European Journal of Plant Pathology (2005) 111: 19–28

Infection of wheat spikes by *Fusarium avenaceum* and alterations of cell wall components in the infected tissue

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Accepted 31 May 2004

Key words: degradation of cell wall components, *Fusarium avenaceum*, infection process, *Triticum aestivum*

Abstract

The infection process of *Fusarium avenaceum* on wheat spikes and the alteration of cell wall components in the infected host tissue were examined by means of electron microscopy and cytochemical labelling techniques following spray inoculation at growth stage (GS) 65 (mid-flowering). Macroconidia of the pathogen germinated with one to several germ-tubes 6–12 h after inoculation (hai) on host surfaces. The germ-tubes did not penetrate host tissues immediately, but extended and branched on the host surfaces. Hyphal growth on abaxial surfaces of the glume, lemma and palea was scanty 3–4 days after inoculation (dai) and no direct penetration of the outer surfaces of the spikelet was observed. Dense mycelial networks formed on the inner surfaces of the glume, lemma, palea and ovary 36–48 hai. Penetration of the host tissue occurred 36 hai by infection hyphae only on the adaxial surfaces of the glume, lemma, palea and upper part of ovary. The fungus penetrated the cuticle and hyphae extended subcuticularly or between the epidermal wall layers. The subcuticular growth phase was followed by penetration of the epidermal wall, and hyphae spread rapidly inter- and intracellularly in the glume, lemma, palea and ovary. During this necrotrophic colonization phase of the wheat spike, a series of alterations occurred in the host tissues, such as degeneration of cytoplasm and cell organelles, collapse of host cells and disintegration of host cell walls. Immunogold labelling techniques showed that cell walls of spike tissues contained reduced amounts of cellulose, xylan and pectin near intercellular hyphae or infection pegs compared to walls of healthy host tissues. These studies suggest that cell wall degrading enzymes produced by *F. avenaceum* facilitated rapid colonization of wheat spikes. The different penetration properties of abaxial and adaxial surfaces of the spikelet tissues as well as the two distinct colonization strategies of host tissues by *F. avenaceum* are discussed. The penetration and colonization behaviour of *F. avenaceum* in wheat spikelets resembled that of *F. culmorum* and *F. graminearum*, although mycotoxins produced by *F. avenaceum* differed from those of the latter two *Fusarium* species.

Introduction

Fusarium head blight (FHB) of wheat (*Triticum aestivum*), also known as scab or ear blight, is one of the most destructive diseases in humid and semi-humid wheat-growing areas (Cook, 1981; McMullen et al., 1997). Epidemics of FHB result in a significant yield reduction and low quality of grains (Boyacioglu and Hettiarachchy, 1995), but also in contamination of grains with mycotoxins produced by the pathogens (Snijders and Perkowski, 1990; Golinski et al., 1996). The mycotoxins have been shown to be harmful to human and animal health (Desjardins and Hohn, 1997). FHB may be caused by 17 different *Fusarium* species, the most common of which are *Fusarium*

graminearum, *F. culmorum*, *F. avenaceum*, *F. poae*, and *Microdochium nivale* (=*Fusarium nivale*) (Wiese, 1987; Parry et al., 1995).

Previous studies have demonstrated that FHB is predominantly a floral disease (Dickson et al., 1921; Anderson, 1948; Strange and Smith, 1971). Spikelets infected by the pathogen first show light brown, water-soaked spots on the glumes. The infected spikelets then lose the water-soaked appearance and become dark brown. The necrosis spreads from the infected spikelet to adjacent spikelets throughout the rachis. Recently, we clarified the infection process and colonization pathway of *F. culmorum* and *F. graminearum* in wheat spikes by means of light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM), and found that these two pathogens exhibit a similar behaviour regarding infection and colonization of wheat spikes (Kang and Buchenauer, 2000a; Wanyoike et al., 2002). Moreover, using enzyme-gold and immuno-gold labelling techniques, we demonstrated that *F. culmorum* and *F. graminearum* produced cell wall-degrading enzymes such as cellulases, xylanases and pectinases at early stages of infection in wheat spikes (Kang and Buchenauer, 2000b; Wanyoike et al., 2002).

The predominant Fusarium head blight species besides *F. culmorum* and *F. graminearum* are *F. avenaceum* and *F. poae*. While *F. culmorum* and *F. graminearum* (teleomorph *Gibberella zeae*) belong to the Fusarium-section Discolor, *F. avenaceum* (teleomorph *G. avenacea*) is a member of section Roseum (Cunfer, 1987; Parry et al., 1995). *Fusarium avenaceum* is widespread throughout the cool temperature cereal-growing areas and produces moniliformin, cyclic peptides (for instance enniatins) and fusarin as secondary toxic metabolites (Desjardins and Proctor, 2001).

The pathogenicity of *F. avenaceum* is generally lower than that of *F. culmorum* and *F. graminearum*. *Fusarium avenaceum* has been reported as one of the most frequently isolated Fusarium species from cereals in Norway (Kosiak et al., 1997; Langseth et al., 1997), Finland (Yla-Mattila et al., 2002), Poland (Golinski et al., 1996), England (Parry et al., 1995) and Germany (Schütze et al., 1997). Because information on the infection process of *F. avenaceum* in wheat spikes is limited, penetration and colonization of wheat spikes by *F. avenaceum* was examined in this study by SEM and TEM. The degradation of host cell wall components in *F. avenaceum*-infected wheat spikes was investigated by means of enzyme-gold and immunogold labelling.

Materials and methods

Plant and pathogen

The susceptible winter wheat cv. Agent was used. The plants were grown as described (Kang and Buchenauer, 1999). Methods of macroconidial production and preparation of inoculum suspension of *F. avenaceum* (isolate no. 64854, provided by Dr Nirenberg Institute of Microbiology, BBA, Königin-Luise-Strasse 19, Berlin, Germany) were similar to those reported for *F. culmorum* (Kang and Buchenauer, 1999).

Inoculation of wheat spikes

When the plants were at mid-flowering, growth stage (GS) 65 (Zadoks et al., 1974), wheat spikes were inoculated (June 12, 2002) with a conidial suspension (5×10^5 conidial ml^{-1}) of *F. avenaceum*. Control plants were treated with distilled water instead of the macroconidial suspension. Inoculated spikes were covered with plastic bags and the plants were transferred into a growth chamber with 16 h fluorescent light at 23 °C and 8 h darkness at 18 °C. Two days later the plastic bags were removed and the plants were returned to outdoor conditions. During this period the temperature varied between 20 and 23 °C and the relative humidity between 65% and 93%. Pots were kept under shelter during rainy periods.

Scanning electron microscopy (SEM)

Whole single spikelets from inoculated wheat spikes were sampled 6, 12, and 24 h as well as 2, 3, 4, and 6 days after inoculation (dai). Samples were first fixed with 4% (v/v) glutaraldehyde in 50 mM phosphate buffer (pH 6.8) for 6–8 h at 4 °C and rinsed with the same buffer for 2 h. Samples were then postfixed in 1% (w/v) osmium tetroxide in 50 mM phosphate buffer for 1 h. After dehydration in a graded actone series, the samples were critical-point dried, mounted on stubs, sputter coated with gold-palladium, and viewed using a

Zeiss 100 scanning electron microscope operating at 15 kV.

Transmission electron microscopy (TEM)

Inoculated florets were taken 24 and 36 h, as well as 2, 3, 4, 5, 6, 8, and 10 dai. Glumes, lemmas, paleas, ovaries, and the rachis from the florets were excised into pieces and fixed in 3% (v/v) glutaraldehyde in 50 mM phosphate buffer (pH 6.8) for 3–6 h at 4 °C. Thereafter, samples were rinsed thoroughly with 50 mM phosphate buffer (pH 6.8) and post-fixed with 1% (w/v) osmium tetroxide in the same buffer for 2 h at 4 °C. However, for immuno-gold labelling, the samples were fixed with 1% (v/v) glutaraldehyde and 4% (w/v) formaldehyde in 100 mM sodium cacodylate buffer (pH 7.2) for 6 h. Subsequently, all samples were dehydrated in a graded ethanol series, embedded in LR White (TAAB Laboratories, Munich) and polymerized at 50 °C for 2 days. Ultra thin sections of the samples were cut with a diamond knife and collected on 200-mesh copper grids. After contrasting with uranyl acetate and lead citrate, the grids were examined with a Zeiss-EM10 electron microscope at 80 kV.

Cytochemical labelling for cellulose, xylan and pectin

The method of Frens (1973) was used for preparing a colloidal gold solution with particles of 15 nm average diam. The cellulase-gold probe and xylanase-gold probe were prepared using the cellulase of *Trichoderma reesei* (Worthington Biochemical Corporation, Freehold, NJ, USA) and the xylanase of *T. viride*, (Sigma Chemical Corporation, St Louis, MO, USA), respectively, as described by Berg (1990) and Giesbert et al. (1998). For localization of cellulose and xylan, ultrathin sections were quenched with citrate buffer (50 mM, pH 5.4), containing 1% bovine serum albumin (BSA) and 0.5% gelatine (Merk, Germany) for 20 min and then incubated with the respective enzyme-gold solution (1:10) for 20 min. For immuno-gold detection of pectin, ultra thin sections were first blocked with 1% (w/v) of BSA in Tris-buffered saline (TBS, 10 mM Tris–HCl, 150 mM NaCl, pH 7.4) for 20 min, then incubated with monoclonal antibody JIM7 (specifically binding to methyl-esterified pectin; Knox et al., 1990) diluted 1:1 with TBS for 14 h at 4 °C. After rinsing with TBS, the sections were incubated for 2 h with goat anti-rat immunoglobulin linked to 15 nm colloidal gold particles (GAT-gold antibody, EM GATIg G15, Biocell, Cardiff, UK) diluted 1:30 in TBS. All ultrathin sections were then contrasted with uranyl acetate and lead citrate before examination with the electron microscope. Control sections were incubated with uncoated colloidal gold solution or goat anti-rat immunoglobulin solution alone, or incubated with cellulase-gold, xylanase-gold or the monoclonal antibody JIM7, to which carboxymethylcellulose, xylan or pectin from citrus, repectively, was previously added (Kang and Buchenauer, 2000b).

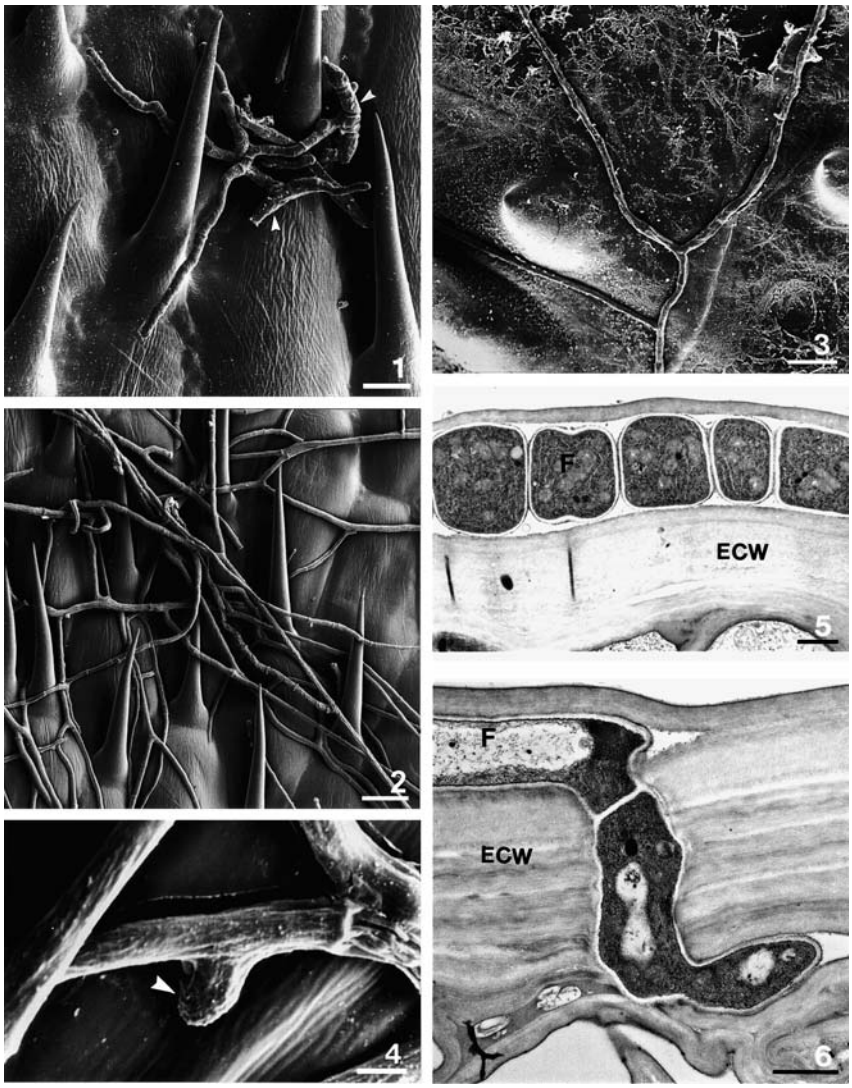
Quantification of labelling

The labelling densities for cellulose, xylan and pectin in the uninoculated and infected wheat spikes were compared by determining the number of gold particles per μm^2 over specified areas on 15–20 photomicrographs. The difference in the number of gold particles between uninoculated healthy and infected wheat spikes was statistically analyzed by the paired *t*-test.

Results

Infection process and colonization pathway

Observations from SEM showed that macroconidia of *F. avenaceum* germinated with one to several germ-tubes on the surfaces of the glume, lemma, palea, ovary and rachis 6–12 hai (Figure 1). The germ-tubes did not penetrate host tissues immediately, but they extended and branched on the host surfaces. However, the pathogen's further development on the abaxial and adaxial surfaces of the spikelet was significantly different. Hyphal growth was limited on the abaxial surfaces of the spikelet, and a relatively scanty hyphal network developed on the outer surface of the glume, lemma, palea and rachis 3–4 dai (Figure 3). During this incubation period no direct penetration of the abaxial surfaces of the spikelet by *F. avenaceum* was observed. The hyphae near the edges of the glume and lemma extended over the edges to reach the adaxial surfaces of the floret. A dense mycelial network was formed on the inner

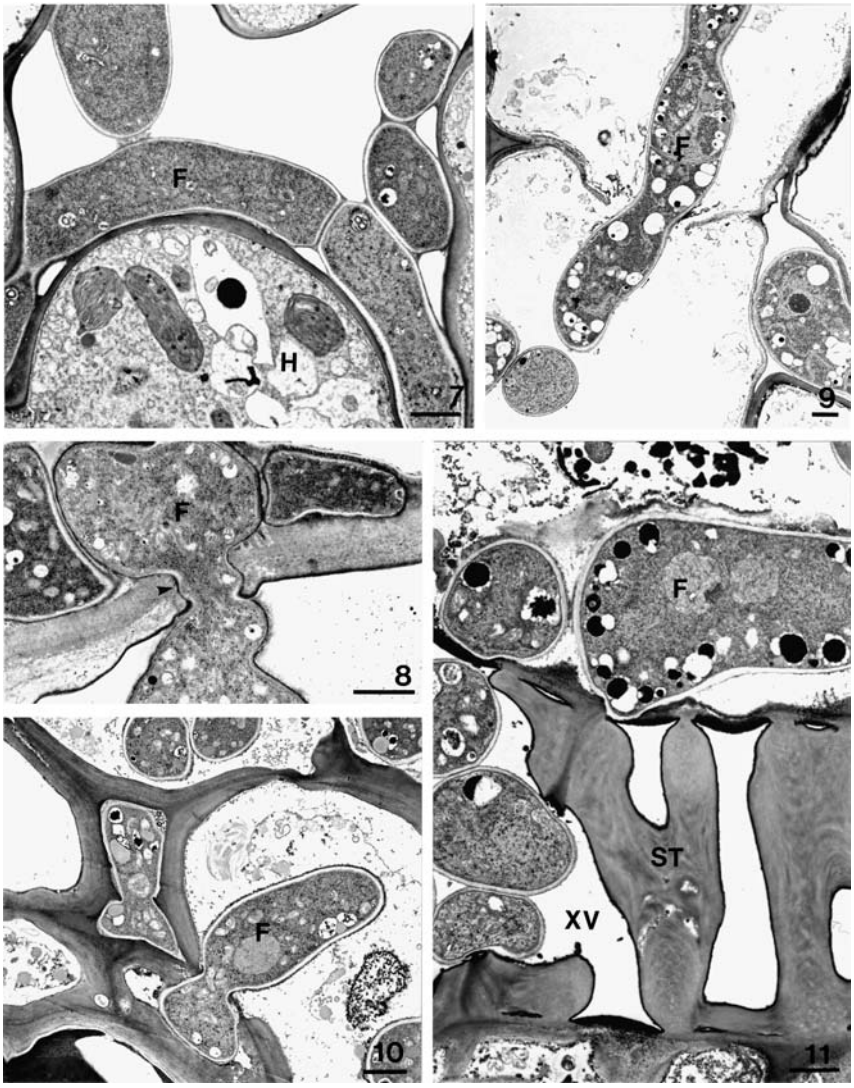


Figures 1–6. Scanning (Figures 1–4) and transmission (Figures 5, 6) electron micrographs of colonization of wheat spike by *Fusarium avenaceum*. (1) *F. avenaceum* macroconidia (arrowheads) germinated with germ-tubes on the adaxial surface of the lemma, 12 h after inoculation (hai) (Bar = 10 μm). (2) Hyphae on the abaxial surface of the glume, 4 dai. Scanty mycelium had developed on the abaxial surface of the glume (Bar = 10 μm). (3) Dense hyphal network on the adaxial surface of the lemma, 2 days after inoculation (dai) (Bar = 20 μm). (4) Infection hypha (arrowhead) developing from a hypha adhering to the adaxial surface of the lemma, 36 hai (Bar = 2 μm). (5) Hyphae (F) extending subcuticularly in the epidermial cell wall (ECW) on the adaxial surface of the lemma, 2 dai (Bar = 1 μm). (6) Hypha (F) growing subcuticularly in the epidermial cell wall (ECW) of the adaxial surface of the lemma penetrating the rest layers of the wall by a penetration peg, 3 dai (Bar = 1 μm).

surfaces of the glume, lemma, palea and on the ovary 36–48 hai (Figure 2). With extended incubation time, hyphal growth on the inner surfaces of the spikelet became denser and 3–4 dai the hyphae had reached the bases of the glume, lemma and palea.

By 36 hai, the hyphae on the adaxial surfaces of the glume, lemma and palea formed short branches, the infection hyphae (Figure 4). The infection hyphae did not produce appressoria, but penetrated the epidermal cell wall directly with a penetration peg. The fungus did not often penetrate the epidermal cell wall completely, penetrating only the cuticle, and hyphae extended beneath the cuticle or between wall layers. With continuing growth, more hyphae could be observed subcu-

ticularly (Figure 5) or inside the epidermal cell wall. During this subcuticular development of *F. avenaceum* hyphae no detrimental effects on host cells were observed. These subcuticular hyphae also produced penetration pegs on the remaining layers of the epidermal cell wall (Figure 6). Hyphae were detected 2 dai in the intercellular space between parenchyma cells in the glume, lemma and palea (Figure 7). During growth in the intercellular spaces, the hyphae induced degenerative alterations in the host cells which were in contact with the hyphae and the necrotrophic phase was initiated. Small appositions appeared between the cell wall and the plasmalemma and vacuoles



Figures 7–11. Transmission electron micrographs of colonization of wheat spike by *Fusarium avenaceum*. (7) Hyphae (F) extending in the intercellular spaces between host cells (H) in the lemma, 2 days after inoculation (dai). Vacuoles increased in the cytoplasm of the host cell (Bar = 2 μm). (8) Penetration of the epidermal cell wall of the ovary by a hypha (F) with a penetration peg (arrowhead), 2 dai. The host cell was degenerated (Bar = 2 μm). (9) Hyphae (F) extending inter- and intracellularly in the pericarp tissue in the ovary, 3 dai. Host cells showed signs of degeneration and the host cell walls became disintegrated (Bar = 2 μm). (10) Hyphae (F) extending inter- and intracellularly in cortical tissue of the rachis, 5 dai. The host cells were degenerated (Bar = 2 μm). (11) Hyphae (F) spreading in the xylem vessels (XV) and paratracheal parenchyma cells, 6 dai. There was a dense coating on the secondary thickenings (ST) of the vessel tissue (Bar = 2 μm).

increased in number in the cytoplasm. Electron dense material was detected in the vacuoles and chloroplasts became swollen. The pathogen spread extensively 3–4 dai in the tissue of the glume, lemma and palea. Hyphae reached the bases of these tissues by inter- and intracellular growth. At this time, typical symptoms of brown, water-soaked spots appeared on the glume or lemma. Pronounced alterations in the host cells occurred to different degrees, such as degeneration of host cytoplasm, disintegration of host organelles and collapse of parenchyma cells.

Pathogen penetration occurred predominantly on the upper part of the ovary. Fungal hyphae extending on the upper surface of the ovary developed infection hyphae about 36 hai. After penetrating the cuticular layer, the fungus first became established by subcuticular hyphal growth or extended between the wall layers. The remainder of the host cell wall was penetrated 2 dai (Figure 8) and hyphae in the epidermis spread intracellularly to the upper pericarp cells. Thereafter, hyphae extended very rapidly by inter- and intracellular growth from the upper part of the pericarp to the base of the ovary (Figure 9). The pericarp cells invaded by the hyphae or in proximity to the intercellular hyphae were degenerated and host cell walls became disintegrated.

The pathogen reached the rachis from the infected glume, lemma, palea and ovary 5–6 dai. In the rachis, hyphae extended upwards and downwards by inter- and intracellular growth in cortical tissue and vascular bundles (Figures 10 and 11). In the infected vascular bundles, hyphae were detected in the xylem vessels, phloem sieves and paratracheal parenchyma cells (Figure 11). A thin layer of electron-dense coating material was often detected on the secondary thickenings of the xylem vessels.

Localization of cellulose, xylan and pectin in healthy and infected wheat spikes

After incubation of ultra-thin sections of the different tissues from the uninoculated wheat spikes with the cellulase-gold probe, the cell walls of the host tissues showed intense and regular labelling, while other structures such as organelles, cytoplasm and plasma membrane were free of significant labelling (Figure 12). Incubation of the ultra thin sections of the infected host tissues with the cellulase-gold probe resulted in depositions of gold particles over the host cell walls, but not over the hyphal walls (Figure 13). However, the labelling density over the host cell walls was markedly reduced and the distribution of the gold particles over the walls was uneven in comparison with corresponding host cell walls in the healthy tissues (Table 1). Host cell walls in contact with or close to the hyphae usually showed less labelling than host cell walls that were distant from the fungal hyphae.

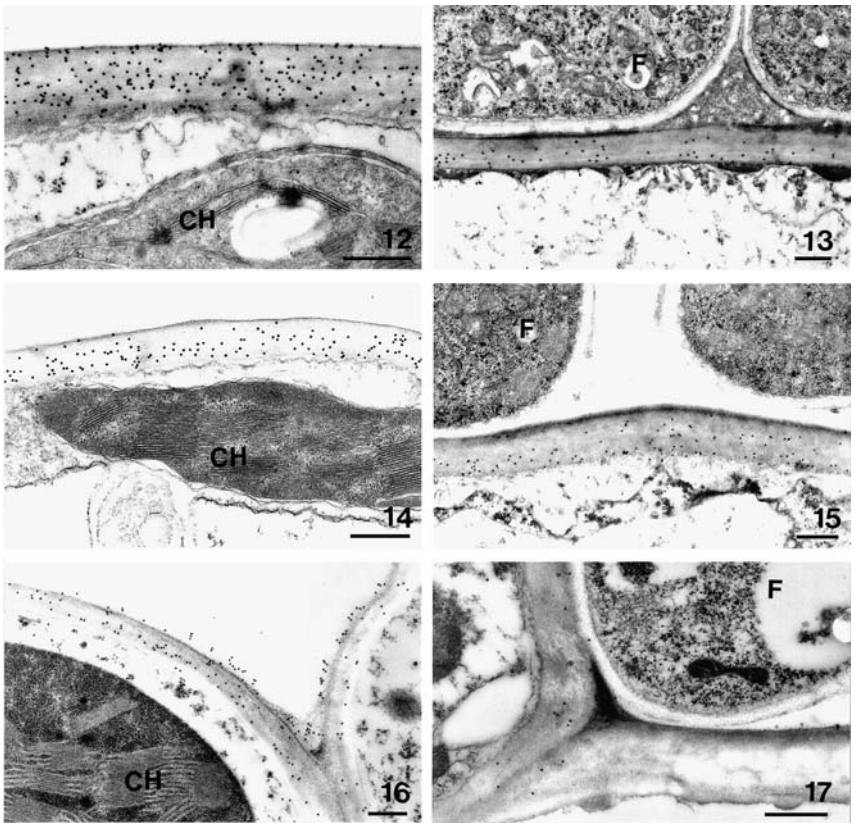
Following incubation of ultra-thin sections of different tissues from the uninoculated wheat spikes with the xylanase-gold probe, intense and regular labelling occurred exclusively over the cell walls of host tissues (Figure 14). Incubation of infected host tissues with the xylanase-gold probe resulted in alterations of density and pattern of gold particles in the host cell walls (Figure 15). Host cell walls, when attached to or close to the hyphae, usually showed less gold particles than host cell walls at a distance from the hyphae. Quantification of gold particles revealed a significantly decreased labelling density over the cell walls of the infected host tissues in comparison with that of the healthy tissue (Table 1).

When uninoculated healthy host tissues were incubated with the JIM7 monoclonal antibody, against methyl-esterified pectin and goat anti-rat gold solution, specific gold labelling occurred over pectin layers of host cell walls and also over the outermost wall layer (Figure 16). Incubation of ultra-thin sections of infected host tissues with monoclonal antibody JIM7 resulted in a reduced and irregular labelling over the cell walls as compared to the results of the uninoculated healthy host tissues (Figure 17, Table 1).

The section of host tissues showed little or no labelling for the controls, either with uncoated colloidal gold solution or goat anti-rat immunoglobulin solution alone, or after incubation with cellulase-gold, xylanase-gold or the monoclonal antibody JIM7, respectively, to which carboxymethylcellulose, xylan or pectin from citrus was previously added.

Discussion

The infection process and colonization pathway of *F. avenaceum* in wheat spikes following spray



Figures 12–17. Cytochemical (Figures 12–15) and immuno-gold (Figures 16–17) labelling for cellulose, xylan and pectin in the parenchyma cell walls in the lemma of uninoculated healthy and *Fusarium avenaceum*-infected wheat spikes. (12) Parenchyma cells of the lemma from healthy wheat spike, incubated with cellulase-gold probe. Cell wall was labelled evenly and intensely with gold particles, but the other host cell constituents (CH = chloroplast) were free of labelling (Bar = 0.5 μm). (13) Parenchyma cells in the infected lemma, 4 days after inoculation (dai), incubated with cellulase-gold probe. The density of gold particles over the host cell wall was markedly reduced and the fungal cell (F) wall was free of gold particles (Bar = 0.5 μm). (14) Parenchyma cells in the lemma of the healthy wheat spike, incubated with xylanase-gold probe. Cell wall showed uniform labelling with dense gold particles (Bar = 0.5 μm). (15) Parenchyma cells of the infected lemma, 4 dai, incubated with xylanase-gold probe. Labelling over the host cell wall was obviously decreased (Bar = 0.5 μm). (16) Parenchyma cells in lemma from healthy wheat spike, incubated with monoclonal antibody JIM7, which labels pectin. Labelling occurred over host cell walls and the outermost wall layer, while host cytoplasm and chloroplast (CH) showed no labelling (Bar = 0.5 μm). (17) Parenchyma cells of the infected lemma, 4 dai, incubated with monoclonal antibody JIM7 labelling pectin. The labelling density over the host cell wall was markedly reduced and the fungal cell (F) wall was free of gold particles (Bar = 0.5 μm).

inoculation at GS 65 (mid-flowering) was studied by SEM and TEM. SEM studies revealed striking differences regarding hyphal growth of *F. avenaceum* on the abaxial (external) surfaces of the glume, lemma and palea and on the adaxial surfaces of lemma and palea. The macroconidia of *F. avenaceum* germinated with one or several germtubes within 6–12 h after inoculation on the abaxial surfaces; mycelium development was scanty and no direct penetration was detected 3–4 dai. The abaxial epidermal cells of the glume, lemma and palea are characterized by highly thickened lignified walls (Pugh et al., 1933) and the underlying sclerenchyma-like hypodermal cells also have thickened lignified cell walls. In addition, silicon is accumulated in the abaxial epidermal cell walls of the glume, lemma and palea of wheat spikes (Hodson and Sangster, 1989). The epidermal cell walls of the rachis of wheat spikes are thickened similar to those of the glume, lemma and palea. These structural components of epidermal cell walls seem to prevent penetration by *F. avenaceum*. SEM studies with *F. culmorum* and *F. graminearum* revealed that both species also

Table 1. Labelling density of cellulose, xylan and pectin with cellulase-gold probe, xylanase-gold probe and monoclonal antibody, JIM7 (which labels pectin), over parenchyma cell walls in the lemma of uninoculated and *Fusarium avenaceum*-infected wheat spikes 4 days after inoculation[a]

	Healthy lemma	Infected lemma	Difference
Cellulose	86.64 ± 4.82[b]	40.42 ± 5.36	s[c]
Xylan	63.74 ± 4.26	44.68 ± 4.12	s
Pectin	38.28 ± 2.48	16.57 ± 2.84	s

[a] Density of labelling was expressed by the number of gold particles per μm^2.
[b] Mean of values (numbers) and ± standard deviation of the gold particles determined per μm^2. The mean densities for uninoculated healthy and infected wheat tissues were compared by the *t*-test, $P = 0.05$.
[c] s = significant.

developed a scanty mycelium on the abaxial surfaces of wheat spikes and these fungal species could not directly penetrate the thick-walled epidermal cells of exterior surfaces of the glume, lemma and palea (Kang and Buchenauer, 2000a; Wanyoike et al., 2002). These observations are in agreement with earlier studies of Strange and Smith (1971) who demonstrated that no infection occurred after placing droplets of spore suspension of *F. graminearum* on the outer surface of the glume.

While the adaxial surface of wheat glumes also has thick-walled epidermal and hypodermal cells, the adaxial epidermal cell walls of the lemma and palea are thin-walled. The inside of the floret cavities greatly facilitates hyphal growth and penetration of the adaxial surfaces of lemma and palea by Fusarium head blight fungi. SEM studies showed that hyphae of *F. avenaceum* grew over the abaxial surface of the glumes to reach the more susceptible sites of the interior of florets. Similar results have been obtained with *F. culmorum* (Kang and Buchenauer, 2000a, c) and *F. graminearum* (Wanyoike et al., 2002). The open floret at flowering stage also presents an avenue for Fusarium head blight spores to reach the interior of spikelets following spray inoculation at flowering stage or natural inoculation conditions. The partially extruded anthers and the crevices between palea and lemma may be further avenues for Fusarium head blight fungi to enter the florets (Strange and Smith, 1971; Lewandowski and Bushnell, 2001).

When spores or hyphae of *F. avenaceum* had entered the inside of florets, the fungus developed an intensive network of mycelium on the adaxial surfaces of the lemma and palea. Pollen may also support hyphal growth of *F. avenaceum* as has been demonstrated for *F. culmorum* and *F. graminearum* (Kang and Buchenauer, 2000a; Ribichich et al., 2000). Hyphae growing on the adaxial surfaces of lemma and palea developed short branches 36 hai, the infection hyphae, which formed penetration pegs. The fungus often penetrated epidermal cell walls partly and hyphae extended subcuticularly or between cell wall layers. During this initial phase of subcuticular growth of *F. avenaceum* (36–72 hai) host cells were not killed and this stage of pathogenesis may be regarded as a biotrophic relationship, which preceded the following necrotrophic phase. The subcuticular hyphae formed penetration pegs to penetrate the remaining cell walls and 2 dai hyphae were detected in the intercellular space between parenchyma cells in the host tissues. Hyphae in contact with host cells induced alterations in the host cells, such as small appositions between cell walls and the plasmalemma. The number of vacuoles increased, electron dense material was detected in vacuoles and during the rapid inter- and intracellular colonization host cytoplasm degenerated, host organelles disintegrated and parenchyma cells collapsed. Electronmicroscopical studies revealed a similar growth habit of *F. avenaceum* in the ovary as observed in the lemma and palea tissues. After penetration (36 hai) a pronounced subcuticular growth was established. Two dai, when hyphae started to spread intercellularly, the necrotic phase was initiated. The subcuticular growth phase of *F. avenaceum* in the ovary seemed to be shorter than in the glume tissues. Subcuticular growth patterns have also been reported for other pathogens of cereals, such as *Rhynchosporium secalis* on barley (Ayres and Owen, 1971), *Pyricularia oryzae* on rice (Koga, 1995) and *Cochliobolus heterostrophus* on maize (Wheeler, 1977). SEM and TEM studies with *F. culmorum* and *F. graminearum* revealed similar modes of penetration and colonization of floret tissues and similar biotrophic and necrotrophic host-pathogen relationships (Kang and Buchenauer, 2000a, c; Wanyoike et al., 2002) to those shown for *F. avenaceum*.

Immunogold labelling techniques showed that cell walls of lemma and palea contained reduced amounts of cellulose, xylan and pectin near intercellular hyphae or infection pegs of *F. avenaceum*. The studies confirmed that corresponding cell wall

degrading enzymes produced by the pathogen supported rapid colonization of wheat spike tissues. These results on activity of cell wall degrading enzymes regarding penetration and spreading resembled those findings obtained in earlier studies on degradation of cellulose, xylan and pectin in wheat spikes infected by *F. culmorum* and *F. graminearum* (Kang and Buchenauer, 2000b; Wanyoike et al., 2002). In several reports production of cuticle and cell wall degrading enzymes *in vitro* by fungal species that cause Fusarium head blight has been described. These enzymes include cutinases (Soliday and Kolattukudy, 1976), cellulases (Matsumoto et al., 1974; Zalewska-Sobczak and Urbanek, 1981; Manka, 1988), pectinases (Mullen and Bateman, 1971; Zalewska-Sobczak, 1985; Urbanek, 1989) and xylanase (Urbanek, 1989; Klechkovskaya et al., 1997).

Fusarium head blight fungi like *F. culmorum* and *F. graminearum* produce deoxynivalenol (DON) and related trichothecene mycotoxins; these toxins may be involved in the transition from the biotrophic to the necrotrophic stage, and these compounds may be regarded as virulence factors during pathogenesis. On the other hand, *F. avenaceum* does not produce trichothecene mycotoxins; the predominant mycotoxins produced by this Fusarium species are moniliformin, cyclic peptides (for instance ennatins) and fusarin (Desjardins and Proctor, 2001). The role of mycotoxins produced by *F. avenaceum* as possible factors of virulence during infection and colonization by the fungus in wheat spikes has still to be determined.

Acknowledgements

The authors wish to thank Dr K. Robert (John Innes Centre, UK) for kindly supplying the monoclonal antibody JIM7, and Mrs H. Brandl for excellent technical assistance. Mrs G. Moll is gratefully acknowledged for preparing the photographs. This study has been financed by the Institute of Phytomedicine, University of Hohenheim and partly supported by a Nature Science Foundation of China grant (No. 30125031) to Z. Kang.

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