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Fine structure of the early interaction of lily roots with *Fusarium oxysporum* f.sp. *lilii*

R.P. Baayen[1] and F.H.J. Rijkenberg[2]
[1]*DLO Research Institute for Plant Protection, P.O. Box 9060, 6700 GW Wageningen, The Netherlands; Present address: Plant Protection Service, P.O. Box 9102, 6700 HC Wageningen, The Netherlands (Fax: 31.317.421701; E-mail: R.P.Baayen@PD.AGRO.NL);* [2]*University of Natal, Faculty of Agriculture, Private Bag X01, Scottsville 3209, South Africa*

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Abstract

The early interaction of lily roots with the cortical rot pathogen *Fusarium oxysporum* f.sp. *lilii* was studied using roots of lily bulblets grown in Hoagland's solution, inoculated with the pathogen, and sampled up to 48 h later. Conidia produced germ tubes within 6 h, which extended towards and into the mucilage covering the root elongation zone, and along and into the anticlinal grooves and middle lamellae of epidermal cells. By 24–48 h, infecting hyphae had reached the periclinal walls and intercellular spaces between the epidermis and the outermost cells of the cortex. Penetration of intercellularly growing hyphae directly across host cell walls was not observed; invasion of the cell lumen only occurred by gradual infringing of hyphae upon successive primary wall layers. Non-cellulosic wall appositions rich in vesicles and covered by a cellulosic protective-like layer were formed in response to approaching hyphae in resistant cv. Connecticut King, but rarely in susceptible cv. Esther which seemed more susceptible to plasmolysis and rot. Finger-like projections of the appositions into the host cell cytoplasm likely represent early stages of transfer cell formation.

Introduction

The production of lily (*Lilium* sp.) bulbs and flowers is under serious threat from basal rot caused by the soil-borne fungus *Fusarium oxysporum* Schlecht.: Fr. f.sp. *lilii* Imle (Löffler et al., 1990). The fungus is not a vascular parasite, but colonizes, plasmolyzes and degrades the cortical tissues (Imle, 1940, 1942a,b; Linderman, 1981; Baayen and Förch, 1999), resulting in severe root rot along with destruction of the basal plate of the bulb. Bulb scales are eventually severed from the rotting basal plate; additional to infection out of the basal plate scales may also be infected through stomata in the epidermis (Baayen, 1996; Baayen and Förch, 1999).

Root rot is well-known in bulb rot diseases caused by *F. oxysporum*. Daffodil root tips are thought to be particularly sensitive to penetration by *F. oxysporum* f.sp. *narcissi* (Langerak, 1985; Nicholson et al., 1989). *F. oxysporum* f.sp. *tulipae* may infect undamaged tulip roots, and proceed through the root cortex into the basal plate (Bergman, 1965). This is also the case with infection of iris by *F. oxysporum* f.sp. *gladioli* (f.sp. *iridis* auct.) (Linderman, 1981). Root rot phenomena are not restricted to bulb rot diseases, however, but have also been reported in association with vascular colonization for wilt disease of flax (Kroes et al., 1998) and carnation (Baayen et al., 1989). Root cortex colonization and rot have recently even been reported for tomato roots colonized by

a non-pathogenic biocontrol strain of *F. oxysporum* (Olivain and Alabouvette, 1997).

The present study focusses on the first 48 h of the interaction of lily roots with *F. oxysporum* f.sp. *lilii*. We have used two cultivars of which the bulb tissue exhibits different levels of partial resistance to the rotting process (Straathof et al., 1993); however, it is not known whether or not such differences also pertain to the roots. Aim of this study was to investigate the fine structure of the early interaction of lily roots with *F. oxysporum* f.sp. *lilii*, and to resolve what factors may be involved in partial resistance to bulb and root rot.

Materials and methods

Fungal material. Isolate Fol-4 of *Fusarium oxysporum* f.sp. *lilii* was received from H.J.M. Löffler, DLO Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Wageningen, The Netherlands. Fol-4 is a representative member of the single clonal lineage within *F. oxysporum* f.sp. *lilii* (VCG 0190; Baayen et al., 1998). Spore suspensions were prepared by filtering through cheese cloth 12-day-old shake cultures grown in Czapek Dox liquid medium (Oxoid) at 22 °C; the obtained suspension was centrifuged at 7500 rpm in a Sorvall RC2-B for 12 min, and the pellet of conidia suspended in fresh Hoagland's solution to yield a concentration of 4×10^6 ml^{-1}.

Plant material. Virus- and fungicide-free bulbs (3–4 cm in diameter) of the Asiatic hybrid lily (*Lilium* section *Sinomartagon*) cvs Esther (susceptible to *Fusarium oxysporum* f.sp. *lilii*) and Connecticut King (partially resistant) were obtained from CPRO-DLO, Wageningen. Individual scales from these bulbs were allowed to produce bulblets, roots, and leaves in steamed soil in an illuminated (16 h day^{-1}) greenhouse, maintained at 20 °C. After 30 days, the scale-borne bulblets (hereafter called 'plants') were carefully dug out and their roots washed in running tap water.

In a first experiment, plastic Petri dishes (150×20 mm) were covered with aluminum foil; the latter was perforated, and one plant per Petri dish was suspended through the perforation so that its roots were in contact with 50 ml Hoagland's solution (Hoagland and Arnon, 1950). In this manner, eight plants from each cv. were maintained in the greenhouse for 25 days. The Hoagland's solution was then drained from six plants of each lot and replaced with 50 ml of a spore suspension, the non-treated plants serving as controls. Roots of inoculated plants and controls were harvested at 24 and 48 h post-inoculation.

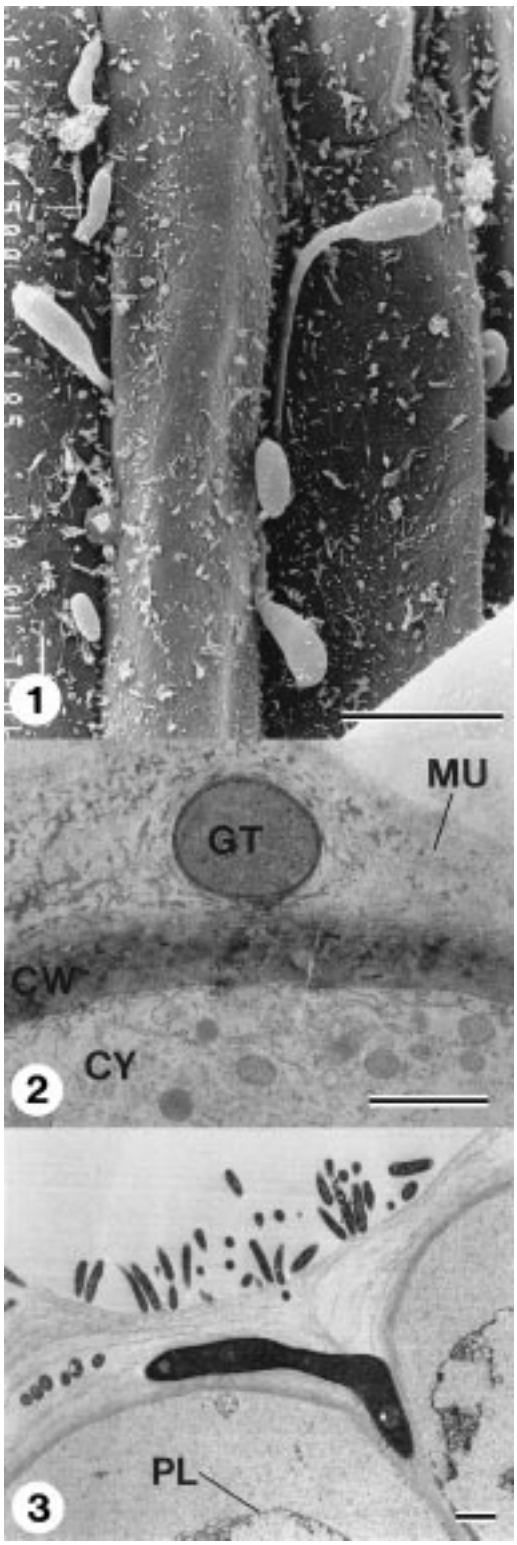
In a second experiment, plants were grown on Hoagland's solution in an opaque, $450 \times 300 \times 80$ mm polyethylene tray with a tight-fitting transparent lid. In this tray, plants were supported on aluminium-foil-covered wire netting laid upon 2 cm long supports. Plants were inoculated as described above. The roots of two plants of each cv. were sampled at 6, 12, 24 and 48 h.

Transmission electron microscopy (TEM). Control and treated roots of each cv. were fixed for 5 h in 5% glutaraldehyde in 0.02 M phosphate buffer at pH 7.2, rinsed in buffer, post-fixed overnight in 2% osmium tetroxide, rinsed in buffer, dehydrated in an ethanol series and embedded in Spurr's resin (Spurr, 1969). Cross sections, generally cut just behind the root tip, mainly in the zone of elongation, were stained with 2% uranyl acetate and Reynolds' lead citrate (Reynolds, 1963), and examined with a Philips CM 12 transmission electron microscope. Ordinarily, samples from 4 roots per harvested plant were examined.

To label cellulose, we used a commercial cellulase (Sigma C-8546) with affinity for $\beta(1 \rightarrow 4)$-D-glucans, directly conjugated to colloidal gold following the method described by Nicole et al. (1992). Gold particles of 15 nm average diameter were prepared according to Frens (1973). Sections mounted on nickel grids were floated on a drop of 0.01 M phosphate-buffered saline (PBS) (pH 6.5) containing 0.02% polyethylene glycol (PEG) (20000) for 10 min, then incubated for 30 min with the enzyme–gold complex diluted in PBS-PEG. Specificity of the labelling was verified by incubating sections on the cellulase–gold complex that was previously adsorbed with $\beta(1 \rightarrow 4)$-D-glucans from barley (Sigma G-6513), which entirely quenched the labelling response.

Scanning electron microscopy (SEM). Three roots per plant harvested at 24 h were fixed and dehydrated as for TEM. After passing through a dilution series of ethanol to amyl acetate, the roots were critical-point dried and examined in a JEOL 35C scanning electron microscope.

Light microscopy (LM). Additional roots, fixed and dehydrated as for TEM, were embedded in



polyethylene glycol methacrylate (Technovit 7100), and sectioned longitudinally at 3 µm with a Jung 2040 rotary microtome equipped with Ralph glass knives. Sections were stained with 0.05% toluidine blue in 0.025 M phosphate buffer at pH 6.8, and photographed with a Zeiss Axioplan light microscope with MC-100 camera. Polysaccharides with free uronic acid groups were differentiated from lignin and suberin by their orthochromatic (red) rather than metachromatic (turquoise) staining reaction (Baayen et al., 1996).

Results

Control plants. Roots and shoots of uninoculated control plants remained healthy throughout the experiments. No plasmolysis nor any other damage was encountered.

Macroscopic symptoms. Inoculated plants of both cultivars showed progressive softening and disintegration of the root system over 2–3 weeks. In susceptible cv. Esther, leaves were markedly yellow 15 days post-inoculation and plants were dead after 23 days. In partially resistant cv. Connecticut King, yellowing and death of leaves were slightly slower. In view of the similar disease development, root pathogenesis is described in the following sections, except the last, without further reference to cultivar identity.

Contact of germ tubes with epidermal cells. The fungal conidia had germinated by 6 h after inoculation. Their germ tubes extended towards the root and into the anticlinal grooves, along which they sometimes grew for a long distance at 24 h (Figure 1). The germ

Figures 1–3. (1) Germ tubes and developing hyphae of microconidia of *Fusarium oxysporum* f.sp. *lilii* aligned in anticlinal grooves of a lily root. 24 h. Bar = 20 µm. (2 and 3) Germ tubes in the mucilagenous layer of epidermal cells in the zone of elongation. Bars = 2.0 µm. (2) Displacement of mucilage (MU) by a germ tube (GT) growing over the cell wall (CW). The host cytoplasm (CY) seems largely unaffected. 24 h. (3) A germ tube growing through the mucilagenous layer penetrates anticlinally between epidermal cells. Root cells show severe plasmolysis (PL – plasma membrane) and disorganized cytoplasm. The mucilagenous layer consists of fibrillar material oriented along the epidermal cell wall, and interrupted at anticlinal grooves. Bacteria have also pervaded into the mucilage. 48 h.

tubes and developing hyphae penetrated the mucilagenous layer covering both the epidermal cells in the zone of elongation immediately behind the root cap (Figures 2 and 3) and the root cap cells (not shown). The mucilagenous layer consisted of wall-like fibrillar material, oriented in parallel with the outer cell wall and interrupted at cell wall junctures (Figures 3 and 4); it labelled for cellulose and was continuous or confluent with the cell wall proper, suggesting that it may have formed by distension of the latter (Figure 4). Extension of fungal hyphae through the mucilagenous layer appeared to further distend it (Figure 2). The outer fungal wall layers that infringed upon the mucilage also labelled highly for cellulose, in sharp contrast to other portions of hyphal walls that were free of a host wall interface (Figure 5, arrows).

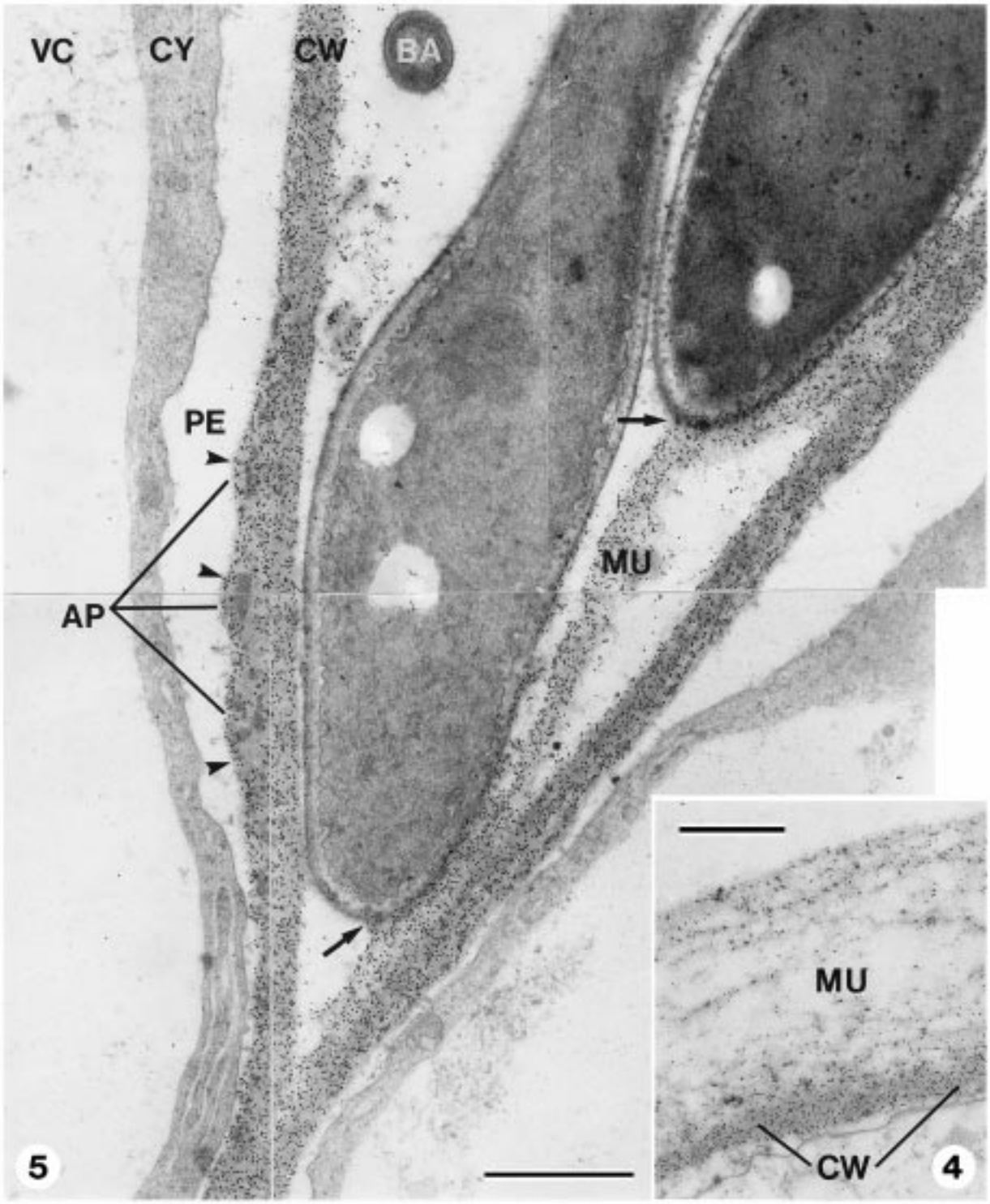
At points of hyphal contact with the walls of neighboring plant cells, some cells plasmolyzed (Figure 3) within 12–24 h, but generally appositions of various materials formed against the epidermal cell wall opposite the hyphal cell (Figures 5–9). Appositions stained in an uneven, often electron-dense manner and contained many vesicles. Vesicles and other type materials were also abundant in the enlarged periplasmic space next to appositions, which otherwise was largely electron-lucent (Figure 8). The appositions were sometimes irregular in outline, at times appearing as inner finger-like projections (Figures 8 and 9). Developing appositions were poorly labelled for cellulose or not at all, except for a protective-like inner covering layer separating them from the periplasmic space (Figures 5 and 6). The cytoplasm of the cells involved contained abundant endoplasmic reticulum and many large mitochondria (Figures 7 and 8), suggesting intense metabolic activity. Plasmolyzed cells did not develop wall appositions.

Penetration of the root. From the anticlinal grooves, the developing hyphae grew into the middle lamellae of anticlinal walls of adjacent epidermal cells, and penetrated the host root. The penetrating fungal hyphae distended the anticlinal epidermal cell walls (Figures 9–11), resulting in void spaces that connected with enlarged and also colonized intercellular spaces (Figure 12; for comparison a normal intercellular space is shown in Figure 13). Occasionally at 24 h, but certainly at 48 h, infecting hyphae had attained the periclinal wall between the epidermis and the outermost cell layer of the cortex. Appositions were commonly formed next to invaded intercellular spaces. However, plasmolyzed areas lacking the wall appositions were rapidly colonized and suffered wall degradation.

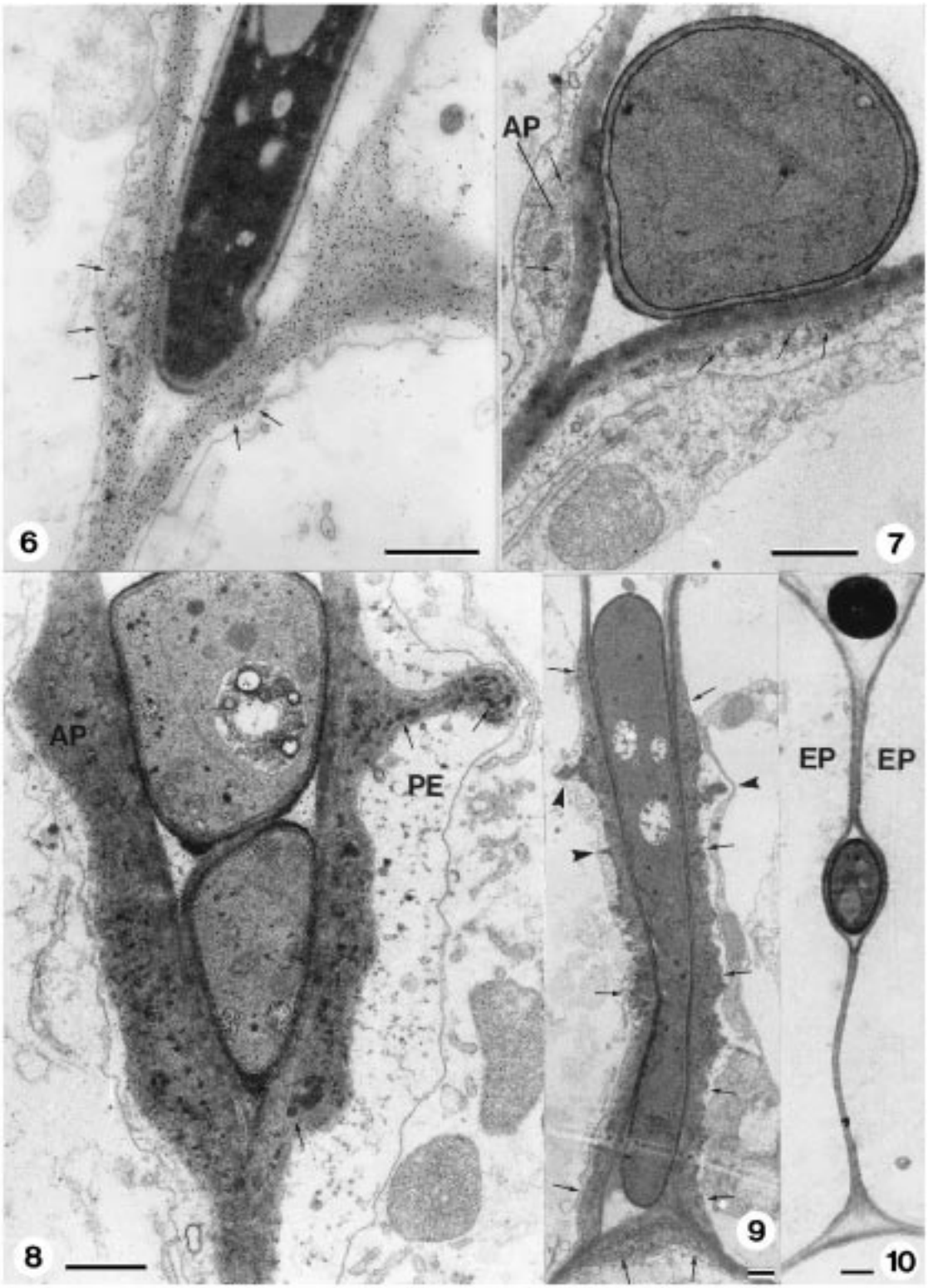
Colonization and rot of plasmolyzed tissues. In plasmolyzed tissues, fungal hyphae infringed upon the primary wall layers and disrupted these (Figures 14 and 15), allowing the fungus to invade the neighbouring cells (Figure 15). The barely perceptible host wall layer surrounding the penetrating fungal cells could be more easily visualized, however, by labelling for cellulose (Figure 16). Colonized cell walls disintegrated into distinct layers that still labelled for cellulose, indicating the removal of predominantly non-cellulosic wall materials (Figures 16 and 17). In the light microscope, the walls of such cells no longer stained with toluidine blue (not shown). Penetration of intercellularly growing hyphae perpendicularly across host cell walls was never observed; invasion of the cell lumen only occurred by gradual infringing of hyphae upon the primary wall layers, as shown above and as also indicated by the fact that hyphae within cell lumina were always positioned parallel to the cell wall (Figure 18). Fungal hyphae were relatively abundant in or against the walls of the plasmolyzed cells (Figure 19). At 48 h, the walls of cells in the outermost cortex layer had in rare cases disintegrated entirely, resulting in a sub-epidermal cavity and a blister-like swelling of the epidermis in that area (not shown).

Wall appositions in non-plasmolyzed tissues. Surrounding colonized intercellular spaces in non-plasmolyzed areas, fairly voluminous wall appositions were formed both in cortical and in epidermal cells (Figures 20–23). Vesicle-containing layers at times alternated with cellulosic protective-like layers (Figure 22). A single observation was made of a fairly large apposition containing a fungal hypha internally (Figure 24). The loose texture of the apposition involved suggests that it was either not fully formed or became altered, likely concomitantly with passage of the fungal hypha into the cell lumen. The same explanation could also apply to Figure 18, in which an apposition appears to have disintegrated in the vicinity of the fungus, as witnessed by the remaining vesicles and inner covering cellulosic layer.

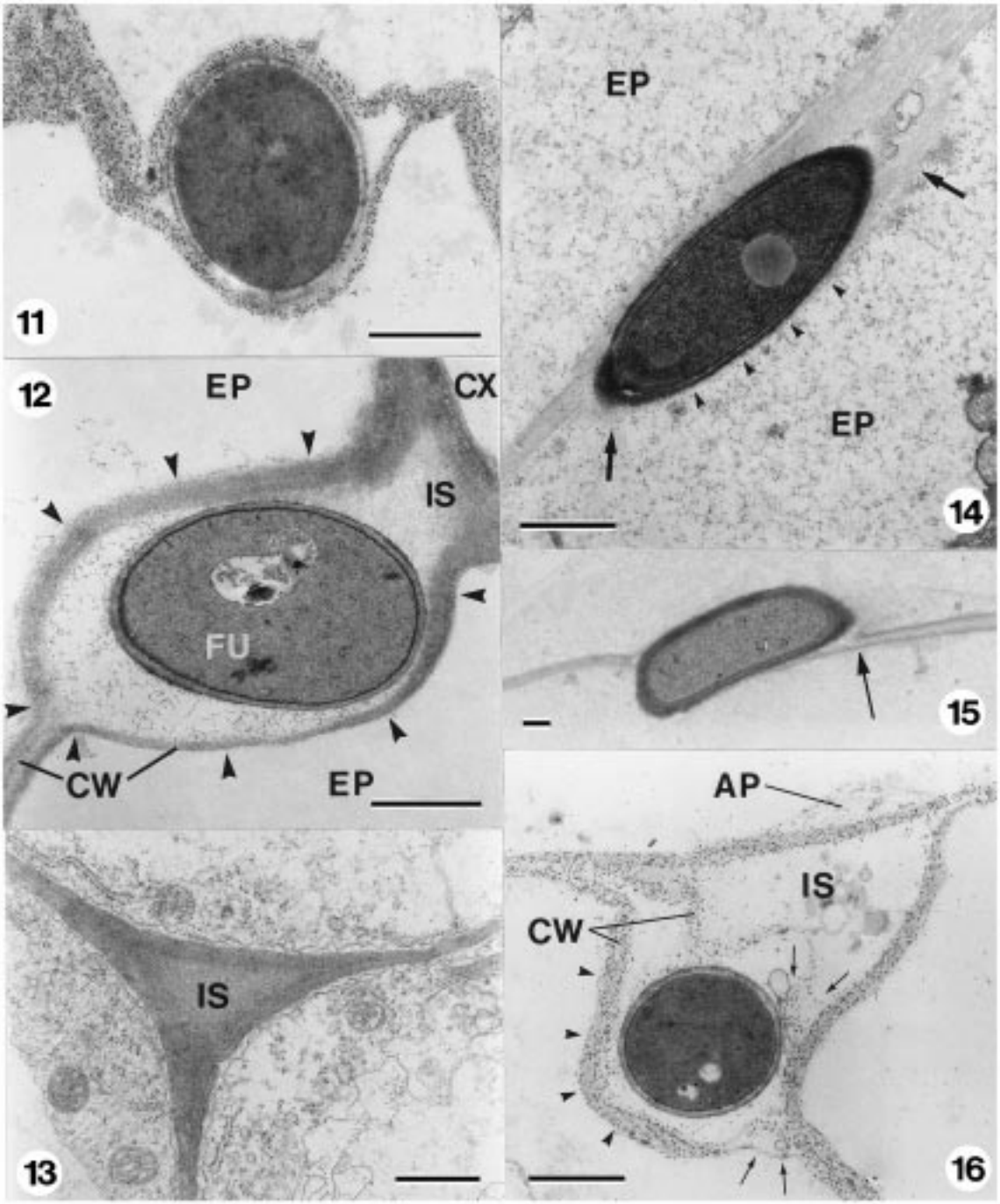
In several cases, the fungal wall was covered by an extracellular sheath consisting of tubular-like structures (white arrowheads in Figure 21; see also Figures 18, 20, 22–24) that radiated out of the fungal wall into the matrix of the intercellular space, and towards or



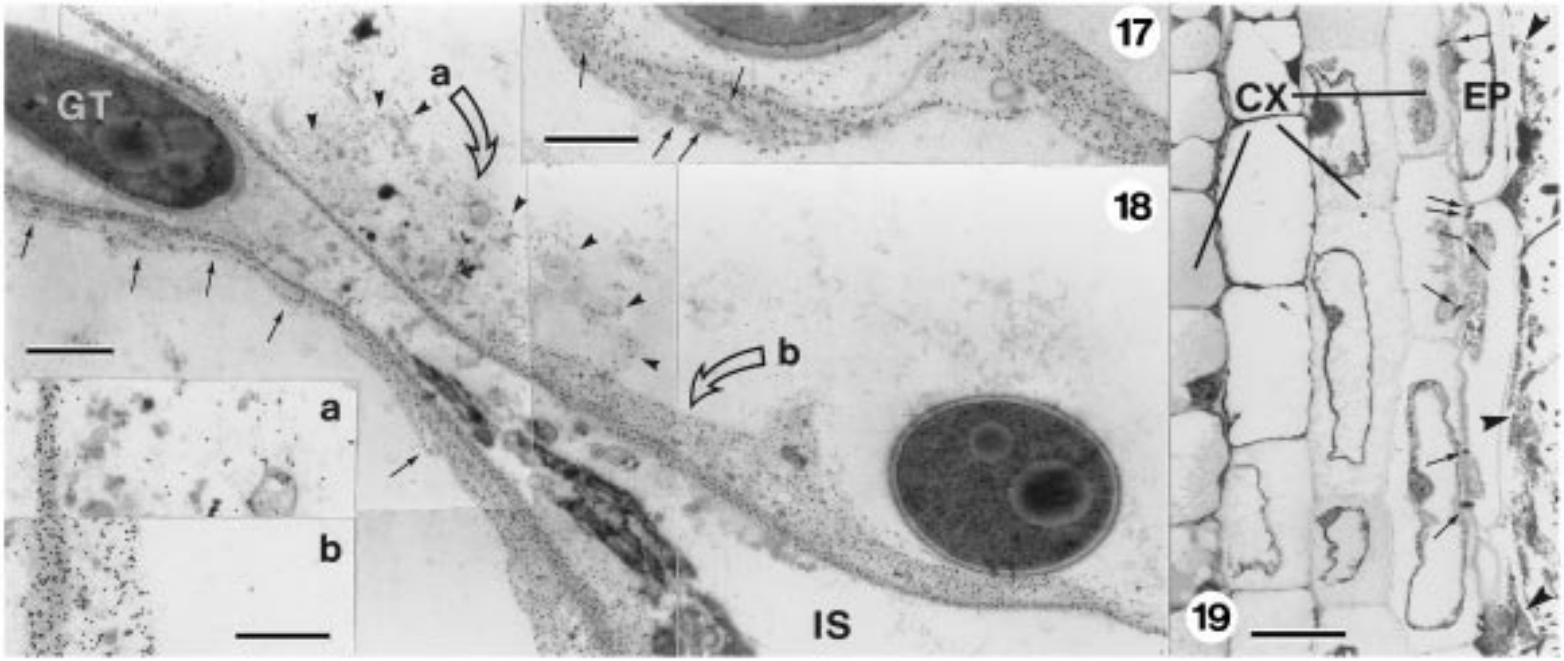
Figures 4,5. (4) The mucilagenous layer (MU) consists of wall-like fibrillar material labelling for cellulose, oriented in parallel and confluent with the outer cell wall (CW). (5) Apposition of novel material (AP) in the periplasmic space (PE) against the cell wall proximal to a fungal hypha. The protective-like layer covering the developing apposition labels for cellulose (arrowheads). The cellulose-labelled mucilage covering the epidermis is locally incorporated into the outer, electron-opaque fungal cell wall (arrows), which otherwise does not label for cellulose. BA – bacterium; CY – cytoplasm; VC – vacuole. 48 h. Bars = 1.0 µm.



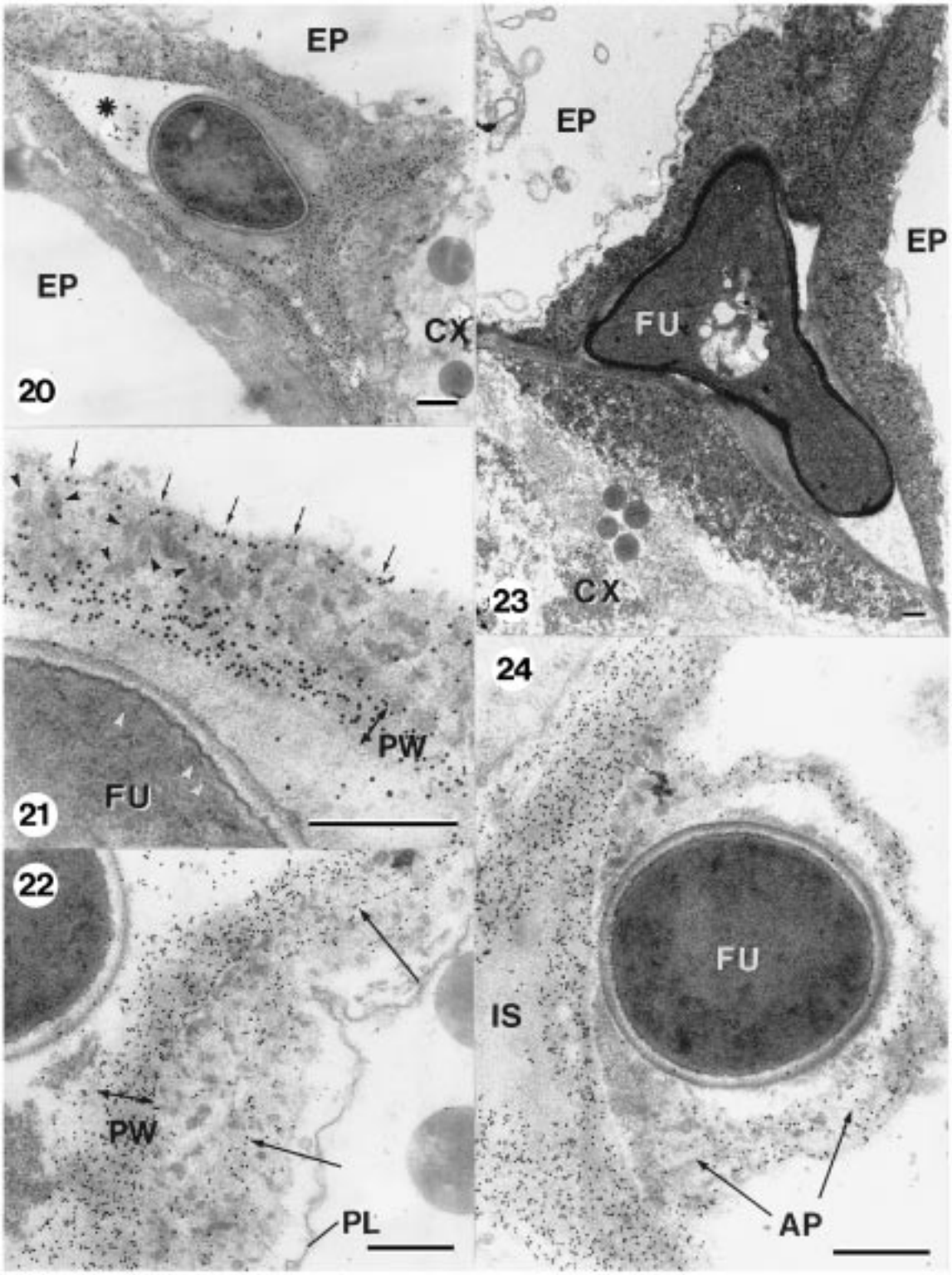
Figures 6–10. (6) Developing appositions covered by a cellulose-labelled protective-like layer (arrows) in two epidermal cells at the site of contact with the fungus. (7 and 8) Host cell wall appositions (AP) containing vesicles (arrows) opposite to hyphae in anticlinal grooves. Similar vesicles are also present in the periplasmic space (PE), between the host plasmalemma and the appositions. The cells involved have active cytoplasm. Note finger-like protrusion of apposition in Figure 8. (9) Penetration of a hypha through an anticlinal wall between epidermal cells towards the cortex. Note the finger-like protrusions (arrowheads) from the appositions (arrows) formed next to the hypha. (10) A hypha advancing between anticlinal walls obliquely or parallel, instead of perpendicularly, to the root axis. The epidermal cells (EP) are plasmolyzed, and appositions have not been formed. 48 h. Bars = 1.0 µm.



Figures 11–16. (11) A hypha growing through the middle lamella between two cells splits the wall. Section probed for cellulose. (12) Splitting (arrowheads) of the wall (CW) allows the fungus (FU) to reach an intercellular space (IS) between epidermis (EP) and cortex (CX). (13) Section from an uninoculated plant, showing the normal dimensions of intercellular spaces. (14) Excentric growth of a hypha through an anticlinal primary wall between two epidermal cells. On one side of the wall, the fibrillar wall material is thinning out (arrows), and the fungal cell is covered only by a very thin, almost imperceptible layer of host wall material (arrowheads). (15) Passage of hyphae through successive host cell wall layers allows them to move out into the cell lumen. Note the near-rupture where the cell wall has been thinning out (arrow). (16) Growth of a fungal hypha through a cell wall next to an intercellular space. The wall disintegrates into layers of fibrillar, cellulose-labelled material. While on one side the fungus is very close to the intercellular space and is separated from the cell lumen by a largely normal wall (arrowheads), on the other side the wall has split up in the opposite way (arrows). Note the juvenile apposition (AP) next to the intercellular space. 48 h. Bars = 1.0 µm.



Figures 17–19. (17) Details of Figure 16 showing disintegration of the colonized wall into fibrillar layers that label for cellulose. Note the vesicles in between some of these layers (arrows). Bar = 0.5 µm. (18) Next to a germ tube (GT) in the anticlinal groove, the fungus is present inside a plasmolyzed epidermal cell, against its wall. Developing appositions along the epidermal wall in the lower cell have a cellulose-labelled inner covering layer (arrows). In the upper cell, a disintegrating apposition (a) may be recognized both by its cellulose-labelled outline (arrowheads), and by the presence of vesicles. 48 h. Bar = 1.0 µm. Insets a and b, details of the disintegrating (upper cell) and intact (lower cell) apposition layers, respectively. Bar = 0.5 µm. (19) Intercellular proliferation of the fungus (arrows) in between the epidermis (EP) and cortex (CX) has induced plasmolysis of neighboring and more distant cells, and decreased staining of the involved cell walls with toluidine blue. Note remainders of the mucilagenous layer (arrowheads) on top of the root surface. 48 h. Bar = 50 µm.



Figures 20–24. (20) Wall appositions formed in epidermal (EP) and cortical (CX) cells surrounding intercellular spaces (IS) colonized by the fungus (FU). 48 h. Bars = 0.5 µm. (20) Enlarged (∗) colonized intercellular space, entirely circumscribed by appositions. (21) Detail of the apposition in the upper epidermal cell of Figure 20. The apposition contains vesicles (black arrowheads) and is covered by a cellulose-labelled protective-like layer (arrows). Note tubular-like structures (white arrowheads) radiating in a sheath-like manner out of the fungal wall into the matrix of the intercellular space, towards the host primary wall (PW). Similar extracellular sheath-like materials are visible in Figures 18, 20, 23 and 24. (22) Apposition in a cortical cell showing sequential deposition of alternating non-cellulosic and cellulosic (arrows) layers. PL – plasma membrane. (23) Voluminous appositions in epidermal and cortical cells. (24) Presence of the fungus inside a dissolving apposition. The cellulose-labelled protective-like layer appears to be relatively resistant to degradation.

even into the host primary wall. Materials pervading from the fungal wall into the host walls may possibly account for its higher opacity next to penetrating hyphae (Figures 7, 8 and 23).

Comparison of cvs. Esther and Connecticut King. Cell plasmolysis, colonization and rot were encountered both in susceptible cv. Esther and, slightly later and to a lesser extent, in partially resistant cv. Connecticut King. In general, cortical cells of the susceptible cultivar appeared to plasmolyze just in advance of the hyphal front (Figure 19). In cv. Esther, appositions were observed incidentally in epidermal cells but never in the cortex, while in cv. Connecticut King they were formed both in the epidermis and in the cortex.

Discussion

Fungal penetration of lily roots took place just behind the root cap, as is usual (Smith et al., 1981; Bishop and Cooper, 1983; Nicholson et al., 1989; Turlier et al., 1994), by hyphal growth through the anticlinal epidermal walls. Direct, intracellular penetration as reported for flax, cotton and tomato (Turlier et al., 1994; Rodríguez-Gálvez and Mendgen, 1995; Olivain and Alabouvette, 1997) was not observed. Once inside the root, fungal growth was largely confined to the middle lamella, the hyphae splitting the adjoining primary walls. Intracellular growth of the pathogen was only observed secondarily, through gradual infringing of hyphae on the primary wall layers, until the cell lumen was reached.

Hyphal passage out of the wall into the cell lumen only occurred in plasmolyzed cells, in association with progressive colonization and the onset of rot. In such tissues, plasmolysis of cells often occurred in front of the advancing hyphae. Plasmolysis and apparent death of host cells in the vicinity of the fungus as presently reported also characterize progressive colonization of the cortex of the basal plate and the scales during the necrotrophic phase of the disease (Imle, 1942b; Baayen, 1996; Baayen and Förch, 1999). Plasmolysis is unlikely to be an artifact, because other cells in the same roots were unaffected and had been able to produce wall appositions. Moreover, other cortical rot fusaria such as *F. avenaceum* also induce severe plasmolysis, often attributable to the action of fungal phytotoxins (Herrmann et al., 1996; Kalc Wright, 1993). *F. oxysporum* produces several toxins such as fusaric acid, fusaproliferin and beauvericin (A. Logrieco and A. Moretti, personal communication). Corm rot of gladiolus, induced by *F. oxysporum* f.sp. *gladioli*, is determined at least partially by the production of toxic levels of fusaric acid *in planta* (Remotti, 1996). Fusaric acid, one of the toxins produced by *F. oxysporum* f.sp. *lilii*, is indeed toxic to lily bulblets (Löffler and Mouris, 1992).

Host cell walls in the zone of progressive colonization and rot no longer stained orthochromatically with toluidine blue, indicating that uronic-acid-containing wall constituents had been altered or removed. The crystalline cellulose fraction of walls was relatively unaffected, however, as judged from labelling with a gold-conjugated probe for cellulose by TEM. The present results resemble observations made on transformed carrot roots infected by *F. oxysporum* f.sp. *chrysanthemi*, where the occurrence of exoglucanase-gold particles was indicative of the presence of degraded host cell walls that were no longer discernible ultrastructurally (Benhamou et al., 1994). The genus *Fusarium* is known to contain prolific producers of extracellular cell wall-degrading enzymes (Urbanek, 1989; Yoshida et al., 1989). For example, production of pectin-degrading enzymes is correlated quantitatively to the extent of disease development in the case of *F. oxysporum* f.sp. *dianthi* (Baayen et al., 1997). The incipient disintegration of host cell walls reported in this study refers only to the first 48 h after inoculation. Eventually, the colonized cortical tissue is completely degraded by the fungus (Imle, 1942b; Baayen, 1996), the dissolving cell walls typically falling apart into cellulose-labelled but otherwise indiscernible layers (Baayen and Förch, 1999).

The present study showed that a close contact was established from the beginning between the hyphal tips and the host cell wall. Hyphal tips were closely appressed to the host cell wall, and appeared to incorporate extraneous cellulosic material into their own outer wall. Cellulose is not a normal constituent of the walls of *F. oxysporum* (Benhamou et al., 1987, 1994; Nicole et al., 1994). Similar observations on incorporation of host wall material (cellulose, pectin) into the walls of *F. oxysporum* have been made for carnation and sumac (G.B. Ouellette, personal communication) using among others a highly specific exoglucanase probe for cellulose that is widely used by the scientific

community. Further support for the specificity of the cellulase–gold complex used in the present study for $\beta(1\rightarrow4)$-D-glucans has been obtained by testing this complex in comparison with labelled antibodies specific for $\beta(1\rightarrow3)$-D-glucans on callose from lettuce sieve plates, which was exclusively labelled by the antibodies but not the cellulase–gold complex (R.P. Baayen, unpublished data).

Apart from incorporation of extraneous host materials, fungal hyphae also seemed to produce extracellular sheath-like materials (including the tubular-like structures reported for such sheaths in various fungi) pervading to or into the host wall (Nicole et al., 1994; Cole et al., 1996). Extracellular sheath-like materials have previously been reported for *F. oxysporum* f.sp. *vasinfectum* (Nicole et al., 1994), and similar observations have been made for f.sp. *dianthi* and f.sp. *rhois* (G.B. Ouellette, personal communication).

In non-plasmolyzed cells, the close interaction of fungal hyphae with the host cell wall induced the apposition of vesicular materials against the wall. Wall-degrading or other signal proteins present on extracellular fungal sheaths (Nicole et al., 1994) may well play a role in the induction of such responses. Appositions, interrupted and covered by successive cellulosic layers, became fairly voluminous within 48 h and were associated with intense cytoplasmic activity. Finger-like projections from the wall appositions were present as early as 48 h after inoculation. Such wall ingrowths likely represent early stages of the formation of transfer cells, which are commonly formed in lily roots and bulbs in response to fungal invasion (Baayen and Förch, 1999). Transfer cells, characterized by extensive wall ingrowths proximal to the colonized intercellular spaces, are likely involved in sugar secretion towards the fungus which acts as a sink for the assimilates. Transfer cells are considered specialized in short-distance transport of assimilates between symplast and apoplast (Pate and Gunning, 1972; Eschrich, 1995); in lily, for example, transfer cells also occur in sugar-secreting pistil cells (Rosen and Thomas, 1970). The presence of transfer cells in lily roots likely represents an early biotrophic endophytic phase in the disease, which ends when fungal biomass has increased to an extent that fungal cell wall degrading enzymes and/or toxins damage the colonized tissue (Baayen and Förch, 1999). Compared with necrotrophs, fungi with a transient biotrophic phase would be able to absorb and utilize most of the nutrients of the host without competition by other soil-borne fungi or bacteria. The wall ingrowths eventually are degraded by the fungus along with the cell wall proper during the necrotrophic phase of the disease (Baayen and Förch, 1999). Early induction of transfer cells, later followed by degradation of the host tissues, is also known from mycorrhizal root infection (Ashford and Allaway, 1985; Strobel and Sinclair, 1992).

Finger-like wall apposition projections similar to those presently reported for lily are produced early after infection (24 h) in cotton roots in response to infection by *F. oxysporum* f.sp. *vasinfectum*, and have also been considered indicative of a short endophytic or biotrophic infection phase (Rodríguez-Gálvez and Mendgen, 1995). However, the latter authors also encountered these structures surrounding intracellular hyphae growing inside the cytoplasm of otherwise healthy-looking cells. In contrast, we have never observed growth of hyphae inside the host cell cytoplasm in lily (Baayen and Förch, 1999, and present results), even though we did observe this in flax roots infected by *F. oxysporum* f.sp. *lini* (Kroes et al., 1998). Penetration, colonization and host defense responses clearly differ from one host–pathogen system to another.

Plasmolysis, progressive colonization and rot were observed both in susceptible cv. Esther and in partially resistant cv. Connecticut King. However, plasmolysis and rot in cv. Esther were more rapid than in cv. Connecticut King, and consequently fewer appositions were formed in the former than in the latter cultivar. Cortical cells produced appositions only in the latter cultivar. Our observations suggest that cv. Connecticut King is less sensitive to fungus-induced plasmolysis, resulting in a prolonged biotrophic phase and thus also in a retarded onset of the necrotrophic phase of the disease. In the present trials, which involved a high inoculum dosage applied to fully exposed roots, differences in pathogenesis were small, and both cvs. suffered progressive rot within several weeks. Under natural conditions, however, the development of basal rot takes several months. Therefore, minor differences in the balance between the biotrophic and necrotrophic phases of the disease as presently reported may have profound effects on the levels of partial resistance. This supports the notion (Straathof et al., 1993; Baayen and Förch, 1999) that partially resistant cultivars temporarily retard, but never fully inhibit, colonization and disease.

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