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Immunocytochemical Localization of Hydroxyproline-Rich Glycoproteins Accumulating in Melon and Bean at Sites of Resistance to Bacteria and Fungi

R. J. O'Connell,[1] I. R. Brown,[2] J. W. Mansfield,[2] J. A. Bailey,[1] D. Mazau,[3] D. Rumeau,[3] and M.-T. Esquerré-Tugayé[3]

[1]Department of Agricultural Sciences, University of Bristol, Agricultural and Food Research Council Institute of Arable Crops Research, Long Ashton Research Station, Bristol, BS18 9AF, U.K.; [2] Biochemistry and Biological Sciences Department, Wye College, Ashford, Kent, TN25 5AH, U.K.; and [3]Université Paul Sabatier, Centre de Physiologie Végétale, NA 241 CNRS, 31062 Toulouse Cédex, France.

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Immunocytochemistry was used to locate hydroxyproline-rich glycoproteins (HRGPs) during incompatible interactions in hypocotyls of *Phaseolus vulgaris* inoculated with *Colletotrichum lindemuthianum*, and in leaves of *Phaseolus vulgaris* and *Cucumis melo* inoculated with *Pseudomonas syringae* pv. *phaseolicola* or with *P. fluorescens* (a saprophyte). Postembedding immunogold labeling with antibodies to melon $HRGP_{2b}$ showed that HRGPs accumulated in walls of living plant cells adjoining dead hypersensitive cells during resistant reactions to both the fungus and bacteria. HRGPs were also detected in papillae encasing intracellular fungal hyphae and in small papillae adjacent to bacterial colonies. Intercellular material encapsulating cells of *P. fluorescens* also contained HRGPs. The highly localized accumulation of HRGPs at sites where the growth of bacteria and fungi is arrested supports the involvement of these structural glycoproteins in disease resistance.

Additional keywords: hypersensitive reaction.

The active resistance of plants to infection by pathogenic microorganisms involves the rapid, coordinated induction of a series of genes, leading to the synthesis and accumulation of various plant secondary metabolites (Bell *et al.* 1986; Ryder *et al.* 1986), including phytoalexins (Bell 1981), hydrolytic enzymes (Schlumbaum *et al.* 1986), and also cell wall components such as lignin (Ride 1983) and hydroxyproline-rich glycoproteins (HRGPs) (Showalter *et al.* 1985; Mazau and Esquerré-Tugayé 1986). HRGPs (extensin) are normally present in small amounts in the cell walls of higher plants (Esquerré-Tugayé and Lamport 1979; O'Neill and Selvendran 1980). The unusual, highly conserved structure of plant HRGPs has received much attention. The protein backbone is rich in basic amino acids and contains the repeating pentapeptide sequences Ser-$(Hyp)_4$. Carbohydrate comprises about two thirds of the total mass, with the unique hydroxyproline arabinosides (Hyp-Ara_{4-1}) predominating. The molecule has a helical conformation that is stabilized by the carbohydrate moiety. It has been suggested that the linear, rodlike molecules strengthen the cell wall by forming, via isodityrosine residues, an extensively cross-linked insoluble matrix interwoven with the mesh of cellulose microfibrils (Cooper *et al.* 1984; Stafstrom and Staehelin 1986).

The involvement of these structural glycoproteins in plant defense is suggested by reports that HRGP concentrations increase in some dicotyledonous plants by between 50 and 900% after infection by fungi, bacteria, and viruses (Mazau and Esquerré-Tugayé 1986), and that this accumulation occurs earlier and reaches higher levels in resistant plants (Hammerschmidt *et al.* 1984; Showalter *et al.* 1985). Enrichment of cell walls with HRGPs may restrict penetration by fungal hyphae by strengthening the wall (Esquerré-Tugayé *et al.* 1979). Having a high isoelectric point, HRGPs may also inhibit the growth of microbes by agglutination (Leach *et al.* 1982; Mellon and Helgeson 1982). However, any such role in defense requires that HRGPs accumulate at those sites at which microbial growth is restricted. Currently, there is no information on the precise location of HRGPs within infected plant tissues.

Here we report the use of ultrastructural immunocytochemistry to identify the sites of HRGP accumulation in plants infected with bacteria and fungi, using antibodies raised against an HRGP isolated from melon (*Cucumis melo* L.) callus (Mazau *et al.* 1988). Five different plant-microbe interactions were studied: melon inoculated with either *Pseudomonas fluorescens* Migula (a saprophytic bacterium) or *P. syringae* pv. *phaseolicola* (Burk.) Dows (a pathogen of bean), and French bean (*Phaseolus vulgaris* L.) infected with *P. fluorescens, P. s.* pv. *phaseolicola*, or the anthracnose fungus, *Colletotrichum lindemuthianum* (Sacc. and Mag.) Briosi and Cav. Our results show that HRGPs accumulate at the microsites where fungal and bacterial growth is restricted by plants, thus providing additional information regarding the possible role of these structural glycoproteins in disease resistance.

MATERIALS AND METHODS

Plants. Seeds of bean cultivar Tendergreen and melon cultivar Sweetheart (Sutton and Sons, Torquay, U.K.) were sown in a soilless compost, two seeds per 15-cm-diameter pot. Plants were initially grown in a glasshouse at 18–24°

Address reprint requests to R. J. O'Connell.

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C and then transferred to a growth room (22° C; 16-hr photoperiod, 40 $W \cdot m^{-2}$ at soil level) for inoculation. Seedlings of bean cultivar Kievitsboon Koekoek were grown in vermiculite in a controlled environment (25° C ± 1° C; 80% relative humidity; 16-hr photoperiod, 40 $W \cdot m^{-2}$ at soil level). After 6 days, a segment (7-cm) was excised from the center of the hypocotyl and the cut ends were sealed with wax. Hypocotyl segments were placed horizontally on glass supports over moist paper inside plastic boxes.

Bacteria and fungi. The race 3 isolate 1301A of *P. s.* pv. *phaseolicola* was obtained from J. D. Taylor (Institute of Horticultural Research, Wellesbourne, Warwick, U.K.). *P. fluorescens* (NCPPB 1964) was obtained from the National Collection of Plant Pathogenic Bacteria, Harpenden, Herts, U.K. Bacteria were maintained on nutrient agar, and suspensions of 5×10^8 cells per milliliter in sterile distilled water were prepared and inoculated into fully expanded primary leaves of bean cultivar Tendergreen as previously described (Harper *et al.* 1987). Expanded melon leaves were similarly inoculated with bacterial suspensions when plants had reached the four- to five-leaf stage.

Hypocotyls of bean cultivar Kievitsboon Koekoek were inoculated with droplets of a spore suspension ($5 \times 10^5 \cdot ml^{-1}$) of *C. lindemuthianum* race β (ATCC 62984) and incubated at 17 ± 1° C (O'Connell *et al.* 1984).

Preparation of tissue for electron microscopy. For immunocytochemistry, tissue was cut into strips that were 1-mm-wide, 0.5-mm-thick, and 3–5-mm-long (hypocotyls) or 1–2-mm squares (leaves) and fixed in 3% (v/v) glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.0). Hypocotyl tissue was fixed at 22° C for 2 hr including 10 min of vacuum infiltration, while leaf tissue was fixed at 4° C for 16 hr without vacuum infiltration. After rinsing in buffer (3 × 5 min), tissues were postfixed in 1% (v/v) buffered osmium tetroxide for 1 hr, rinsed in distilled water (5 × 5 min), and dehydrated in graded concentrations of ethanol. Tissue was infiltrated for 4 days with LR White acrylic resin (London Resin Co., Basingstoke, Hampshire, U.K.), which was then polymerized at 60° C for 24 hr. Ultrathin sections were cut with a diamond knife and mounted on uncoated gold grids (300 or 400 mesh).

For morphological studies, inoculated tissue was fixed and embedded in epoxy resin using the methods described previously (O'Connell *et al.* 1985; Brown and Mansfield 1988). The addition of 0.05% ruthenium red to the glutaraldehyde fixative helped to visualize acidic polysaccharides, notably bacterial extracellular polysaccharides (EPSs) and plant pectic polysaccharides.

Preparation and characterization of antibodies. HRGPs were solubilized at low pH from melon callus homogenate and purified by ion exchange chromatography on CM-Sepharose, followed by affinity chromatography on Heparin-Ultrogel (Mazau *et al.* 1988). The major glycoprotein fraction, designated $HRGP_{2b}$, was used to prepare antisera in rabbits, from which IgG was purified (Mazau *et al.* 1988). The IgG solution was stored in aliquots at −20° C. Antibody specificity was characterized by Ouchterlony double diffusion and immunoprecipitation followed by sodium dodecyl sulfate (SDS)-PAGE (Mazau *et al.* 1988).

Immunocytochemistry. Sections were treated with rabbit anti-$HRGP_{2b}$ IgG diluted 1:100, 1:200, or 1:500, followed

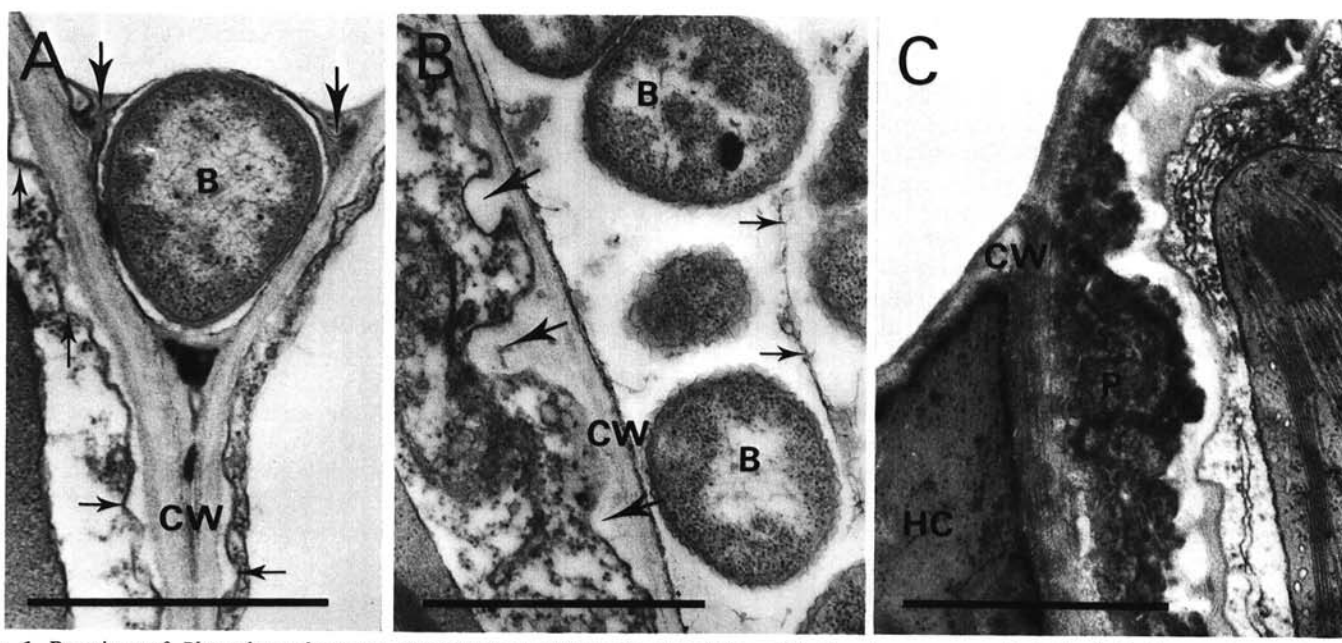

Fig. 1. Reactions of *Phaseolus vulgaris* cv. Tendergreen leaf tissue to inoculation with *Pseudomonas* spp. **A**, Cell of *P. fluorescens* attached to the cell wall (CW) at the junction between spongy mesophyll cells 12 hr after inoculation. Note the encapsulation of bacteria (B) by amorphous material (large arrows) and slight convolution of the host plasma membrane (small arrows). **B**, Cells of *P. syringae* pv. *phaseolicola* race 3 between spongy mesophyll cells 6 hr after inoculation. Note fibrillar extracellular polysaccharides (small arrows) around bacteria (B), and small electron-lucent papillae (large arrows) between the host plasma membrane and cell wall (CW). **C**, Junction between the dead hypersensitive cell and living mesophyll cell 96 hr after inoculation with *P. s.* pv. *phaseolicola* race 3. Note the disorganized electron-opaque contents of the hypersensitive cell (HC) and large electron-opaque papillae (P) in the adjacent living cell. Bars = 1 μm.

by a 1:10 dilution of goat anti-rabbit IgG conjugated with either 10- or 15-nm-diameter colloidal gold particles (Auroprobe EM GAR10/GAR15, Janssen Pharmaceutical, Wantage, Oxford) using the procedure of O'Connell *et al.* (1986). All sections were counterstained with alkaline lead citrate before examination either in the AEI Corinth 500 or Hitachi H-7000 transmission electron microscope. The specificity of antibody labeling was determined by replacing the primary antibody with 1) buffer, 2) nonimmune rabbit IgG, or 3) anti-$HRGP_{2b}$ IgG preadsorbed with an excess of pure melon $HRGP_{2b}$ (Mazau *et al.* 1988) for 2 hr at 20° C.

RESULTS

Morphological observations. The saprophyte, *P. fluorescens*, produced no visible symptoms in either melon or bean leaves, and ultrastructural studies showed that the bacterium did not multiply significantly in either plant (Brown and Mansfield 1988). Cells of *P. fluorescens* within intercellular spaces were encapsulated in a thin layer of amorphous material close to the plant cell wall. In both melon and bean, the plant plasma membrane adjacent to some cells of *P. fluorescens* became convoluted (Fig. 1A).

The pathogen *P. s.* pv. *phaseolicola* race 3 induced a hypersensitive reaction (HR) in both melon and the resistant bean cultivar Tendergreen. Infected tissues collapsed, becoming brown and desiccated by 30 hr after inoculation, and no further expansion of the lesions occurred (Brown and Mansfield 1988). Cells of *P. s.* pv. *phaseolicola* race 3 formed small colonies within the intercellular spaces of melon and bean leaves, particularly at junctions between spongy mesophyll cells. Bacterial cells were not in close contact with the plant cell wall and became surrounded by fibrillar bacterial EPSs, rather than by the amorphous material that surrounded the saprophyte. Within 3 hr of inoculation of bean and melon leaves with *P. s.* pv. *phaseolicola* race 3, the plasma membrane of mesophyll cells adjacent to bacterial colonies appeared convoluted, and by 6 hr small deposits of electron-lucent material (papillae) were present between the plasma membrane and the cell wall (Fig. 1B). After 12 hr the tonoplast of many responding cells appeared ruptured, and by 24 hr such cells contained few recognizable organelles (Brown and Mansfield 1988). Forty-eight to 96 hr after inoculation of bean leaves with *P. s.* pv. *phaseolicola* race 3, living mesophyll cells adjoining dead hypersensitive cells often contained large electron-opaque papillae between the plasma membrane and cell wall (Fig. 1C).

Conidia of *C. lindemuthianum* race β germinated to form appressoria on the surface of hypocotyls of bean cultivar Kievitsboon Koekoek and penetrated epidermal cells as described previously (O'Connell *et al.* 1985). In this incompatible interaction, restriction of fungal growth was typi-

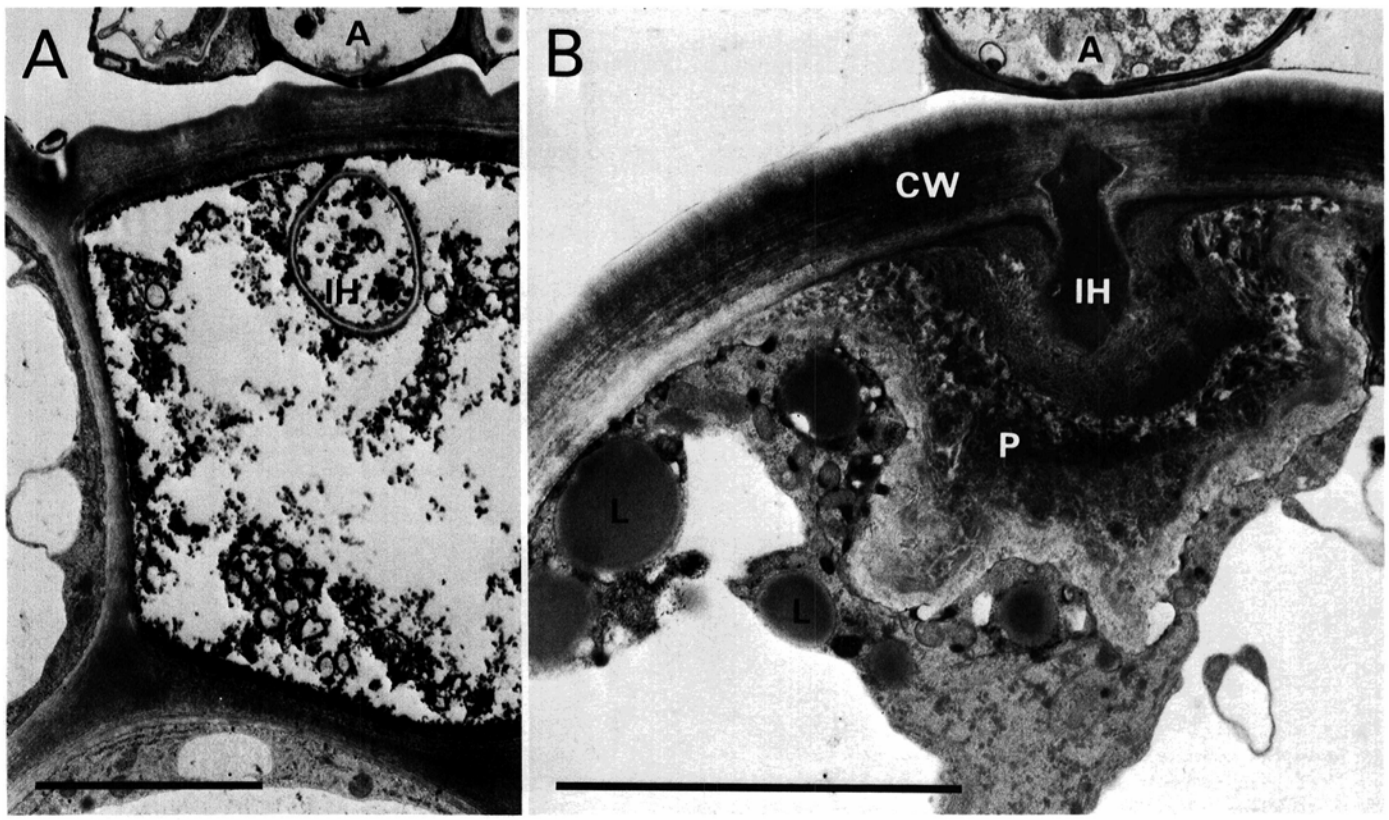

Fig. 2. Reactions of *Phaseolus vulgaris* cv. Kievitsboon Koekoek hypocotyl tissue to infection by *Colletotrichum lindemuthianum* race β 96 hr after inoculation. **A**, Hypersensitive reaction. An epidermal cell penetrated by a small infection hypha (IH) has a disorganized electron-opaque cytoplasm, whereas adjacent uninfected cells have intact cytoplasm. **B**, Papilla reaction. A small infection hypha (IH) within an epidermal cell is encased by a multilayered papilla (P). Note that the epidermal cell wall (CW) above the papilla is electron-opaque and that lipid globules (L) are abundant. Bars = 5 μm. (A, appressorium.)

cally associated with the rapid death and browning (HR) of single penetrated epidermal cells (Fig. 2A). A small proportion of intracellular hyphae became encased in a large deposit of host wall-like material (papilla) and did not develop further (Fig. 2B). Papillae had a heterogeneous, multilayered structure. At 72 hr, papillae were composed of electron-lucent material with small irregular electron-opaque inclusions. Subsequently, electron-opaque material was deposited both in the papilla and in the adjoining region of the epidermal cell wall (Fig. 2B). Host cells undergoing the papilla response remained alive (O'Connell and Bailey 1986). Previous studies have shown that the HR is associated with the race-specific resistance of bean to *C. lindemuthianum*, whereas papilla production is not a race-specific response (O'Connell *et al.* 1985).

Immunogold labeling of HRGPs. Antigenic determinants were well-preserved in tissues embedded in LR White resin; however, structural preservation was inferior to that of tissues embedded in epoxy resin and membranes were poorly contrasted. Indirect immunogold labeling on sections of uninoculated melon and bean tissues using antibodies specific for $HRGP_{2b}$ resulted in labeling of the cell wall, which decreased in intensity with distance from the plasma membrane (Fig. 3A). Cytoplasm, vacuoles, and intercellular spaces were not labeled. In uninoculated bean hypocotyl tissue, even though a higher concentration of primary antibody was used for immunocytochemistry, cell walls were consistently labeled less strongly than those of bean and melon leaves. Gold labeling of uninoculated bean hypocotyl tissue was similar to gold labeling of cortical cells remote from sites of *C. lindemuthianum* infection as shown below.

In melon leaves 6 hr after inoculation with *P. fluorescens* (Fig. 3B), antibodies bound to the region between the plant plasma membrane and cell wall at sites of membrane convolution in mesophyll cells adjoining bacteria. Some gold label was also present within intercellular spaces associated with the amorphous material encapsulating the bacterial cells. A similar distribution of gold label was observed in bean leaves inoculated with *P. fluorescens* (Fig. 3C).

In melon and bean leaves 3 hr after inoculation with *P. s.* pv. *phaseolicola*, gold label was present between the plasma membrane and wall of mesophyll cells at sites of membrane convolution adjacent to bacterial colonies. Antibodies also bound to the electron-lucent papillae present at these sites after 12 hr (Fig. 4A). The EPS surrounding bacterial cells within intercellular spaces was not labeled. At 48–96 hr, the cell walls and paramural papillae in living bean cells adjoining dead hypersensitive cells were intensely labeled, and some gold label also bound to the disorganized cytoplasm of hypersensitive cells (Fig. 4B).

In mature papillae produced in bean hypocotyls inoculated with *C. lindemuthianum*, the electron-lucent layers nearest the host plasma membrane were more intensely labeled than the inner electron-opaque regions (Fig. 5A). The walls of cells containing papillae (Fig. 5B) were labeled more strongly than cell walls in uninoculated hypocotyl tissue. The walls of living uninfected cells adjoining dead hypersensitive cells (Fig. 6A) were also labeled more intensely than walls in uninoculated tissue. As with bacterial infections of bean leaves, most gold particles bound to the inner surface of the cell wall and to the region between the wall and retracted plasma membrane. Increased labeling of the cell wall extended around the entire circumference of uninfected cells, but was confined only to cells contiguous with the hypersensitive epidermal cell. Gold labeling of cell walls more distant from the site of infection (Fig. 6C) was similar to that of cell walls in uninoculated tissue.

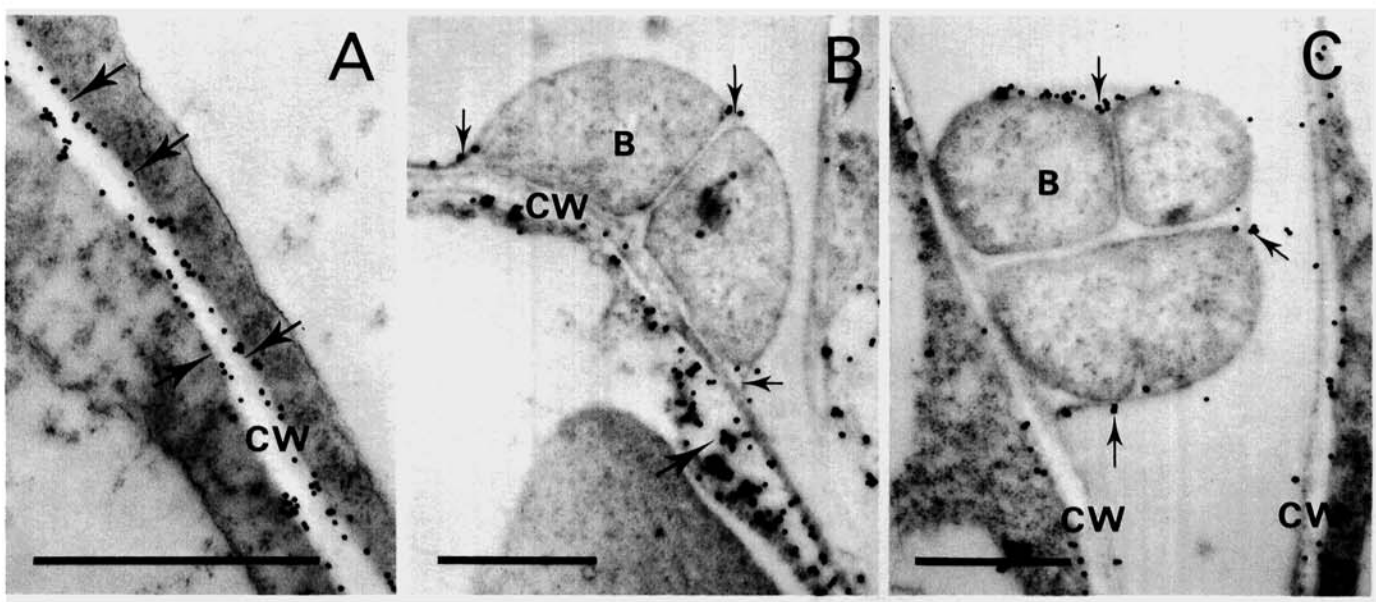

Fig. 3. Immunogold labeling (15-nm particles) of hydroxyproline-rich glycoproteins in leaf mesophyll cells. **A**, Uninoculated *Phaseolus vulgaris* cv. Tendergreen leaf tissue. Note gold particles associated with the cell wall (CW) close to the plasma membrane (arrows). Primary antibody diluted 1:200. **B**, *Cucumis melo* leaf tissue 6 hr after inoculation with *Pseudomonas fluorescens*. Note gold particles associated with amorphous material (small arrows) around bacteria (B), and intense labeling of the region between the plasma membrane and cell wall (large arrow). Primary antibody diluted 1:500. **C**, Bean cultivar Tendergreen leaf tissue 24 hr after inoculation with *P. fluorescens*. Note labeling of amorphous material (arrows) around bacteria (B). Primary antibody diluted 1:500. Bars = 1 μm.

When antibodies to $HRGP_{2b}$ were preabsorbed with excess antigen or replaced with buffer or normal rabbit serum (Fig. 6B), a low level of nonspecific binding was observed as a few gold particles randomly scattered over both tissue and clear resin.

DISCUSSION

Higher plant HRGPs are highly conserved molecules, and those from melon and bean cell walls are very similar, if not identical, in structure. HRGPs from the two plants are glycosylated with the same hydroxyproline arabinosides in the same proportions (Mazau and Esquerré-Tugayé 1986), and they show extensive homology in their amino acid sequences and in the nucleotide sequences of their corresponding genes and cDNAs (Showalter and Varner, in press). It is therefore very likely that antibodies to melon $HRGP_{2b}$ recognize shared epitopes in bean HRGPs and that the pattern of immunogold labeling observed in the present study corresponds to the distribution of HRGPs in both melon and bean tissues.

Due to the low electrophoretic mobility of intact HRGPs, western blot analysis of antibody specificity could not be performed. The possibility of cross-reaction with other cell wall glycoproteins/polysaccharides of melon and bean cannot therefore be excluded. However, several lines of evidence suggest that the antibodies are specific for the extensin-like $HRGP_{2b}$. Previous experiments, using Ouchterlony double diffusion and immunoprecipitation followed by SDS-PAGE (Mazau *et al.* 1988), showed that the antibodies recognize both glycosylated and unglycosylated epitopes in melon $HRGP_{2b}$ and do not cross-react with melon arabinogalactan proteins ($HRGP_1$), which are also rich in hydroxyproline and arabinose. The hydroxyproline arabinosides Hyp-Ara_3 and Hyp-Ara_4, characteristic of $HRGP_{2b}$, compete very efficiently with the antigen-antibody reaction in diffusion assays, and they are probably the major epitopes recognized by the antibodies (Mazau *et al.* 1988). In the present study, cell walls of infected tissues, known to contain enhanced levels of $HRGP_{2b}$ (Mazau and Esquerré-Tugayé 1986), were labeled more strongly than the walls of uninoculated tissue. Finally, preincubation of the antibodies with purified $HRGP_{2b}$ completely eliminated immunogold labeling of tissue sections.

Cell walls in fully expanded bean primary leaves were labeled more strongly than those in rapidly elongating bean hypocotyls, although labeling of the different tissues was not performed simultaneously. This finding is consistent with the increase in wall-bound HRGPs that occurs at the end of cell expansion (Van Holst *et al.* 1980; Roberts *et al.* 1985). Using immunogold cytochemistry, Cassab and Varner (1987) and Stafstrom and Staehelin (1988) similarly reported lower levels of HRGP labeling in the cell walls of young soybean seed coats and carrot roots compared to mature tissues. The latter researchers found that HRGP was uniformly distributed across the walls of carrot phloem parenchyma cells. In contrast, our results from both healthy and infected melon and bean tissues indicate that HRGP is concentrated close to the plasma membrane. HRGP was less abundant in the middle lamella region, as was reported for carrot tissue (Stafstrom and Staehelin 1988).

Our results provide the first evidence that HRGPs are present in the wall-like papillae produced by plant cells in response to infection by fungi and bacteria, although protein has been detected in papillae using histochemistry (Wolf and Fric 1981; Keon and Hargreaves 1984). Comparative studies of newly formed and mature papillae (unpublished data) suggested that HRGPs were present at all stages of development but became masked by the

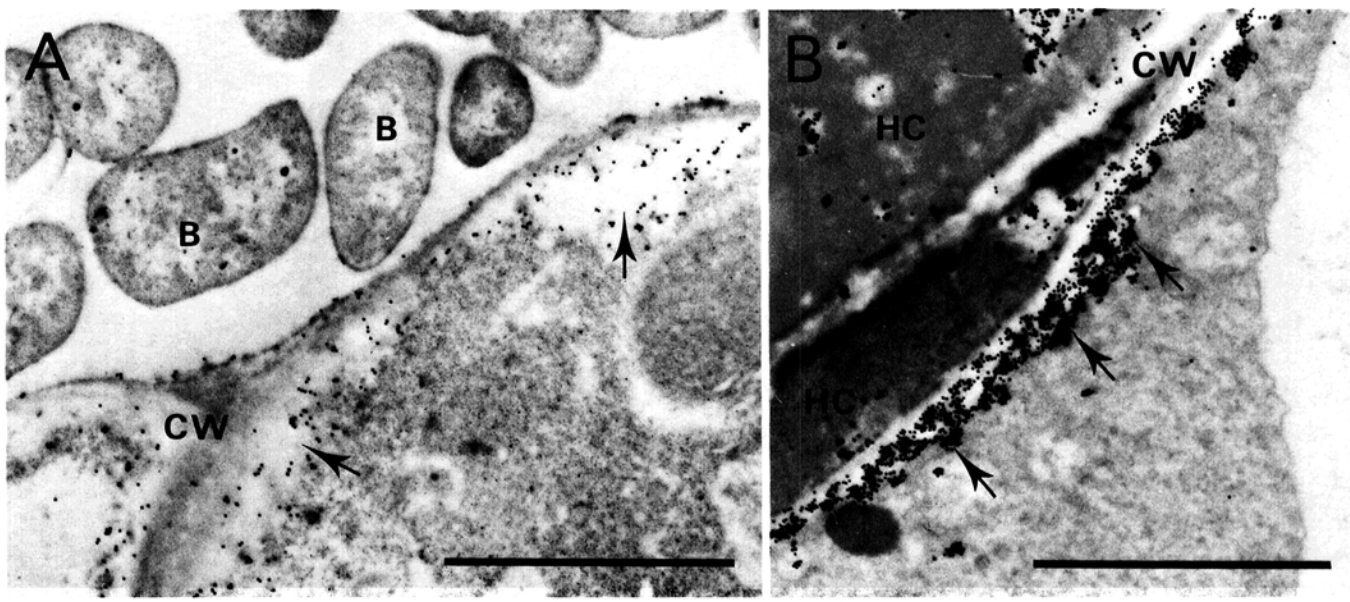

Fig. 4. Immunogold labeling of hydroxyproline-rich glycoproteins in mesophyll cells of *Phaseolus vulgaris* cv. Tendergreen leaves inoculated with *Pseudomonas syringae* pv. *phaseolicola* race 3. **A**, Twelve hours after inoculation. Note labeling of electron-lucent papillae (arrows) adjacent to bacteria (B). Primary antibody diluted 1:500, 10-nm particles. **B**, Forty-eight hours after inoculation. Note gold particles associated with disorganized cytoplasm of hypersensitive cells (HC), and intense labeling of the cell wall and papillae (arrows) in the adjoining living cell. Primary antibody diluted 1:200, 15-nm particles. Bars = 2 μm. (CW, cell wall.)

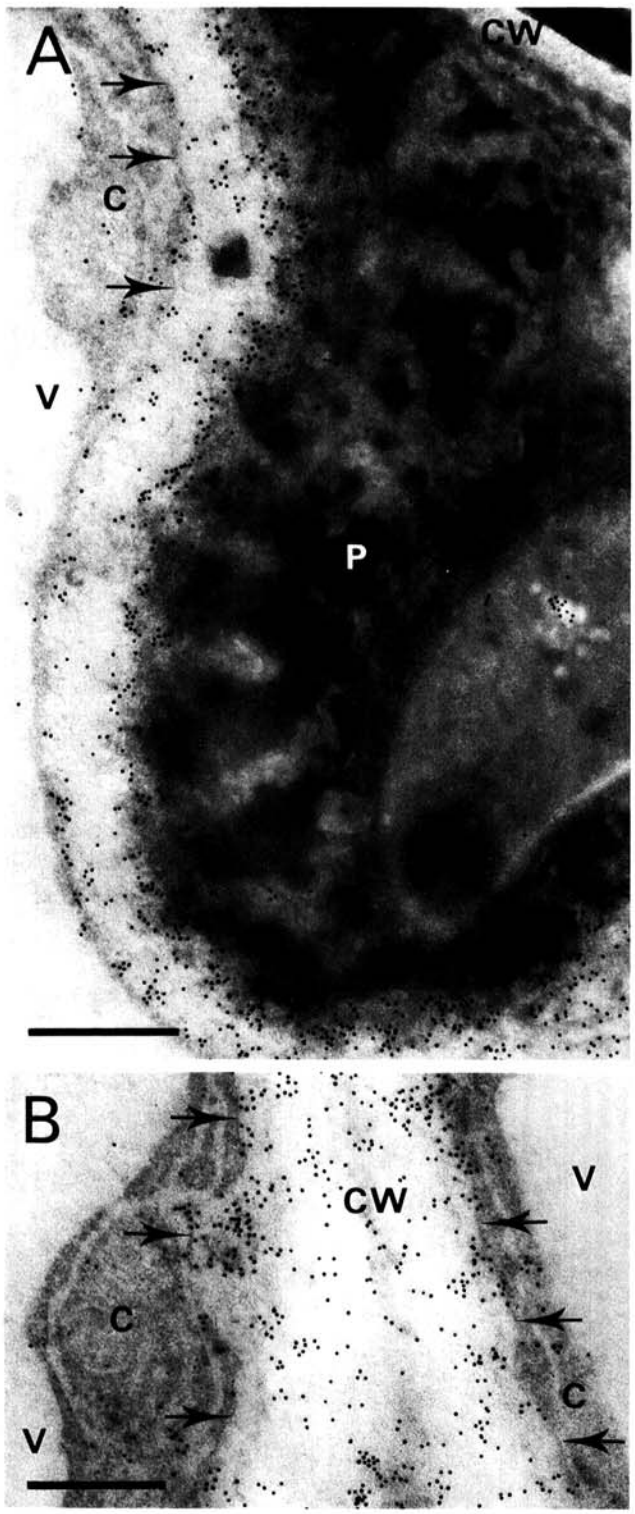

Fig. 5. Immunogold labeling (10-nm particles) of hydroxyproline-rich glycoproteins (primary antibody diluted 1:100) in a hypocotyl epidermal cell of *Phaseolus vulgaris* cv. Kievitsboon Koekoek infected with *Colletotrichum lindemuthianum* race β (96 hr after inoculation). **A**, Near-median section through a papilla (P). Note that most gold particles are associated with outer electron-lucent layers. Arrows indicate the plasma membrane. **B**, Cell containing the papilla shown in **A** (left) and an adjacent uninfected cell (right). Note the intense labeling of the cell wall (CW) and the region between the plasma membrane (arrows) and cell wall. Bars = 0.5 μm. (C, cytoplasm; V, vacuole.)

deposition of electron-opaque material. The extent of intermolecular cross-linking of the HRGPs in papillae is unknown, but it is possible that they increase the mechanical strength of papillae and so present a mechanical barrier to penetration by fungal hyphae. The recent finding that in soybean seeds HRGPs are concentrated in sclerenchyma cell walls (Cassab and Varner 1987) supports the suggestion that these glycoproteins generally have a mechanical function in higher plants. HRGPs in papillae could also provide a template for the subsequent deposition of phenolic materials such as lignin, which have been implicated in the restriction of pathogen development (Whitmore 1978; Ride 1983).

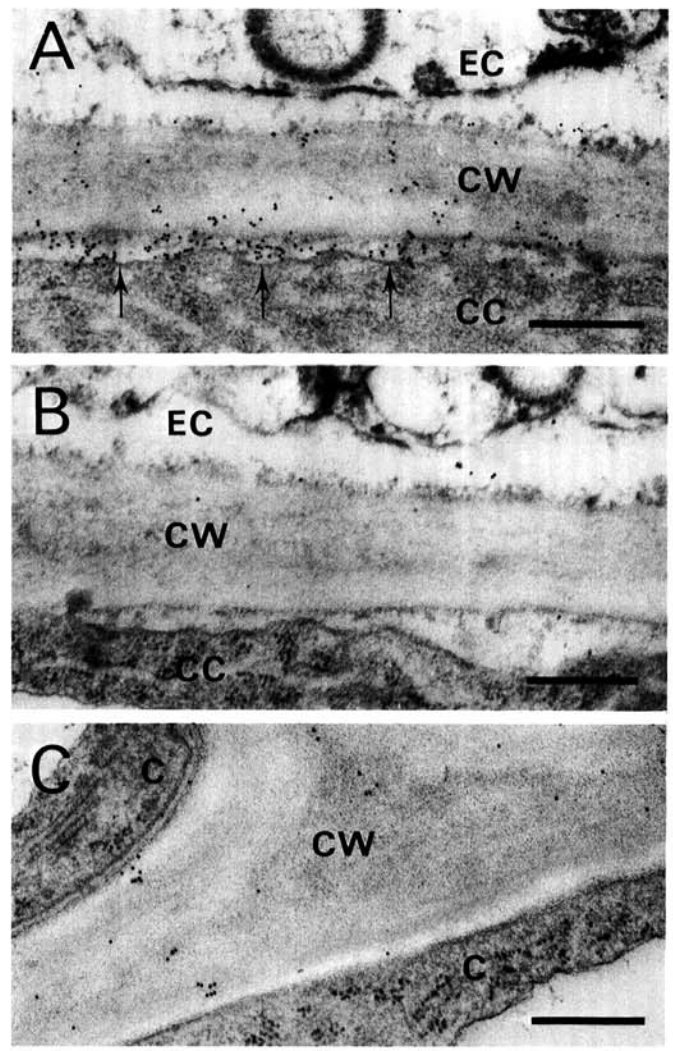

Fig. 6. Immunogold labeling (10-nm particles) of hydroxyproline-rich glycoproteins (primary antibody diluted 1:100) in hypocotyl cells of *Phaseolus vulgaris* cv. Kievitsboon Koekoek infected with *Colletotrichum lindemuthianum* race β (96 hr after inoculation). **A**, Part of a dead hypersensitive epidermal cell (EC) and an underlying living cortical cell (CC). Note that most gold particles are associated with the cell wall (CW) and the region between the cell wall and plasma membrane (arrows). **B**, Section near-adjacent to that shown in **A** treated with normal rabbit serum. Note the few gold particles scattered over the section. **C**, Uninfected cortical cells two cell layers beneath the epidermal cell shown in **A**. Note that the cell wall is labeled less intensely. The cytoplasm (C) lacks gold particles but has densely stained ribosomes. Bars = 0.5 μm.

During the hypersensitive reactions of bean hypocotyls to *C. lindemuthianum* and of melon and bean leaves to *P. s.* pv. *phaseolicola*, HRGPs accumulated in the walls and paramural papillae of living cells adjoining dead cells. A very similar HRGP distribution was observed by Stafstrom and Staehelin (1988) at the junction of living and dead cells in uninfected carrot tissue. Accumulation of HRGPs at these sites is unlikely to contribute to restricting growth of either intracellular hyphae within the lumina of dead cells or bacteria within intercellular spaces. The accumulation of diffusible inhibitors such as phytoalexins, the synthesis of which is coordinately induced with that of HRGP in bean (Showalter *et al.* 1985; Dixon *et al.* 1986), is probably more significant in resistance to *C. lindemuthianum* (Bailey *et al.* 1980) and *P. s.* pv. *phaseolicola* (Lyon and Wood 1975). However, HRGP accumulation in cell walls may be effective in restricting other pathogenic fungi having intercellular, intramural, or subcuticular modes of growth. In addition, the enrichment of cell walls with HRGP around dead hypersensitive cells could be important as a barrier to secondary infections of the necrotic tissue.

The early appearance of HRGPs in material encapsulating cells of *P. fluorescens* is consistent with the involvement of these glycoproteins in agglutination and immobilization of saprophytic bacteria on plant cell walls. This finding also indicates that secreted HRGPs are able to permeate the primary plant cell wall and enter intercellular spaces.

The correlation between HRGP accumulation and the expression of disease resistance in plants has been well-established (Esquerré-Tugayé *et al.* 1979; Hammerschmidt *et al.* 1984; Showalter *et al.* 1985; Mazau and Esquerré-Tugayé 1986). The present immunocytochemical study shows that this accumulation is highly localized to the sites of resistance at which microbial growth is restricted. Further work is now required to determine the precise mechanism by which these structural glycoproteins interact with bacteria and fungi.

ACKNOWLEDGMENTS

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