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Effects on growth and comparison of root tissue colonization patterns of *Eucalyptus viminalis* by pathogenic and nonpathogenic strains of *Fusarium oxysporum*

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SUMMARY

Soilborne pathogens, especially *Fusarium oxysporum*, are responsible for damping-off and root necrosis in *Eucalyptus* nurseries. New technologies are increasingly considering strategies for plant disease control other than chemical fungicides. Among these, natural fungal antagonists, which are colonizers of the root cortex, are potential biocontrol agents. An *in vitro* system was used: (1) to test the pathogenic effects of *F. oxysporum* strain Foeu1 which was recovered from a forest nursery soil; (2) to explore the potential of the nonpathogenic *F. oxysporum* strain Fo47, which is known for its efficiency in biological control, to suppress damping-off of *Eucalyptus* seedlings; (3) to compare the patterns of root colonization and host response to invasion by the two *Fusarium* strains inoculated separately in a time-course study. Root inoculation of *E. viminalis* with *F. oxysporum* strain Foeu1 caused damping-off in young seedlings *in vitro*, whilst disease symptoms were not visible in plants inoculated with *F. oxysporum* strain Fo47 or when both strains (Foeu1 + Fo47) were inoculated simultaneously. Each strain showed similarities in patterns of root tissue colonization, and in the processes of root penetration and initial colonization. Differential effects on root tissue were observed with fungal development within the cortex: ingress of strain Foeu1 was accompanied by severe host-cell alterations whilst no tissue damage occurred with development of strain Fo47.

Key words: *Fusarium oxysporum*, *Eucalyptus viminalis*, colonization, damping-off, biological control.

INTRODUCTION

Soilborne pathogens are responsible for damping-off and root necrosis in forest nurseries around the world. Although these diseases are regularly controlled by the use of chemical treatments, crop rotation or sanitary practices, recolonization of soil by pathogens is a general problem. The fungi most commonly isolated from damped-off seedlings in forest nurseries in the Province of Buenos Aires (Argentina) are *Pythium* and *Fusarium* species. The three most frequently isolated species of *Fusarium* are *F. oxysporum*, *F. equiseti* and *F. solani*. Attempts have been made to control damping-off of *Eucalyptus viminalis* by solarization in forest nurseries in the Province of Buenos Aires but root necroses were observed after three months in seedlings. *Fusarium oxysporum* was the only pathogenic fungus isolated from the diseased roots (Salerno *et al.*, 1999).

Fusarium oxysporum is distributed worldwide in soil, and is frequently associated with roots or lower parts of plants. Some fungal populations are highly pathogenic, causing wilts (Armstrong & Armstrong, 1981) and root rots (Bloomberg & Lock, 1974; Bloomberg, 1976, 1979; Graham & Linderman, 1983) in many plants of economic importance, whereas others appear to be vigorous saprophytes (Rouxel *et al.*, 1979; Gordon *et al.*, 1989). Nonpathogenic or less virulent *Fusarium* species have been reported to suppress the development of several forms of *F. oxysporum* in different crops (Alabouvette *et al.*, 1987; Cugudda & Garibaldi, 1987; von Mattusch, 1990). The mechanisms by which nonpathogenic strains of *F. oxysporum* suppress diseases are not well understood (Olivain & Alabouvette,

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1997) and those cited include nutritional, saprophytic and/or parasitic competition (Alabouvette *et al.*, 1979; Schneider, 1984; Lemanceau, 1989), as well as the possible existence of induced resistance (Olivain *et al.*, 1995; Duijff *et al.*, 1997; Duijff *et al.*, 1998).

The potential for exploiting nonpathogenic forms of *F. oxysporum* as biological control agents in the field is continuously being explored, but experiments have resulted in various degrees of disease suppression (Postma & Rattink, 1992). Knowledge of the patterns of root colonization by pathogenic and nonpathogenic isolates of *F. oxysporum* could contribute to an understanding of the mechanisms of disease suppression (Olivain & Alabouvette, 1997, 1999) and might help to improve the success of biological control.

Nothing is known about root colonization of *E. viminalis* by *F. oxysporum* nor about the possibility of using nonpathogenic *Fusarium* species to control pathogen attack. As a first step towards answering these questions, we have investigated the ability of a *F. oxysporum* strain (Foeu1), recovered from a forest nursery in Argentina, to cause damping-off of *E. viminalis* seedlings, analysed the potential of the nonpathogenic *F. oxysporum* strain Fo47, known for its bioprotective effect on other plants (Alabouvette *et al.*, 1987), to suppress damping-off in *E. viminalis* seedlings, and compared the patterns of root colonization and host responses to invasion by the two *Fusarium* strains inoculated separately in a time-course study. An *in vitro* system was used to ensure controlled conditions for these studies, and the effects of each strain inoculated independently (Fo47 or Foeu1) or co-inoculated (Fo47 + Foeu1) on disease incidence and on plant growth response of eucalypt seedlings were analysed. The development and intensity of root colonization by each fungal strain, and tissue responses of *E. viminalis* seedlings to root infection were studied histochemically and by autofluorescence.

MATERIALS AND METHODS

Fusarium oxysporum *strains*

Fusarium oxysporum Schlechtend.: Fr. strain Foeu1 was kindly provided by G. Lori (UNLP-CIC, La Plata, Argentina). This strain was isolated from a forest nursery soil in Saladillo, Argentina, where *Eucalyptus* species are grown regularly, and where root rot or damping-off diseases are known to occur. Nonpathogenic *F. oxysporum* strain Fo47 (kindly provided by C. Alabouvette, INRA, Dijon, France) was recovered from a horticultural soil in Chateaurenard, France, where *Eucalyptus* species have never grown. Both strains were cultured on potato-dextrose-agar (PDA) (Difco Laboratories, Detroit, MI, USA).

Plant growth and inoculation in vitro

Seeds of *Eucalyptus viminalis* Labill. were surface-disinfected for 20 min with sodium hypochlorite (NaOCl) (12% active chloride), rinsed three times in sterile distilled water and pregerminated on 0.6% water agar for 3 d at 27°C in the dark. When roots were 0.5 cm long, seedlings were gently transferred to Petri dishes vertically half-filled with Crush and Hay medium containing 5 g l^{-1} active C (Pons *et al.*, 1983). The seedlings, two per Petri dish, were incubated vertically in a growth cabinet at 25°C (12:12 h, day:night). Three days later, when seedlings were 6 d old and roots were 1.5 cm long, 2 mm^2 agar plugs of mycelium of *F. oxysporum* strain Foeu1 or strain Fo47, or both strains together (Foeu1 + Fo47), were placed on both sides of the root tip 0.5 cm from the root surface. The mycelium of strain Fo47 was placed closer to the root system than strain Foeu1 when they were inoculated simultaneously. Plants were grown in the same conditions as already described. Control seedlings were not inoculated.

Disease incidence and plant growth were followed for one month in a total of 10 seedlings from each treatment (Fo47, Foeu1, Fo47 + Foeu1 and controls). Plant height and root length were measured after 8, 10, 14, 20 and 30 d, and root or shoot increment was calculated (mm d^{-1}) for each treatment between 20 and 30 d. Disease symptoms developing during this time were recorded.

Data were subjected to ANOVA, and the treatment means, corresponding to each time point, were compared separately by the Student-Newman-Keuls Test ($P < 0.05$), using the SAS program (SAS Institute Inc., Cary, NY, USA).

Light microscopy of root infection and host responses

Eucalyptus viminalis seedlings were grown *in vitro* and inoculated with *F. oxysporum* strain Fo47, with strain Foeu1, with both strains or left non-inoculated, as described above. Ten seedlings from each treatment were sampled 3, 4, 5, 6 or 30 d after inoculation for individual strains and after 30 d for the co-inoculated treatment. Two different 0.5-cm-long root zones were cut from each seedling: beginning 0.5 cm and 1.5 cm behind the apex to give 5 mm-long root pieces. Root pieces were immersed overnight at 4°C in 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, then samples were dehydrated in a graded ethanol series (30–95%). Four to five randomly selected root segments were embedded in LR White resin (Oxford Instruments, Orsay, France), as described by Gianinazzi & Gianinazzi-Pearson (1992), for each treatment, time point and root region.

Five to 10 semi-thin sections (0.5 μm) of *c.* 50 root fragments from each treatment were mounted on Polylysine-coated glass slides, stained with toluidine

blue (Feder & O'Brien, 1968) and viewed with a Leitz (Bron, France) optical microscope. The general distribution of the fungi in root tissues was rated as: 0, absent; 1, up to 25% colonization; 2, up to 50% colonization; 3, up to 75% colonization; 4, 100% colonization. Results were expressed as an average percentage of root infection observed in five replicate root samples from each treatment, time point and root region combination.

Root responses of *Eucalyptis viminalis* to invasion by *F. oxysporum* strain Foeu1 and strain Fo47 were compared with those of noninoculated controls. Semi-thin sections of LR White-embedded roots were stained for 30 s with 0.1% toluidine blue in 1% sodium carbonate, pH 11 (Feder & O'Brien, 1968). Unstained sections were observed under blue light for autofluorescence (excitation filter BP 420–490 nm, barrier filter 515 nm) to detect the presence of phenolic compounds (Fernandez & Heath, 1986).

RESULTS

Effect of Fusarium oxysporum *strains Foeu1 and Fo47 on plant growth and disease incidence*

Macroscopic observations of *in vitro*-grown *E. viminalis* seedlings inoculated with *F. oxysporum* strain Foeu1, or strain Fo47, or co-inoculated with both *Fusarium* strains (Foeu1 + Fo47) showed that hyphae had reached the root surface 3 d after inoculation, especially behind the root tip (0.5 cm behind the apex). In all cases, the whole root systems of *E. viminalis* seedlings were covered with an extensive growth of mycelium within a few days.

All the noninoculated control plants continued growing up to 30 d *in vitro*, at which time they had reached the two-to-four-leaf stage and had normally developed root systems (Fig. 1a). Plants inoculated with *F. oxysporum* strain Foeu1 had reduced growth compared with that of the control, (Fig. 1a) and wilting, with symptoms of damping-off at the base of the hypocotyl, were visible. All seedlings inoculated with *F. oxysporum* strain Fo47 alone, or combined with strain Foeu1, developed much better than those inoculated with strain Foeu1 alone and resembled noninoculated plants (Fig. 1a). Disease symptoms did not appear in these plants, even 30 d after inoculation.

Average height of noninoculated control plants after 30 d growth did not differ significantly ($P < 0.05$) from that of plants inoculated with *F. oxysporum* strain Fo47 alone, or with Foeu1 (Foeu1 + Fo47) (Table 1). When roots of *E. viminalis* were inoculated with *F. oxysporum* strain Foeu1, slower increase in plant height was already evident after 14 d. After 30 d, growth of these plants was significantly decreased ($P < 0.05$) in comparison with that of noninoculated plants and those inoculated with strain Fo47 alone or in combination with strain Foeu1 (Table 1). Mean height increment (mm d^{-1}) between 20 and 30 d of growth was significantly lower ($P < 0.05$) in seedlings inoculated with *F. oxysporum* strain Foeu1 than in noninoculated seedlings and those inoculated with strain Fo47 alone or combined with Foeu1 (Table 2). Differences for mean root length (cm) were not significant between treatments, although roots tended to be shortest and lateral root production less in plants inoculated with strain Foeu1. Between 20 and 30 d, root length increment was greatest in noninoculated plants, intermediate in Fo47-inoculated plants and 0 in those inoculated with Foeu1 (Table 2).

Infection processes

Noninoculated roots had intact epidermal and hypodermal tissues in both the 0.5 cm and 1.5 cm zones behind the apex, and the parenchymal cortex was compact (Fig. 1b). The most common points of entry of *F. oxysporum* strains Foeu1 and Fo47 were through root hairs and at the junction between adjacent epidermal cells, from where they penetrated into the outer cells or developed intercellularly down to the hypodermis and parenchymal cortex (Fig. 1c,d,g). Fungal penetration could also be observed at the point of emergence of a lateral root (not shown).

Both strains of *F. oxysporum* proliferated either intercellularly or intracellularly within the root parenchymal cortex and could colonize the central cylinder, phloem and/or xylem (Fig. 1d,g). Ingress towards the cortex by strain Foeu1 coincided with a progressive alteration of this tissue accompanied by cell collapse and tissue maceration which was already evident in the cortex 3 d after inoculation (Fig. 1c). The phloem as well as the cortical tissue was damaged when this fungus reached the central cylinder 6 d after inoculation (Fig. 1d). In some cases, tissue disorganization was seen in cortical cells or phloem tissue before colonization reached these tissues (Fig. 1c). Hypodermal cells frequently appeared collapsed in the presence of the strain Foeu1 and these collapsed cells formed a layer, separating the epidermis from the cortex, that autofluoresced under blue light (Fig. 1e) and stained red with basic fuchsin (Fig. 1f). However, this phenomenon appeared randomly (Fig. 1e) and was also seen in control tissues at the point of emergence of a secondary root (not shown).

When *F. oxysporum* strain Fo47 infected root systems, root tissues did not show signs of damage with progression of the fungal infection at any time point, even when hyphae heavily colonized the parenchymal cortex and extended beyond the endodermis into the central cylinder (Fig. 1g). The collapsed hypodermal tissue was also observed in the presence of strain Fo47 (not shown).

Root colonization by strains Foeu1 and Fo47 inoculated simultaneously was examined at only one

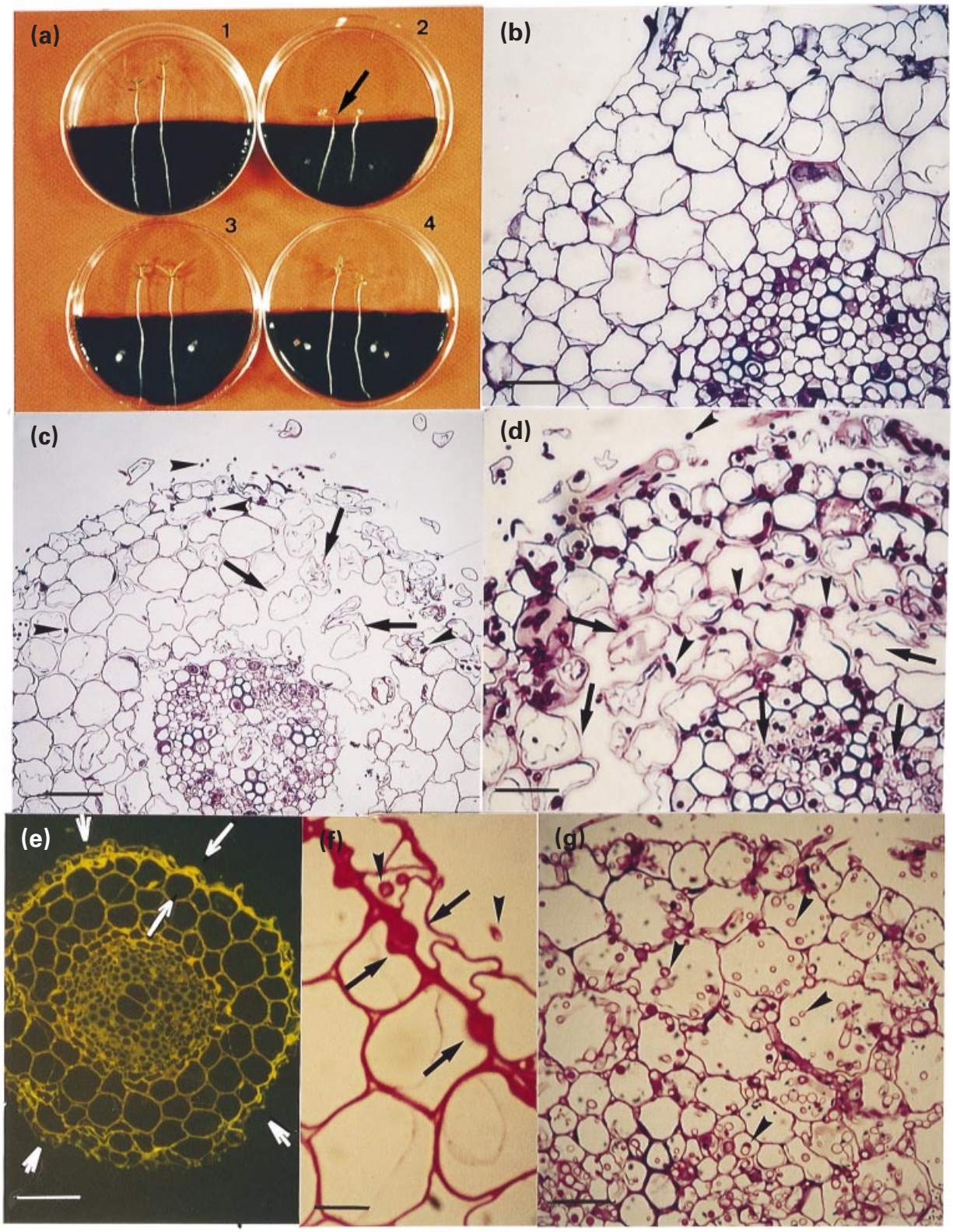


Fig. 1. (a) Thirty d-old noninoculated control plants of *Eucalyptus viminalis* (1), *E. viminalis* seedlings inoculated with *Fusarium oxysporum* strain Foeu1 and showing symptoms of damping-off (hypocotyl wilt, arrow) (2), asymptomatic *E. viminalis* seedlings inoculated with nonpathogenic *F. oxysporum* strain Fo47 (3), and *E. viminalis* seedlings co-inoculated with nonpathogenic *F. oxysporum* strain Fo47 and strain Foeu1, showing no disease symptoms (4). (b) Cross section of a noninoculated control root of *E. viminalis*. Bar, 30 μm. (c,d) Transverse sections of an *E. viminalis* root. (c) 3 d after inoculation with *F. oxysporum* strain Foeu1. Bar, 45 μm. (d) 6 d after inoculation with *F. oxysporum* strain Foeu1. Bar, 30 μm. Progressive tissue damage is observed in the parenchymal cortex and phloem (arrows) as fungal colonization progresses from the external cortical cells (c) to the parenchymal cortex (arrowheads) and inside phloem cells (arrows) (d). (e) Autofluorescence under blue light of a transverse section of an *E. viminalis* root 6 d after inoculation with

Table 1. *Height of* Eucalyptus viminalis *seedlings* in vitro

	Height (cm)					
	Days after inoculation					
Treatment	6	8	10	14	20	30
Noninoculated	0.74	1.02	1.14	1.62	2.01 a	2.57 a
Foeu1	0.77	0.95	1.13	1.31	1.55 b	1.69 b
Fo47	0.88	1.03	1.16	1.47	1.72 ab	2.15 a
Foeu1 + Fo47	0.84	1.10	1.20	1.53	1.80 ab	2.22 a

Values in columns followed by the same letter are not significantly different ($P < 0.05$).

time point after 30 d. Intercellular and intracellular hyphae were observed in epidermal, hypodermal and cortical tissue, but it was not possible to distinguish between the two strains of *F. oxysporum*. Disorganization of the root cortical tissue, characterized by cell collapse, was occasionally observed, and phloem and xylem remained intact (data not shown).

Quantification of root tissue colonization by Fusarium oxysporum *strains Foeu1 and Fo47*

Light-microscope examination of root samples inoculated with *F. oxysporum* strain Foeu1 taken at 0.5 cm behind the root tip showed that the fungus was mainly on the root surface 3 d after inoculation, whilst it was not yet in contact with the root at 1.5 cm behind the apex (data not shown). Four days after inoculation, the fungus had penetrated epidermal cells in the root zone 0.5 cm behind the apex and to a lesser extent at 1.5 cm (Fig. 2a). After 5 d, hyphae had invaded much of the cortex and some had reached the central cylinder in the more apical zone, whilst they were still restricted to epidermal and hypodermal cells in the 1.5 cm root region (Fig. 2b).

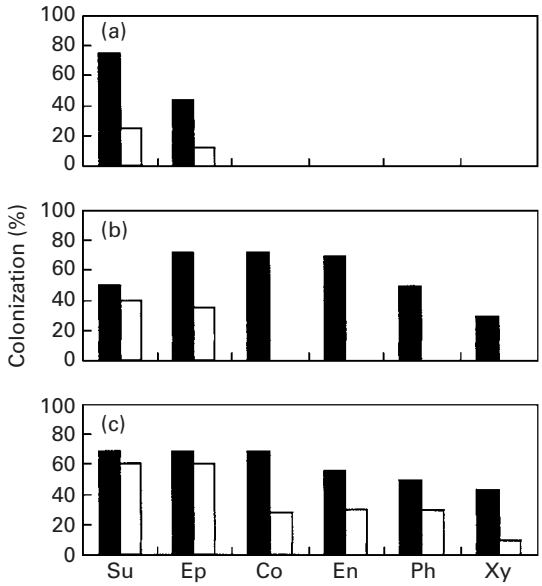


Fig. 2. Colonization of different root tissues o*f Eucalyptus viminalis* seedlings by *Fusarium oxysporum* strain Foeu1 at 0.5 cm (solid bars) and 1.5 cm (open bars) behind the apex, 4 d (a), 5 d (b) and 6 d (c) after inoculation. Su, surface; Ep, epidermis; Co, cortex; En, endodermis; Ph, phloem; Xy, xylem.

Table 2. *Mean growth increment of* Eucalyptus viminalis *seedlings* in vitro *20–30 d after inoculation*

Treatment	Mean height increment (mm d^{-1})	Mean root increment (mm d^{-1})
Noninoculated	0.56 a	0.64 a
Foeu1	0.14 b	0.00 b
Fo47	0.43 a	0.35 a
Foeu1 + Fo47	0.42 a	0.37 a

Values in columns followed by the same letter are not significantly different ($P < 0.05$).

By 6 d after inoculation, colonization by strain Foeu1 was intense in all root tissues just behind the apex, and at 1.5 cm the fungus had reached much of the cortex, with some hyphae colonizing the central cylinder (Fig. 2c).

Transverse sections of root systems inoculated with *F. oxysporum* strain Fo47 from the two zones at 0.5 cm and 1.5 cm behind the apex showed that the fungus had not entered the root system 3 d after inoculation (data not shown). In samples taken 4 d after inoculation, the fungus was found mainly at the root surface and in the epidermis, but in some cases it also extended through the cortex and into the

F. oxysporum strain Foeu1. Hyphae (arrowheads) are restricted to the epidermis. Collapsed cells form a layer separating the epidermis from the cortex (arrows). Bar, 50 μm. (f) Cross section of an *E. viminalis* root colonized by *F. oxysporum* strain Foeu1 and stained with basic fuchsin. Collapsed cells form a layer separating the epidermis from the cortex (arrows) and hyphae are restricted to the epidermis (arrowheads). Bar, 10 μm. (g) Cross section of an *E. viminalis* root 6 d after inoculation with nonpathogenic *F. oxysporum* strain Fo47 and showing no tissue damage although hyphae (arrowheads) are intensely invading all the root tissues. Bar, 30 μm.

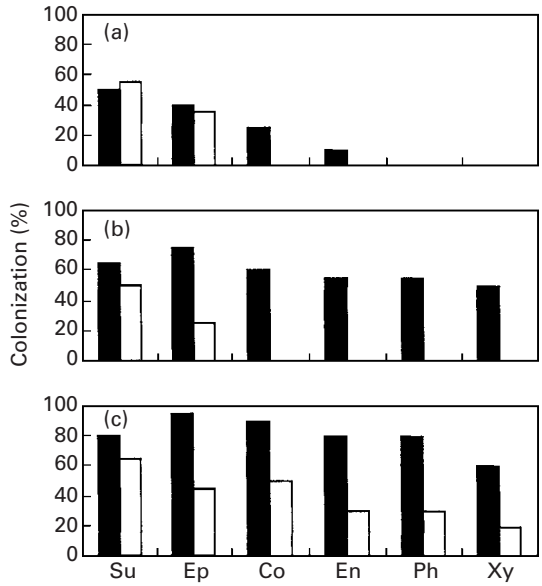


Fig. 3. Colonization of different root tissues of *Eucalyptus viminalis* seedlings by nonpathogenic *Fusarium oxysporum* strain Fo47 at 0.5 cm (solid bars) and 1.5 cm (open bars) behind the apex, 4 d (a), 5 d (b) and 6 d (c) after inoculation. Su, surface; Ep, epidermis; Co, cortex; En, endodermis; Ph, phloem; Xy, xylem.

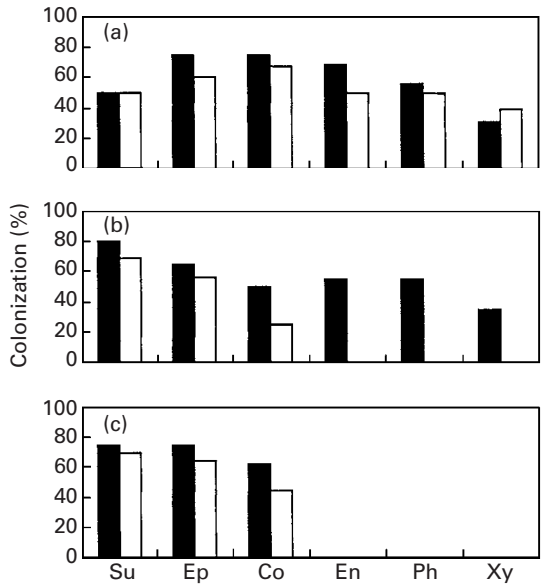


Fig. 4. Colonization of different root tissues of *Eucalyptus viminalis* seedlings by *Fusarium oxysporum*, at 0.5 cm (solid bars) and 1.5 cm (open bars) behind the apex, 30 d after inoculation by (a) strain Foeu1, (b) strain Fo47 and (c) both strains (Foeu1 + Fo47). Su, surface; Ep, epidermis; Co, cortex; En, endodermis; Ph, phloem; Xy, xylem.

endodermis in the 0.5 cm zone (Fig. 3a). After 5 d, strain Fo47 had colonized all the root tissues in the zone just behind the apex whilst it was mainly restricted to epidermal cells in the 1.5 cm zone, in a way similar to strain Foeu1 (Fig. 3b). Likewise, hyphae invaded all tissues intensely in zones at 0.5 cm, and to a lesser extent at 1.5 cm, behind the apex 6 d after inoculation (Fig. 3c).

Both strains of *F. oxysporum* continued to invade newly produced root tissues. Colonization by strain Foeu1 of the central cylinder and other cell types remained high 30 d after inoculation, both just behind the apex and in the 1.5 cm zone (Fig. 4a). *Fusarium oxysporum* strain Fo47 continued to colonize all types of newly produced subapical tissues (0.5 cm zone) 30 d after inoculation, but hyphae were not observed in vascular tissues of the older root zones 1.5 cm behind the apex (Fig. 4b). When strains were co-inoculated, hyphae developed in epidermal tissues and in the cortex of subapical and older root zones of main roots (Fig. 4c) but they were not observed in newly produced lateral roots.

DISCUSSION

Fusarium oxysporum strain Foeu1 caused damping-off in young seedlings of *E. viminalis in vitro* but disease symptoms were not visible in plants inoculated with strain Fo47 alone or in combination with strain Foeu1. This indicates that *F. oxysporum* strain Fo47 is nonpathogenic on *E. viminalis* and can provide a protective effect against the pathogenic strain Foeu1 in eucalypt seedlings. These results are in agreement with those demonstrating that the nonpathogenic *F. oxysporum* strain Fo47 is able to suppress fusarium wilts in tomato plants (Eparvier & Alabouvette, 1994; Fuchs *et al.*, 1997; Duijff *et al.*, 1998) and in carnation (Rattink, 1987; Tramier *et al.*, 1987; von Mattusch, 1990; Lemanceau *et al.*, 1992; Postma & Rattink, 1992). In the present study both pathogenic and nonpathogenic strains of *F. oxysporum* successfully grew and developed on the root surface of *E. viminalis* seedlings, suggesting that either organism responds to similar stimuli from the host and that these stimuli are probably not species-specific (Goodman *et al.*, 1986). Both strains were also able to penetrate all root tissues of *E. viminalis* successfully, indicating that there are no apparent barriers that interfere with the penetration process of either organism. The fact that *F. oxysporum* strain Fo47 colonizes all root tissues of *E. viminalis* agrees with results obtained by Olivain & Alabouvette (1997), who reported that another nonpathogenic strain of *F. oxysporum* (Fo5a4) is able to colonize all root tissues of tomato plants, except the xylem vessels.

The sites of most rapid colonization by either pathogenic or nonpathogenic *F. oxysporum* inoculated independently were just behind the root apex (at 0.5 cm), and points of emergence of lateral roots provided additional infection sites. Strain Fo47 apparently spread into root tissues more quickly, in accordance with the findings of Paulitz *et al.* (1987) who indicated that, in general, nonpathogenic *Fusarium* isolates are highly competitive saprophytes. After penetration, both the pathogenic and nonpathogenic strains of *F. oxysporum* spread from

the infection site to colonize the root cortex and central cylinder. Both fungi developed within the intercellular spaces of root tissues of *E. viminalis*. Intercellular spaces probably provide easy routes for those hyphae able to survive in the extracellular environment (Ride, 1983). Both fungi were also seen inside *E. viminalis* root cells, indicating that hyphae were able to breach the plant cell walls and colonize the cell lumen. Such similar patterns of root colonization by pathogenic and nonpathogenic strains of *F. oxysporum* have also been reported in tomato roots (Olivain & Alabouvette, 1999).

Root colonization by nonpathogenic *F. oxysporum* strain Fo47 was only observed transiently after 6 d in the central cylinder at 1.5 cm behind the apex, and root colonization of these tissues was not found after 30 d. This might be explained by the development of a localized plant resistance in response to fungal invasion (Fuchs *et al.*, 1997) in seedlings. Conversely, phloem and xylem cells continued to be colonized by the strain Foeu1 after 6 d in this root zone and increased up to 30 d. Colonization of phloem and xylem by hyphae was not observed in this root zone when strain Foeu1 was inoculated simultaneously with strain Fo47, suggesting that the nonpathogenic *F. oxysporum* strain Fo47 might limit extension of the pathogenic strain beyond the cortex.

Although *F. oxysporum* was found in xylem vessels it might not be a true vascular pathogen of *Eucalyptus* seedlings, since colonization of the xylem tissue could have been a consequence of widespread fungal infection in the weakened young seedlings. Young seedlings generally have only low resistance to infection; all tissues of young seedlings are juvenile and lack mature plant resistance up to a certain critical age (Garret, 1970). The capacity of *Fusarium* species to colonize less resistant seedlings has previously been reported (Parry & Pegg, 1985).

Even though both the pathogenic and nonpathogenic strains of *F. oxysporum* were found in the root cortex, light-microscope observations indicate that the root tissues of *E. viminalis* react differently to infection by strain Foeu1 and by strain Fo47. Progressive ingress of the pathogenic strain Foeu1 towards the cortical tissue coincided with extensive tissue disorganization which also occurred progressively in phloem tissue as the central cylinder became invaded, whilst root infection by the nonpathogenic strain Fo47 was associated with conservation of structural integrity in all the colonized host tissues. Little tissue damage was observed in cortical cells when strain Foeu1 was co-inoculated with strain Fo47 and the central cylinder remained intact. A visible response of host tissues to fungal colonization by either *F. oxysporum* strain was the presence of completely collapsed hypodermal root cells. However, this was a completely random phenomenon and was also associated with emergence of a lateral root observed in noninoculated control roots. It might, therefore, represent a general response to stress of root tissues in *E. viminalis*.

The fact that *F. oxysporum* strain Fo47 colonized the same root tissues as strain Foeu1 in *E. viminalis* shows that both nonpathogen and pathogen occupy the same ecological niche, and suggests that competition for infection sites might be one of the modes of action involved in bioprotection by strain Fo47, as also evoked by Olivain & Alabouvette (1997, 1999). Furthermore, Paulitz *et al.* (1987) have proposed that, in general, nonpathogenic *Fusarium* isolates are efficient biocontrol agents because they are highly competitive saprophytes and successfully colonize the cortical tissue of roots. However, competition for infection sites alone does not necessarily fully explain all the biocontrol activity of nonpathogenic strains of *F. oxysporum* (Olivain & Alabouvette, 1997). The presence of the pathogen *F. oxysporum* f. sp. *lycopersici* in tomato plants induces increases in chitinase and β-1,3-glucanase activities, as well as elicitation of an additional β-1,3-glucanase isoform, compared with nonpathogenic *F. oxysporum*-infected plants, which indicates a differential response to the pathogenic and nonpathogenic strains (Recorbet *et al.*, 1998). Several authors have suggested that induced resistance contributes to biocontrol effects (Postma & Luttikholt, 1996) and that nonpathogenic isolates of *F. oxysporum*, in addition to competition for infection sites, penetrate intact roots inducing systemic resistance in the plant (Mandeel & Baker, 1991; Duijff *et al.*, 1998). Evidence for a systemic induced response in *Eucalyptus* roots was reported by Albrecht *et al.* (1994) in comparisons of pathogenic and ectomycorrhizal infections. The authors suggest that a systemic signal is generated at the infection sites in roots which is presumably translocated throughout the plant in both symbiotic and pathogenic infections, the plant response to pathogenic infection being much higher (Albrecht *et al.*, 1994).

The high efficacy of the nonpathogenic *F. oxysporum* strain Fo47 in protecting plants of *E. viminalis* against the pathogenic strain Foeu1 is certainly intriguing. Although patterns of root colonization by each strain are rather similar at the tissue level, there are clear cytological differences in host responses to them. Ultrastructural analyses are presently being carried out in order to understand more fully the nature of *E. viminalis–F. oxysporum* interactions, and to gain insight into the possible implication of plant defence reactions in the bioprotective effect of nonpathogenic *F. oxysporum* in *E. viminalis* seedlings.

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