J Gen Plant Pathol (2004) 70:145–152
DOI 10.1007/s10327-004-0107-x

FUNGAL DISEASES

**Maria-Isabel Salerno · Silvio Gianinazzi
Christine Arnould · Vivienne Gianinazzi-Pearson**

Ultrastructural and cell wall modifications during infection of *Eucalyptus viminalis* roots by a pathogenic *Fusarium oxysporum* strain

Received: May 28, 2003 / Accepted: November 14, 2003

Abstract *Fusarium* species are soil-borne fungal pathogens that produce a variety of disease symptoms when attacking crop plants. The mode of root colonization of *Eucalyptus viminalis* seedlings by a pathogenic *F. oxyporum* strain (Foeu1) at the ultrastructural level and changes in cell wall pectin during host pathogen interactions are described. Root systems of *E. viminalis* plants were inoculated with *F. oxysporum* in an in vitro model system. Hyphae of *F. oxysporum* adhered to the outer epidermal cell walls through fibrillar material, and after penetration they spread into the internal tissues. They developed intercellularly and intracellularly in the root cortex and invaded vascular tissues. Papillae were induced, and the host plasma membrane ruptured in colonized cells, causing rapid host tissue and cell damage. Changes in distribution and occurrence of nonesterified and methyl-esterified pectins were evaluated after root colonization by *F. oxysporum* using two monoclonal antibodies, JIM 5 and JIM 7, respectively. Nonesterified pectin in control roots was mainly localized in the epidermal cell walls and middle lamellae in parenchymal cortex, whereas methyl-esterified pectin accumulated more in primary cell walls of the cortex and phloem. Decreases in immunodetected nonesterified and methyl-esterified pectins were associated with extensive plant tissue degradation after root colonization by the pathogenic fungus.

Key words Root pathogenesis · *Fusarium oxysporum* · *Eucalyptus viminalis* · Ultrastructure · Pectin

M-I. Salerno · S. Gianinazzi · C. Arnould ·
V. Gianinazzi-Pearson (✉)
UMR 1088 INRA/5184 CNRS/Université de Bourgogne PME,
INRA-CMSE, BP 86510, 21065 Dijon Cedex, France
Tel. +33-3-80-69-31-55; Fax +33-3-80-69-37-53
e-mail: gianina@epoisses.inra.fr

M-I. Salerno
CISAUA-Facultad de Ciencias Agrarias y Forestales, UNLP, La Plata, Argentina

Introduction

Fusarium oxysporum Schlechtend.:Fr. is an anamorphic species that attacks crops and produces a variety of disease symptoms in infected plants. A pathogenic form of *F. oxysporum* (strain Foeu1) that causes damping off in *Eucalyptus viminalis* Labill. seedlings has been isolated from forest nursery soil in Argentina. Unlike other *Fusarium* species, *F. oxysporum* strain Foeu1 causes extensive tissue maceration and cell death in the root system of the host plant (Salerno et al. 2000). According to Beckman et al. (1982), the failure to limit spread of *Fusarium* in a susceptible plant could result from an inability to recognize the pathogen, the production of an inhibitor of defense responses, or an innately slow or weak ability to respond to pathogen attack.

Many phytopathogenic microorganisms secrete pectolytic enzymes such as pectin methyl esterases (PMEs), endo- and exo-polygalacturonases (PGs), and pectate lyases (PLs), which are actively involved in the penetration of plant tissues (Elad and Evensen 1995; Mansfield and Richardson 1981) and the degradation of cell walls during subsequent invasion of internal host tissues (Have et al. 1998). These pectinolytic enzymes can also elicit plant defense responses through the oligosaccharides they release (Hahn et al. 1989). PMEs from *F. oxysporum* have been characterized by Rexova-Benkova and Markovic (1976) and PGs and PLs by Guevara et al. (1996), but their role in pathogenesis has remained unclear. Steinberg et al. (1999) demonstrated that both pathogenic and nonpathogenic *F. oxysporum* utilize pectin as a carbon source in pure culture and that this activity is not correlated with the pathogenicity of the fungal strains.

To gain insight into the basis of cell–cell interactions between pathogenic *F. oxysporum* strain Foeu1 and *E. viminalis* roots, we investigated the effects of the pathogenic fungus on host tissue integrity and monitored sites of putative pectinolytic enzyme activities (endopolygalacturonase and pectin methyl esterase). We did this by labeling for changes in the nonesterified and methyl-esterified pectin composition of host walls.

Materials and methods

Fungal strain

The pathogenic form of *Fusarium oxysporum* Schlechtend.: Fr., strain Foeu1, was kindly provided by G. Lori (UNLP-CIC, La Plata, Argentina). This strain was recovered from forest nursery soil in Saladillo, Argentina, where *Eucalyptus* species are grown regularly and where root rot and damping off diseases occur. Strains were cultured on potato dextrose agar (PDA) (Difco, Detroit, MI, USA).

Plant growth and inoculation with *F. oxysporum*

Eucalyptus viminalis Labill. seeds were surface-disinfested with sodium hypochlorite (NaOCl) (12% active chloride) for 20 min and pregerminated on water agar (0.6%) for 3 days at 27°C in the dark. When roots were 0.5 cm long, seedlings were transferred to agar medium in petri dishes as described by Salerno et al. (2000) and incubated in an illuminated growth chamber. When seedlings were 6 days old and roots were 1.5 cm long, roots were inoculated with mycelium plugs of *F. oxysporum* as described by Salerno et al. (2000). Control seedlings were not inoculated.

Tissue processing for electron microscopy

Five seedlings from each of the treatments were sampled 6 days after inoculation, and 5 mm long root pieces cut 0.5 cm behind the apex were prepared for electron microscopy. Root segments (2–3 mm) were immersed overnight at 4°C in 2% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2. Samples were then dehydrated in a graded ethanol series of 30%, 50%, 70%, and 95%. Four or five root segments were selected for each of the treatments and embedded in LR White resin (Oxford Instrument, Orsay, France) as described by Gianinazzi and Gianinazzi-Pearson (1992).

Ultrathin sections (98 nm) were cut on a Reichert-Jung Ultracut ultramicrotome, collected on gold grids, and stained with 1% uranyl acetate (Valentines 1961) for 20 min followed by lead citrate (Reynolds 1963) for 10–15 min. Sections were observed using a Hitachi 600 transmission electron microscope (TEM) at 75 kV.

Immunogold detection of pectin

Pectin was visualized in planta with the monoclonal mouse antibodies (immunoglobulin G) JIM 5 and JIM 7, which recognize nonesterified (NE) and methyl-esterified (ME) pectins, respectively (Knox et al. 1990; VandenBosch et al. 1989). The JIM 5 and JIM 7 antibodies were provided by Dr. Paul Knox (John Innes Centre, Norwich, UK). Ultrathin sections were incubated with the primary antibodies (diluted 1:4000), followed by incubation for 1 h at room temperature with a 15-nm gold-conjugated secondary anti-mouse antibody (Biocell, Cardiff, UK) diluted 1:20, as described by Gianinazzi and Gianinazzi-Pearson (1992). Control sections were prepared by omitting the primary antibodies or by incubating with an uncorrelated polyclonal antibody from the fungus *Acaulospora laevis* (Lemoine et al. 1995). Thin sections were stained with 1% uranyl acetate (Valentines 1961) and observed by TEM at 75 kV. Control sections were incubated in 0.1 N HCl for 10 min before immunolabeling to confirm that there was no masking of the epitopes.

The number of gold particles present in the epidermal, cortical, and phloem cell walls was evaluated per unit area (square micrometers), at 20 000-fold magnification, in five random locations on five sections from each of three plants with the aid of the image analysis system SAMBA (Samba Technologies, Grenoble, France) coupled to a video CCD camera (Tokina Optical Co. Ltd., Tokyo, Japan) equipped with an AF Nikkor 20-mm lens. With analysis of variance (ANOVA) after square root transformation, the treatment means, corresponding to each root tissue, were compared separately by the Student-Newman-Keuls test ($p < 0.05$) using the SAS program.

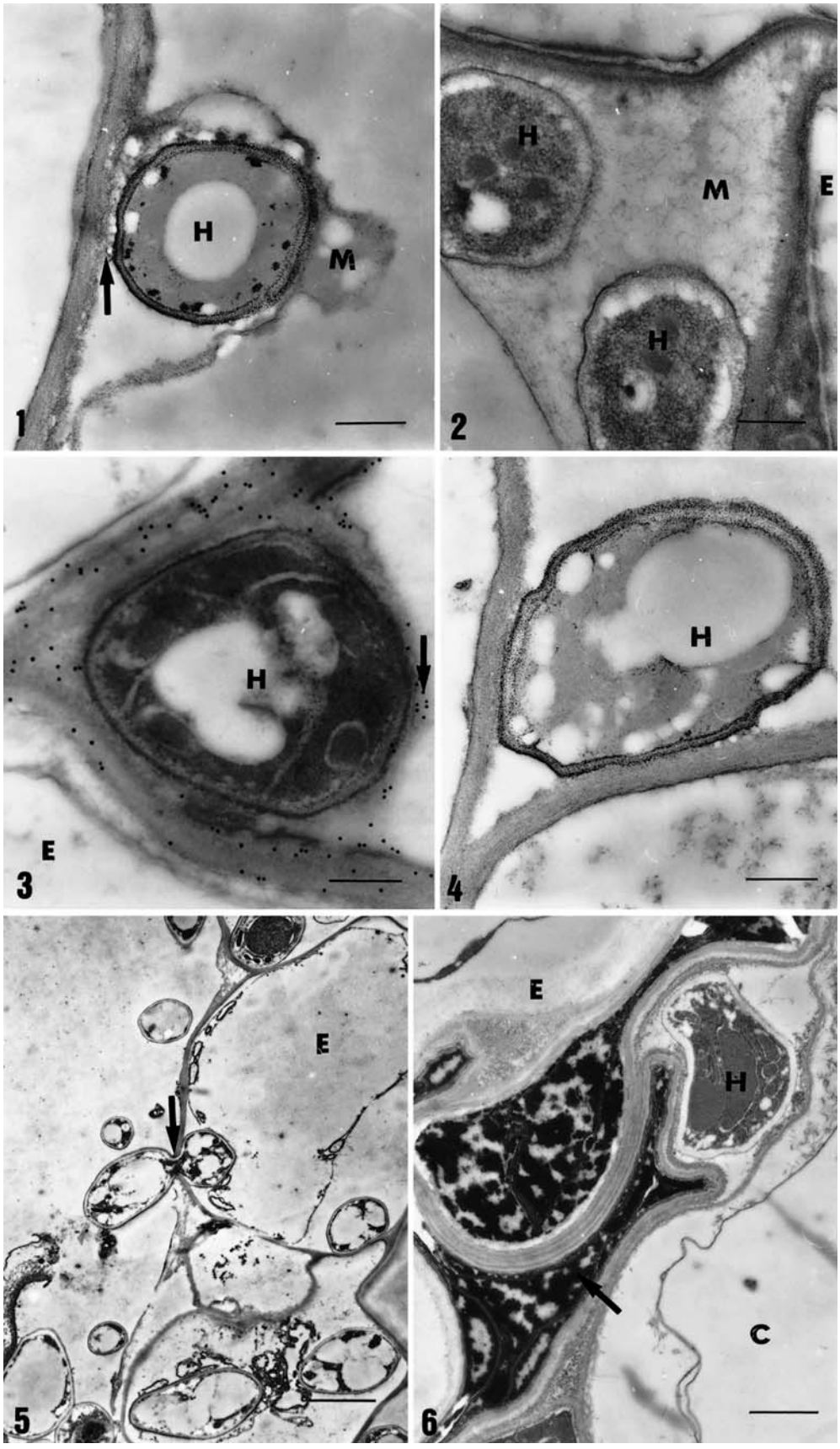
Results

Relations prior to root entry

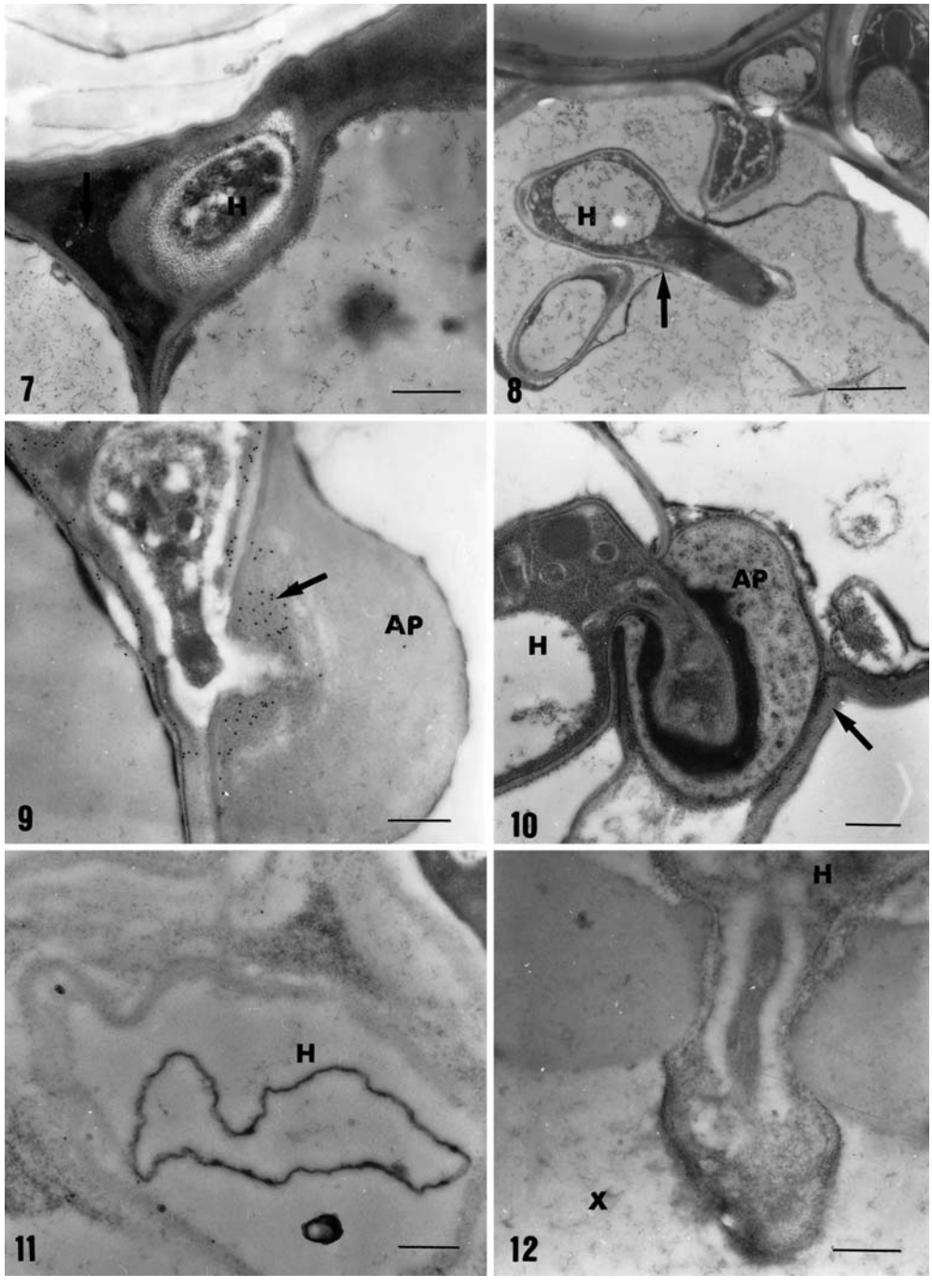
Noninoculated root tissues of *E. viminalis* were compact with a well-defined epidermis, hypodermis, parenchymal cortex, and central cylinder, as described in detail by Salerno et al. (2000). Epidermal cells were covered by a fibrillar, sloughing layer (see Fig. 15, below) and intercellular spaces of the parenchymal cortex had fibrillar or electron-translucent contents (see Fig. 13 and 17, below). *Fusarium oxysporum* Foeu1 grew along the surface of *E. viminalis* roots before penetration, frequently enclosed by a film of material continuous with the outer epidermal cell walls. When hyphae came into contact with the root epidermis, their walls became closely attached to the outer cell walls by fibrillar material (Fig. 1). Hyphae were frequently located at the junction between epidermal cells (Fig. 2), where they were surrounded by a loose fibrillar matrix that was immunolabeled by JIM 5 but not JIM 7 antibody, indicating that it contained an NE pectin component (Fig. 3).

Development of fungal hyphae in root tissues

The most common points of entry of *F. oxysporum* Foeu1 was through root hairs or at the junction between epidermal cells (Fig. 4). Hyphae were able to penetrate directly through the epidermal cell wall and develop inside the epidermal cells (Fig. 5). *F. oxysporum* did not form an appressorium-like structure before infection. Fungal ingress into the epidermis coincided with disorganization of this tissue (Fig. 5). When *F. oxysporum* developed through the hypodermis, the cells collapsed and accumulated electron-dense deposits, forming a compact cell layer separating the epidermis from the cortex (Fig. 6). This phenomenon appeared



Figs. 1–6. Electron micrographs of penetration of *Eucalyptus viminalis* roots by pathogenic *Fusarium oxysporum* strain Foeu1. **1** Hypha (*H*) of pathogenic *F. oxysporum* surrounded by a thin film of material (*M*) and apparently adhering to the external epidermal cell wall of an *E. viminalis* root by the fibrillar material (*arrow*). No antibody control. *Bar* 0.5 μm. **2,3** Hyphae of pathogenic *F. oxysporum* surrounded by a loose fibrillar matrix at the junction of two epidermal cells (E) of an *E. viminalis* root. **2** No immunogold detection of methyl-esterified pectin with JIM 7 monoclonal antibody. *Bar* 0.5 μm. **3** Immunolabeling of nonesterified pectin with JIM 5 monoclonal antibody. *Bar* 0.3 μm. **4** Hypha developing between epidermal cells of an *E. viminalis* root prior to penetration. No antibody control. *Bar* 0.3 μm. **5** Hyphal penetration (*arrow*) by the pathogenic *F. oxysporum* directly through the outer epidermal cell (*E*) wall of an *E. viminalis* root. *Bar* 2.5 μm. **6** Collapsed hypodermal cells with electron-dense contents (*arrow*), separating the epidermis (*E*) from the cortex (*C*) of an *E. viminalis* root in the presence of hyphae of pathogenic *F. oxysporum*. *Bar* 1 μm



Figs. 7–12. Ultrastructural organization of cortical and vascular tissue of *Eucalyptus viminalis* roots infected by pathogenic *Fusarium oxysporum* strain Foeu 1. **7** Hypha (*H*) of pathogenic *F. oxysporum* developing in intercellular spaces with electron-dense contents (*arrow*) in the parenchymal cortex of an *E. viminalis* root. *Bar* 0.7 μm. **8** Hypha of pathogenic *F. oxysporum* in a cortical cell rupturing the host plasma membrane (*arrow*) in a *Eucalyptus viminalis* root. *Bar* 1.7 μm. **9** Hypha of pathogenic *F. oxysporum* developing in the parenchymal cortex of *E. viminalis* roots. Papilla-like wall apposition (*AP*) of a heterogeneous structure forms in response to the invading fungus. Immunolabeling of nonesterified pectin-like material in the papilla (*arrow*). *Bar* 0.5 μm. **10** Hyphal penetration of an endodermal root cell by pathogenic *F. oxysporum*. A papilla-like apposition with heterogeneous content is formed in response to colonization by a hypha. The normal cell wall is labeled by JIM 5 (*arrow*). *Bar* 0.7 μm. **11** Hypha developing in cells of the degraded phloem. *Bar* 0.4 μm. **12** Hypha developing in the xylem (*X*). *Bar* 0.3 μm

randomly in the hypodermis and was also observed in non-inoculated roots at the point of emergence of a lateral root.

Fusarium oxysporum developed along the middle lamella in the parenchymal cortex and was frequently embedded in an electron-dense material in intercellular spaces (Fig. 7). Hyphae penetrated the cortical cells, and the ruptured host plasma membrane was plasmolyzed (Fig. 8). Papillae were sometimes observed when *F. oxysporum* pen-

etrated cells of the parenchymal cortex (Fig. 9). NE pectin accumulated in the inner layer of papillae (Fig. 9), but ME pectin could not be detected (not shown). Tissues of the parenchymal cortex were progressively altered, and cells plasmolyzed and collapsed in the presence of the pathogenic fungus. Papillae with heterogeneous electron-dense and electron-translucent components were generally observed when endodermal cells were infected by *F. oxysporum* (Fig. 10). *Fusarium oxysporum* invaded the central cylinder of *E. viminalis* roots in the region 0.5 cm behind the apex (Salerno et al. 2000). Ingress of the fungus into these tissues coincided with a progressive disorganization of phloem cells (Fig. 11), and hyphae penetrated xylem vessels directly (Fig. 12).

Modifications of methyl-esterified and nonesterified pectin in root tissues

Methyl-esterified pectin detected with JIM 7 antibody was localized in wall domains in all cells and tissues of noninoculated roots but to a lesser extent in epidermal cell walls (Table 1). No immunolabeling was observed in xylem vessels. Immunolocalization of NE pectin in *E. viminalis* roots with JIM 5 antibody produced a similar labeling pattern (Table 1). Tissue colonization by *F. oxysporum* Foeu1 resulted in a substantial decrease in immunolabeling with both JIM 7 and JIM 5 antibodies in cell walls of the parenchymal cortex. These epitopes were absent from cell walls of the heavily colonized, disorganized phloem (Table 1).

At the ultrastructural level, ME pectins were localized with JIM 7 antibody throughout the primary cell walls of the parenchymal cortex (Fig. 13) and phloem of noninoculated *E. viminalis* roots, whereas epidermal cell walls tended to have weaker immunolabeling (Table 1). Radial walls of hypodermal cells, endodermal cells, and xylem vessels were not immunolabeled with JIM 7 antibody. There was an overall decrease in binding with JIM 7 antibody in the cortical cell walls of roots colonized by *F. oxysporum* Foeu1 (Table 1, Fig. 14), and ME could not be detected in degraded cell walls of colonized phloem tissues (Table 1). Pretreatment of root sections with 0.1 N HCl did not affect these results, showing that the decreased immunolabeling was not due to masking of antigenic sites.

Ultrastructural visualization of NE pectin in noninoculated roots confirmed that it was mainly located in epidermal cell walls (Fig. 15), middle lamellae, wall surfaces at intercellular spaces in the parenchymal cortex (Fig. 16), and phloem cell walls. In roots heavily colonized by *F. oxysporum*, an important loss of JIM 5 antibody binding occurred at epidermal cell walls (Fig. 17) and decreased in cortical cell walls, especially those in contact with the fungal pathogen (Fig. 18, Table 1). NE pectin epitopes were not detected in disorganized phloem tissue colonized by hyphae (Table 1). Again, control sections pretreated with 0.1 N HCl did not affect immunolabeling, indicating that the observed decreases did not result from masking of epitopes.

Table 1. Density of immunolabeling of nonesterified (JIM 5 antibody) and methyl-esterified (JIM 7 antibody) pectin in cell walls of noninoculated *Eucalyptus viminalis* and *Fusarium oxysporum* Foeu1-infected ones

Antibody	Tissue	No. of gold particles per unit area (μm^2)	
		Noninoculated control	Pathogenic *F. oxysporum*
JIM 5	Epidermis	1759 ± 255 A	624 ± 204 B
	Cortex	2017 ± 724 A	433 ± 228 B
	Phloem	1745 ± 855 A	0 C
JIM 7	Epidermis	632 ± 342 A	535 ± 117 A
	Cortex	1006 ± 232 A	408 ± 217 A
	Phloem	5145 ± 1161 A	0 B

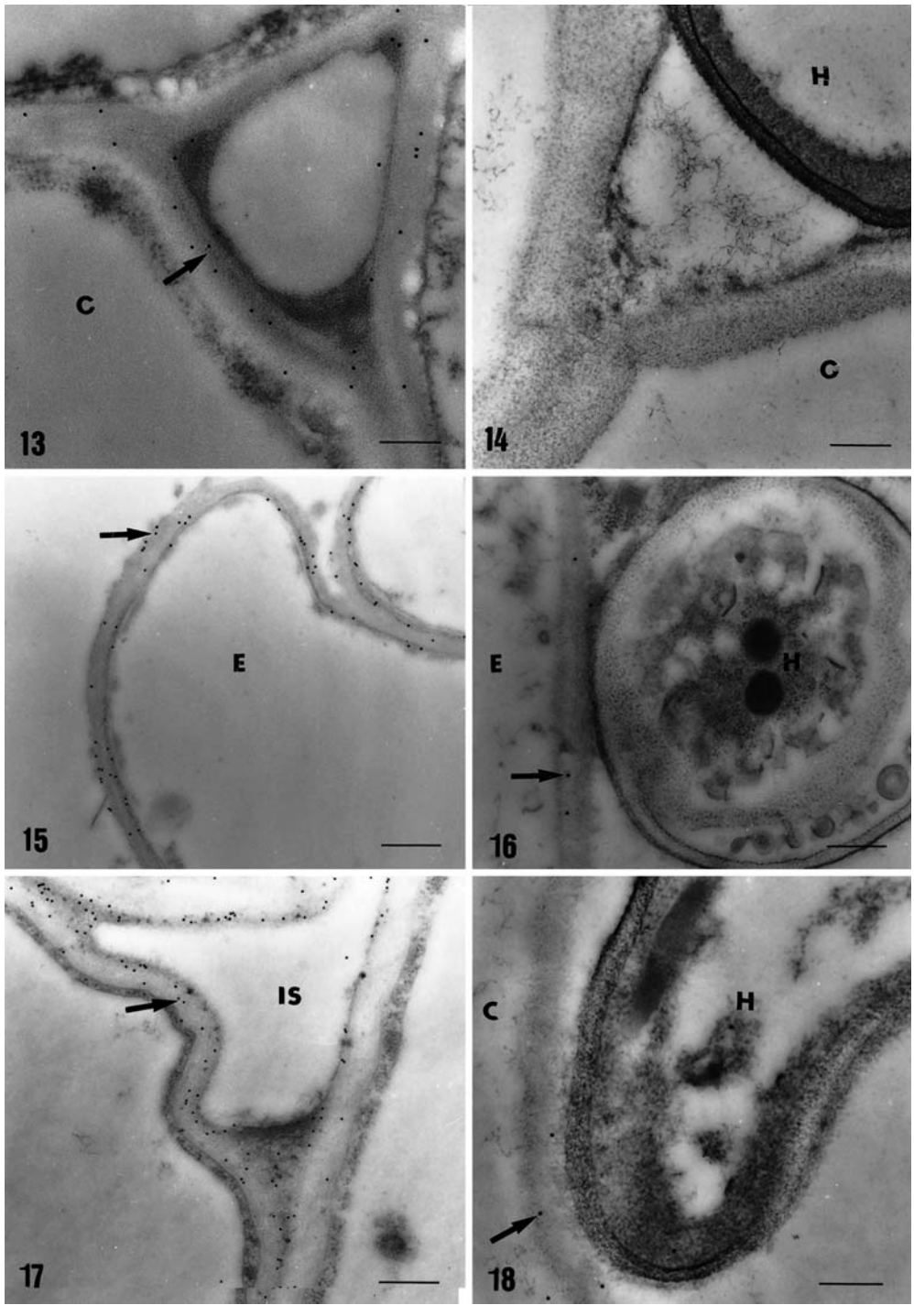
Each value represents replicate counts on five sections from each of three plants
For each antibody, data followed by different letters are significantly different ($p = 0.05$)

Discussion

Previous studies showed that the pathogenic strain Foeu1 of *F. oxysporum* causes damping off and root necrosis of *E. viminalis* seedlings (Salerno et al. 2000). *Fusarium oxysporum* Foeu1, unlike other *Fusarium* species that cause vascular infections, causes extensive tissue disorganization in roots of the host plant. In this study, we investigated in more detail the infection process by this pathogenic *Fusarium* strain, focusing in particular on the responses of *E. viminalis* root tissues at the ultrastructural level.

When hyphae of *F. oxysporum* Foeu1 reach the host root surface, they appear to adhere to the outer epidermal cell walls through fibrillar material. This attachment process could enhance the ability of the fungus to penetrate host cells and may be necessary for infection (Nicholson and Epstein 1991). Extracellular material encloses hyphae at epidermal cell junctions of *E. viminalis* roots, and labeling with the JIM 5 antibody of this material indicates a nonesterified pectin component. Mucilaginous material of a polysaccharide nature has been suggested to aid in binding or adhesion of fungal pathogens to plant cell surfaces (Lapp and Skoropad 1978; Nicholson and Epstein 1991; Pringle 1981). Whether such material is important to the adhesion of *F. oxysporum* Foeu1 to *E. viminalis* root cells has yet to be determined.

No well-defined appressorium-like structure of pathogenic *F. oxysporum* was observed on the root surface of *E. viminalis*. Earlier studies similarly reported that pathogenic *Fusarium* species do not develop appressoria at the site of penetration on other host plants (Bishop and Cooper 1983; McKeen 1977; Olivain and Alabouvette 1997). Direct penetration by fungi that do not differentiate appressoria clearly requires cell wall-degrading enzymes (Mendgen et al. 1996), and the significant decrease in nonesterified pectin in epidermal cell walls of *E. viminalis* in the presence of *F. oxysporum* may be indicative of such enzyme activity by the pathogen. *F. oxysporum* penetrates between two adjacent



Figs. 13–18. Effect of pathogenic *Fusarium oxysporum* strain Foeu1 on the immunolocalization of pectins in *Eucalyptus viminalis* roots. **13** Immunogold detection (*arrow*) of methyl-esterified pectin with the JIM 7 monoclonal antibody in primary cell walls of parenchymal cortex (*C*) and in intercellular spaces of a noninoculated *E. viminalis* root. *Bar* 0.3 μm. **14** Absence of immunolabeling with JIM 7 over the cortical cell wall (*C*) of an *E. viminalis* root in contact with a hypha of pathogenic *F. oxysporum*. *Bar* 0.2 μm. **15** Immunogold detection (*arrow*) of nonesterified pectin with JIM5 monoclonal antibody in epidermal (*E*) cell walls of a noninoculated *E. viminalis* root. *Bar* 0.3 μm. **16** Sparse immunolabeling (*arrow*) with JIM 5 antibody in epidermal cell (E) walls of an *E. viminalis* root in contact with a hypha of pathogenic *F. oxysporum*. *Bar* 0.25 μm. **17** Immunogold labeling (*arrow*) of nonesterified pectin with JIM 5 antibody in the middle lamellae and walls lining intercellular spaces (*IS*) in the parenchymal cortex of a noninoculated *E. viminalis* root. *Bar* 0.3 μm. **18** Weak detection (*arrow*) of nonesterified pectin with JIM 5 antibody in cell walls of parenchymal cortex (*C*) of an *E. viminalis* root in contact with a hypha of pathogenic *F. oxysporum*. *Bar* 0.2 μm

epidermal cells of *E. viminalis*, as shown earlier for other pathogenic *Fusarium* strains (Bishop and Cooper 1983; Malalasekera et al. 1973; McKeen 1977; Parry and Pegg 1985). After penetration, the pathogenic *F. oxysporum* spreads into the internal tissues of *E. viminalis* roots and develops in the intercellular spaces of the root cortex. The presence of electron-dense deposits in intercellular spaces colonized by *F. oxysporum* hyphae may indicate the accumulation of phenolic compounds (Dixon et al. 1983; Kosuge 1969). Phenols have been reported to diffuse from cells in other plants when roots are infected by *F. oxysporum* (Beckman and Mueller 1970).

Root colonization by *F. oxysporum* strain Foeu1 is accompanied by a large amount of damage and irreversible cell wall alterations. Papillae are occasionally observed in *E. viminalis* roots upon infection of cortical cells by the pathogenic fungus. Although callose is a major component of wall appositions and papillae (Aist 1976), it has not been detected in the wall appositions produced by *E. viminalis* root tissues (unpublished data). On the other hand, nonesterified pectins are deposited in the papillae induced by the pathogenic *F. oxysporum* in the parenchymal cortex, similar to observations on tomato and cotton tissues infected by *Fusarium* spp. (Benhamou and Lafontaine 1995; Rodriguez-Gálvez and Mendgen 1995). The electron-dense contents of the papillae in infected endodermal cells in *E. viminalis* roots may reflect accumulation of phenolic substances, but they do not prevent spread of the fungus into the central cylinder tissues (Salerno et al. 2000). The apparently low intensity of host responses and the extensive infection by *F. oxysporum* strain Foeu1 suggest establishment of a susceptible type of interaction in the roots of the *E. viminalis* seedlings.

Fusarium strains break down pectin in pure culture (Steinberg et al. 1999), and it has long been recognized that *F. oxysporum* produces several enzymes that act on the pectic components of cell walls (Paquin and Coulombe 1962). Numerous studies have attempted to implicate these enzymes in events associated with colonizing susceptible tissue, overcoming host resistance, and symptom induction (Bayen et al. 1997). In the present study, we have demonstrated the localization of polygalacturonate substrates in host plants, not the enzymes themselves, to follow the modifications in pectin components that occur during the infection process by pathogenic *F. oxysporum* strains. Decreases in JIM 7-detectable ME pectin in cell walls of the epidermis and parenchymal cortex and the absence in phloem tissues of *E. viminalis* roots colonized by *F. oxysporum* strain Foeu1 may indicate fungal secretion of highly active PMEs, enzymes involved in pectin breakdown during pathogenic infection of plant tissues (Goldberg et al. 1996). Important decreases in binding with the JIM 5 antibody in cell walls of the epidermis, parenchymal cortex, and phloem of *E. viminalis* roots during infection by pathogenic *F. oxysporum* suggest that the fungus has PG activity in host tissues. The role of PGs in fungal infection of plant tissues has been well described by Cervone et al. (1996).

Conclusions

The present study makes a contribution to our understanding of the specific interactions between *E. viminalis*, one of the most economically important plants of Argentina, and a pathogenic strain of *F. oxysporum*. Further studies on pectolytic enzymes and their regulation in *F. oxysporum* strain Foeu1 will help elucidate their contribution to the fungal pathogenicity in host tissues of *E. viminalis*.

Acknowledgments We thank Dr. Paul Knox for providing the antibodies JIM 5 and JIM 7. We thank Gladys Lori for the pathogenic *F. oxysporum* strain. Special thanks go to Charles Schneider and Jean Pierre Caussanel for assistance with the image analysis program and with statistical analyses, respectively. We also thank Denise Dubois for technical assistance.

References

Aist JR (1976) Cytology of penetration and infection by fungi. In: Heitefuss R, Williams PH (eds) Encyclopedia of plant physiology, new series, vol 4: Physiological plant pathology. Springer, Berlin Heidelberg New York, pp 197–221

Bayen RP, Schoffelmeer EAM, Toet S, Elgersma DM (1997) Fungal polygalacturonase activity reflects susceptibility of carnation cultivars to Fusarium wilt. Eur J Plant Pathol 103:15–23

Beckman CH, Mueller WC (1970) Distribution of phenols in specialized cells of banana roots. Phytopathology 60:79–82

Beckman CH, Mueller WC, Tessier BJ, Harrison NA (1982) Recognition and callose deposition in response to vascular infection in fusarium wilt-resistant or susceptible tomato plants. Physiol Plant Pathol 20:1–10

Benhamou N, Lafontaine PJ (1995) Ultrastructural and cytochemical characterization of elicitor-induced structural responses in tomato root tissues infected by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Planta 197:89–102

Bishop CD, Cooper RM (1983) An ultrastructural study of root invasion in three vascular wilt diseases. Physiol Plant Pathol 22:15–27

Cervone F, De Lorenzo G, Aracri B, Bellincampi D, Caprari C, Clark AJ, Desiderio A, Devoto A, Leckie F, Mattei B, Nuss L, Salvi G (1996) The role of polygalacturonase, PGIP and pectin oligomers in fungal infection. In: Visser J, Voragen AGJ (eds) Pectins and pectinases. Elsevier, Amsterdam, pp 191–205

Dixon RA, Dey PM, Lamb CJ (1983) Phytoalexins: enzymology and molecular biology. Adv Enzymol Relat Areas Mol Biol 55:1–136

Elad Y, Evensen K (1995) Physiological aspects of resistance to *Botrytis cinerea*. Phytopathology 85:637–643

Gianinazzi S, Gianinazzi-Pearson V (1992) Cytology, histochemistry and immunocytochemistry as tools for studying structure and function in endomycorrhiza. Methods Microbiol 24:109–139

Goldberg R, Morvan C, Jauneau A, Jarvis MC (1996) Methyl-esterification, de-esterification and gelation of pectins in the primary cell wall. In: Visser J, Voragen AGJ (eds) Pectins and pectinases. Elsevier, Amsterdam, pp 151–172

Guevara MA, Gonzalez-Jaen MT, Estevez P (1996) Pectin lyase from *Fusarium oxysporum* f. sp. *radicis-lycopersici*: purification and characterization. In: Visser J, Voragen AGJ (eds) Pectins and pectinases. Elsevier, Amsterdam, pp 747–760

Hahn MG, Bucheli P, Cervone F, Doares SH, O'Neill RA, Darvill A, Albersheim P (1989) Roles of cell wall constituents in plant-pathogen interactions. In: Kosuge T, Nester EW (eds) Plant–microbe interactions: molecular and genetic perspectives, vol 3. McGraw-Hill, New York, pp 131–181

Have A, Mulder W, Visser J, van Kan JA (1998) The endopolygalacturonase gene *Bcpg*1 is required for full virulence of *Botrytis cinerea*. Mol Plant Microbe Int 11:1009–1016

Knox JP, Linstead PJ, King J, Cooper C, Roberts K (1990) Pectin esterification is spatially regulated both within cell walls and between developing tissues of root apices. Planta 181:512–521

Kosuge T (1969) The role of phenolics in host response to infection. Annu Rev Phytopathol 7:195–222

Lapp MS, Skoropad WP (1978) Nature of adhesive material of *Colletotrichum graminicola* appressoria. Trans Br Mycol Soc 70:221–223

Lemoine MC, Gollotte A, Gianinazzi-Pearson V (1995) Localization of β(1–3) glucan in walls of the endomycorrhizal fungi *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe and *Acaulospora laevis* Gerd. & Trappe during colonization of host roots. New Phytologist 129:97–105

Malalasekera RAP, Sanderson FR, Colhoun J (1973) *Fusarium* diseases of cereals. IX. Penetration and invasion of wheat seedlings by *Fusarium culmorum* and *F. nivale*. Trans B Mycol Soc 60:453–462

Mansfield JW, Richardson A (1981) The ultrastructure of interactions between *Botrytis* species and broad bean leaves. Physiol Plant Pathol 19:41–48

McKeen WE (1977) *Fusarium* in barley and corn roots. Can J Bot 55:12–16

Mendgen K, Hahn M, Deising H (1996) Morphogenesis and mechanisms of penetration by plant pathogenic fungi. Annu Rev Phytopathol 34:367–386

Nicholson RL, Epstein L (1991) Adhesion of fungi to the plant surface: prerequisite for pathogenesis. In: Cole GT, Hoch HC (eds) The fungal spore and disease initiation in plants and animals. Plenum, New York, pp 3–23

Olivain C, Alabouvette C (1997) Colonization of tomato roots by a nonpathogenic strain of *Fusarium oxysporum*. New Phytol 137: 481–494

Paquin P, Coulombe LJ (1962) Pectic enzyme synthesis in relation to virulence in *Fusarium oxysporum, F. lycopersici* (Sacc.) Snyder and Hansen. Can J Bot 40:533–541

Parry DW, Pegg GF (1985) Surface colonization, penetration and growth of three *Fusarium* species in lucerne. Trans Br Mycol Soc 85:495–500

Pringle RB (1981) Nonspecific adhesion of *Bipolaris sorokiniana* sporelings. Can J Plant Pathol 3:9–11

Rexova-Benkova L, Markovic O (1976) Pectic enzymes. Adv Carbohydr Chem Biochem 33:323–385

Reynolds ES (1963) The use of lead citrate at high pH as an electron opaque in electron microscopy. J Cell Biol 17:208–212

Rodriguez-Gálvez E, Mendgen K (1995) Cell wall synthesis in cotton roots after infection with *Fusarium oxysporum*. Planta 197:535–545

Salerno MI, Gianinazzi S, Gianinazzi-Pearson V (2000) Effects on growth and comparison of root tissue colonization patterns of *Eucalyptus viminalis* by pathogenic and nonpathogenic strains of *Fusarium oxysporum*. New Phytol 146:317–324

Steinberg C, Whipps JM, Wood DA, Fenlon J, Alabouvette C (1999) Effects of nutritional sources on growth of one nonpathogenic strain and four strains of *Fusarium oxysporum* pathogenic on tomato. Mycol Res 103:1210–1216

Valentines RC (1961) Contrast enhancement in electron microscopy. Adv Virus Res 8:287–290

VandenBosch KA, Bradley DJ, Knox JP, Perotto S, Butcher GW, Brewin NJ (1989) Common components of the infection threads matrix and the intercellular space identified by immunocytochemical analysis of pea nodules and uninfected roots. EMBO J 8:335–342