, the main action of *T. harzianum* appears to be enzymatic, although the involvement of toxic compounds has not been ruled out.

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