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Stem-base disease and fungal colonisation of winter wheat grown in compost inoculated with *Fusarium culmorum, F. graminearum* and *Microdochium nivale*

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Abstract

Fungal colonisation of winter wheat cv. Cadenza by *Fusarium culmorum, F. graminearum* and *Microdochium nivale* was studied under conditions designed to avoid the splash dispersal of conidia from infested compost, to evaluate the possibility that systemic growth may transfer infection from the stem-base to the head. At decimal growth stages 33, 59, 77–87 and 95 the extent of fungal growth was assessed using a sample of 72 plants, by the recovery of fungal species from the stem-base, from each node and from the ear. Each of the fungi was recovered from stem tissues above soil level in some, apparently symptomless, plants. Symptoms of *Fusarium* foot rot were seen in an increasing proportion of plants during grain-fill and desiccation. There was an inverse relationship between recovery and the height above stem-base from which the stem tissue was excised. *F. culmorum* was the most frequently isolated fungus and it was also recovered from the highest position in plants. Only 3% of plants were colonised above the second node and none of the fungal species were recovered from either the fifth node or the ear. This suggests that colonisation and systemic growth from *Fusarium* infested compost is unlikely to contribute to the development of ear blight symptoms in winter wheat.

Introduction

There are five main species of Fusarium pathogenic to temperate cereal crops: *Fusarium avenaceum* [*Gibberella avenacea*], *F. culmorum, F. graminearum* [*G. zeae*], *F. poae* and *Microdochium nivale* [*Monographella nivalis*] (Parry et al., 1994). These species commonly cause a succession of diseases in wheat and barley: seedling blight, Fusarium foot rot and Fusarium ear blight. For each disease, symptoms may result from infection by either a single species or a disease complex in which two or more species are involved. Environmental factors, particularly temperature and rainfall, influence the distribution of each species with, for example, *F. graminearum* being more commonly associated with warmer, drier conditions while *F. culmorum* and *M. nivale* often occur in cooler, wetter locations. All three pathogens have been implicated in Fusarium ear blight of winter wheat (Jenkins et al., 1988). The mode of transmission of the infection from soil level to the ear has not yet been conclusively demonstrated (Parry et al., 1995). Early work (Bennett, 1928) indicated that diseased ears and grain were due solely to conidia distributed from external sources and a mechanism involving splash-dispersed inocula of *F. culmorum* and *F. avenaceum* has been demonstrated *in vitro* (Jenkinson and Parry, 1994). The gradual upward movement of the pathogen has also been linked to a sequence of symptomless infections which develop following rain splash of conidia from lower to higher leaves (Zinkernagel et al., 1997). Systemic growth of *F. culmorum* has been suggested as a method by which sporodochia may be formed some distance above the stem-base but no evidence was found of systemic growth leading to infected ears (Snijders, 1990). In contrast, Hutcheon and Jordan (1992)

Table 1. Fungal isolates: host, date collected and geographic origin

Fungal species	Code	Host	Date	Location
Fusarium culmorum	Fu5	Wheat, spikelet	1991	Norfolk, UK
Fusarium graminearum	405/1	Wheat	1993	Wankum, Germany
Microdochium nivale	− 356/14b	Wheat, stem-base	1992	Hampshire, UK

claim that ears may become infected through internal systemic colonisation by any one of three *Fusarium* species and *M. nivale*. Systemic infection of winter wheat by *Fusarium* was also reported by Polley and Turner (1995) who isolated *Fusarium* species from the top internode of some stems sampled at GS 73-75. If the disease is predominantly non-systemic, then improved chemical control may be achieved by better disease forecasting and accurate timing of late-season ear sprays (Hutcheon and Jordan, 1992; Parry et al., 1995; Moschini and Fortugno, 1996). However, if systemic growth provides a significant alternative route for ear colonisation then effective seed treatment together with sprays directed at any inoculum produced on stems may be expected to minimise yield losses. Consequently, when devising chemical control strategies for Fusarium ear blight, it is important to determine the degree to which systemic fungal colonisation may contribute to the disease in winter wheat. This study was undertaken to investigate the extent of *Fusarium* colonisation which might occur when wheat plants were grown under conditions designed to avoid the splash dispersal of conidia from infested compost. In addition the effect on colonisation of an application of the fungicide prochloraz was also assessed.

Materials and methods

Fungal cultures and preparation of inocula. Spore suspensions of *F. culmorum*, *F. graminearum* and *M. nivale* were prepared from cultures grown on potato dextrose agar. Cultures were grown from single-spore lines isolated from naturally infected wheat (Table 1).

Cultivation of winter wheat. Untreated seed of winter wheat cv. Cadenza was surface sterilised in 4% sodium hypochlorite (0.5% available chlorine) for 3 min, rinsed in three changes of sterile distilled water, and dried at ambient temperature on sterile filter paper. Seed was sown in trays of autoclaved John Innes No. 2 compost. The compost had previously been autoclaved in small volumes (approx. 12.5 l) for 3 × 55 min at 126 °C and the trays had been sterilised by immersion in 4% sodium hypochlorite for 30 min. Planted trays were incubated in plant growth cabinets (Fitotron or Conviron) operating at 70% r.h. with 16 h day at 15 °C. The temperature was reduced to 10 °C during each dark period. These conditions were maintained throughout the experiment. Seedlings were transplanted at decimal growth stage (GS) 12 (Tottman, 1987) into 72 × 6″ pots containing autoclaved John Innes No. 2 compost, 5 plants per pot.

Inoculation of plants. The fungal spores were applied as a soil drench (2×10^4 spores g^{-1} compost) when the plants were at GS 13. One third of the pots (24) was inoculated with *F. culmorum*, a further third was treated with *F. graminearum* and the final third was exposed to *M. nivale*. From this stage each inoculated pot stood on an individual saucer and was watered only from the base. The treatments were incubated as three randomised blocks. At GS 33, half the pots in each treatment were sprayed with prochloraz (405 g ha^{-1} in 220 l water) using a track-mounted precision pot sprayer.

Sampling and isolation of fungi. At GS 33 (prior to fungicide treatment), GS 59, GS 77–87 and at harvest, one plant was uprooted from each pot and a 3 cm segment of tissue was removed from the stem-base, from each node and from the ear (post emergence). The height, relative to the stem-base, from which each tissue segment was removed, was measured. Segments were surface-sterilised (4% sodium hypochlorite for 3 min), rinsed in sterile distilled water and blotted on sterile filter paper. The segments were bisected longitudinally. One half was plated on potato dextrose agar amended with antibiotics (streptomycin sulphate 10 μg ml^{-1}, neomycin 5 μg ml^{-1} and chloramphenicol 5 μg ml^{-1}) and the other was prepared for scanning electron microscopy. The incubation temperature for the agar plates was varied to reflect the optimum for growth of the species which had been originally used to inoculate the compost (*F. culmorum*, 20 °C; *F. graminearum*, 25 °C; *M. nivale*, 15 °C). After 3–7 days incubation

recovery of each species was assessed by colony morphology, pigmentation and spore morphology.

Data on height within each plant from which fungi were recovered were studied by analysis of variance using Minitab (Minitab Inc., State College, PA, USA). When significant effects were observed, means were compared by the Least Significant Difference test, $P = 0.05$.

Scanning electron microscopy. Bisected stem segments were fixed in 3% glutaraldehyde (0.05 M phosphate buffer, pH 7), dehydrated through an ethanol series to dry acetone and then critical-point dried. Dried specimens were mounted, sputter coated with gold and examined in a Cambridge S200 Stereoscan electron microscope operating at 10 kV. At GS 95, to assess the relationship between symptoms visible on the outside of the culm and internal fungal colonisation, seventy-eight culms were bisected longitudinally and examined under epi-illumination using a Zeiss Jenalab light microscope.

Results

Fungal colonisation of stem tissues. Throughout the experiment most plants showed no disease symptoms. In every plant from which fungi were recovered above the stem-base, the pathogen was isolated from each successive stem segment up to the highest infected node. There were significant differences ($P = 0.001$) in the height of fungal colonisation dependent on either the fungal species infesting the compost or the GS of the wheat plants. Treatment with the fungicide prochloraz at GS 33 had no significant effect on the height of fungal colonisation.

The mean height to which stems were colonised was significantly higher ($P = 0.05$) for plants grown in compost infested with *F. culmorum* than it was for plants grown in compost infested with *M. nivale*. The mean height of stem colonisation by *F. graminearum* did not differ significantly from that of either *F. culmorum* or *M. nivale*. In stems sampled at GS 33 and GS 59, the mean height of fungal colonisation was similar, but after GS 59 it increased significantly ($P = 0.05$) at each successive sampling (GS 77–87 and GS 95).

At GS 33, depending on the species used for inoculation, recovery from stem-base segments varied from 88 to 100% (Figure 1). At this growth stage 14% of plants were colonised above the stem-base but isolation failed to detect infection above the second node.

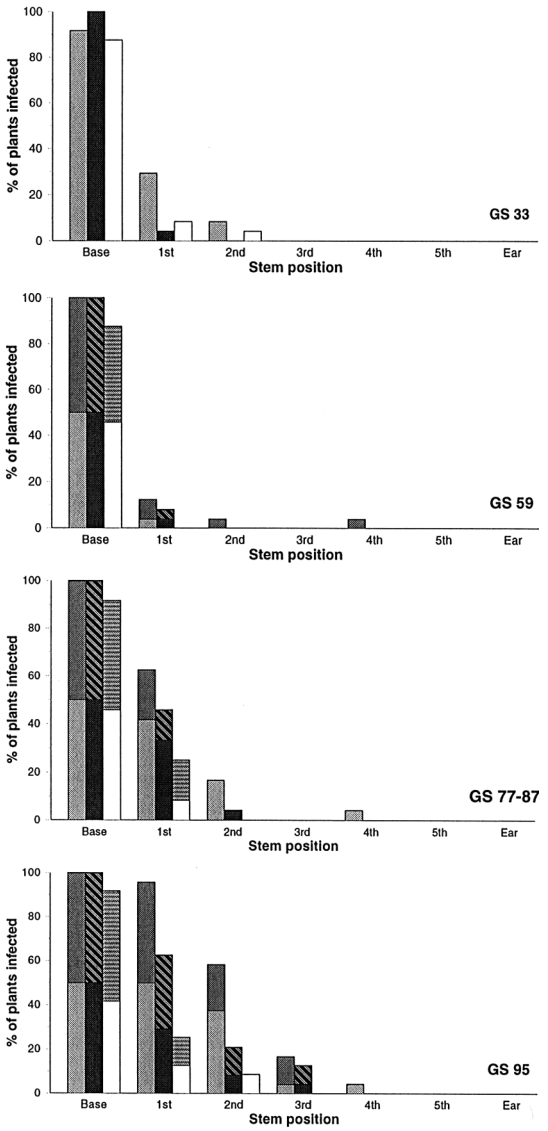


Figure 1. Isolation of *Fusarium culmorum*, *F. graminearum* and *Microdochium nivale* from wheat cv. Cadenza at various growth stages. At each growth stage 24 plants were sampled for each fungal species. *F. culmorum*, ▒; *F. culmorum* fungicide treated, ▓; *F. graminearum*, ■; *F. graminearum* fungicide treated, ▨; *M. nivale*, □; *M. nivale* fungicide treated, ▤.

With all three fungal species there was an inverse relationship between degree of recovery and the height above stem-base from which the tissue was excised. *F. culmorum* was the most frequently isolated fungus (Figure 1) and it was also recovered from the highest position in plants (Figure 2).

In plants sampled at GS 59, stem-base recovery of isolates was almost ubiquitous but less than 6%

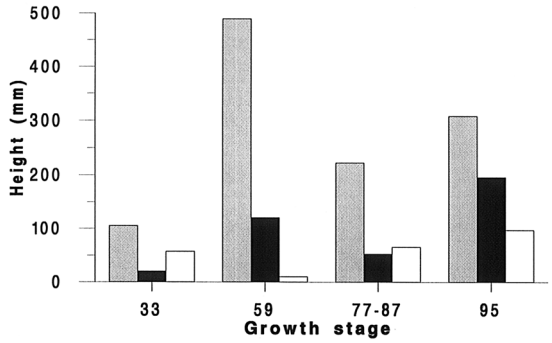


Figure 2. Maximum heights at which *Fusarium culmorum, F. graminearum* and *Microdochium nivale* were isolated from culms of wheat cv. Cadenza at various growth stages. *F. culmorum,* ▩; *F. graminearum,* ■ ; *M. nivale,* □ .

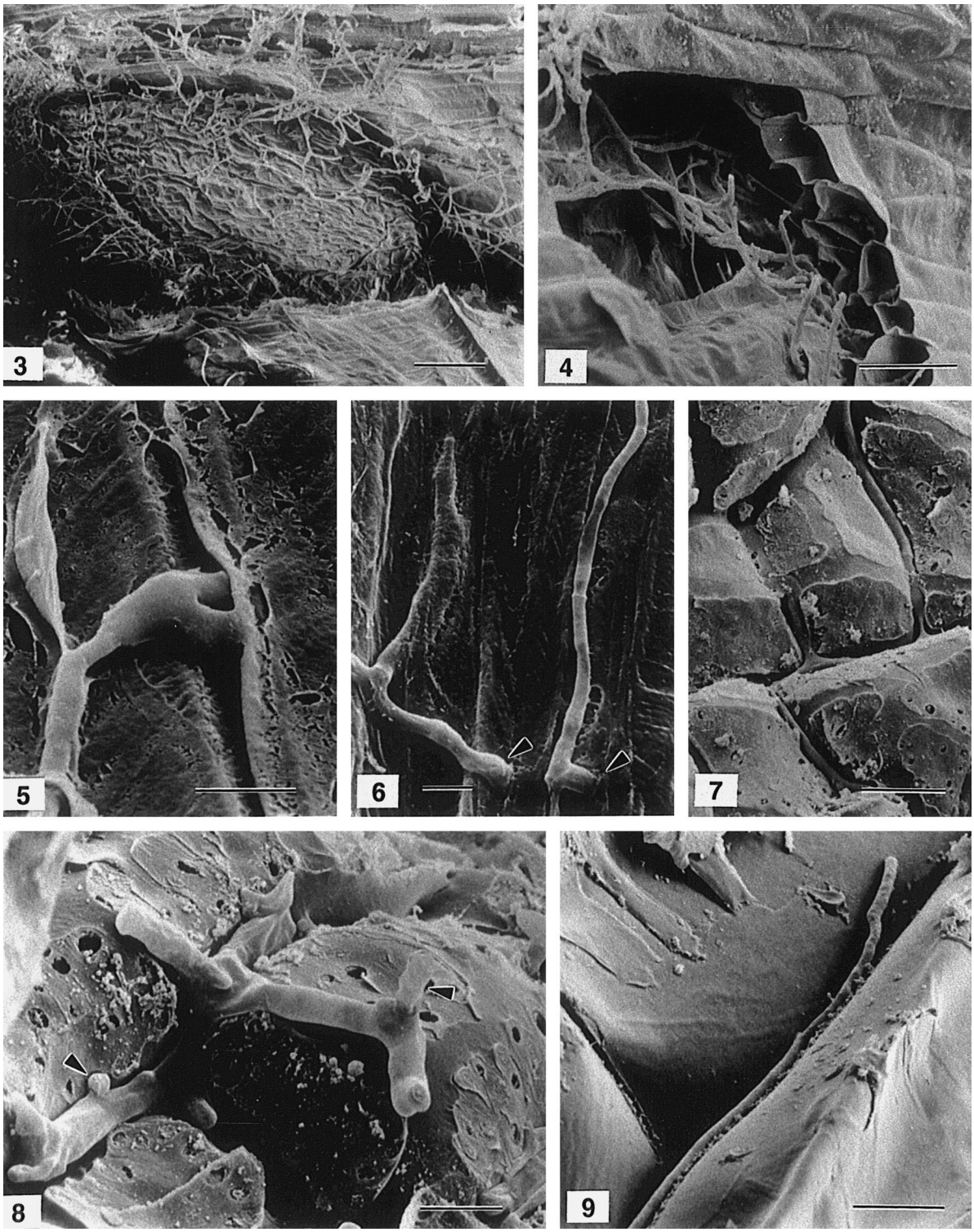
showed colonisation above this height (Figure 1). In this latter group 75% of the plants had been treated with fungicide at GS 33. Of those plants in which there was no vertical colonisation above the stem-base, 46% had been treated with fungicide. *F. culmorum* was again recovered most frequently and in one plant it was isolated from the tissue segment which contained the fourth node (Figures 1 and 2). No species were recovered from tissue segments taken from either the highest node on each plant or the ear.

Stem-base infection remained high at GS 77–87 (Figure 1). The three species were recovered above the stem-base in 44% of plants and, of these, 39% had previously been treated with fungicide. Where no fungal colonisation occurred above the stem-base, 66% of the plants had received a fungicide spray. More than 60% of plants from pots inoculated with *F. culmorum* were infected at the first node and this species was again isolated from one plant at the fourth node (Figures 1 and 2). Again there was no recovery from the highest stem segment sampled or from the ear.

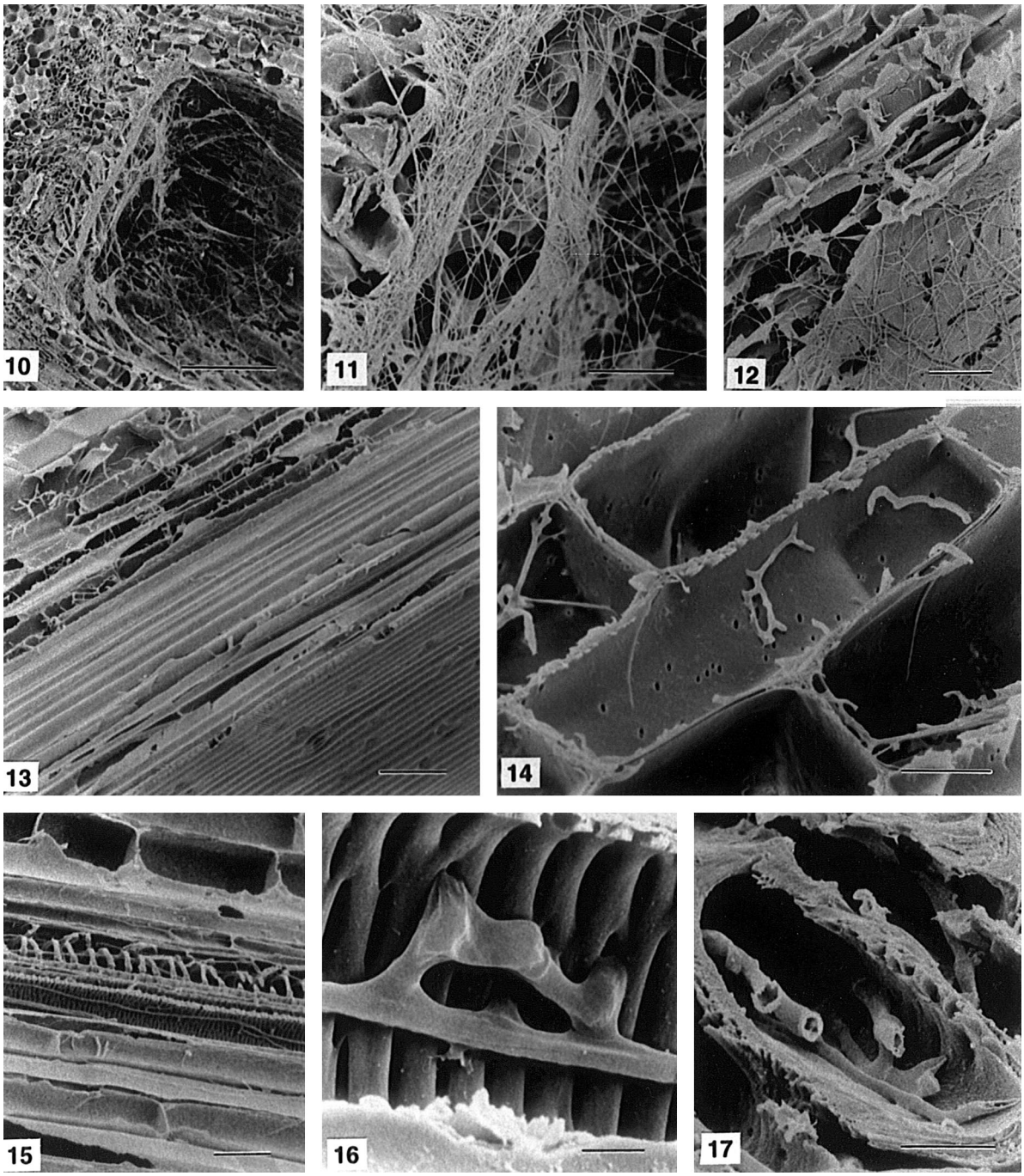
In the final sampling at harvest maturity (GS 95), 53% of plants showed clear symptoms of Fusarium foot rot and 97% of stem-bases were infected (Figure 1). In the group showing clear symptoms, 47% had received fungicide treatment. The frequency of symptom development depended on fungal species with clear foot rot symptoms in 85%, 44% and 18% of plants, infected, respectively, with *F. culmorum, F. graminearum* and *M. nivale*. Vertical colonisation above the stem-base occurred in 61% of plants. Of the remainder, in which there was no recovery of fungi above the stem-base, 44% had been sprayed with fungicide. Seventeen percent and 13% of plants, respectively, from pots inoculated with *F. culmorum* or *F. graminearum*, were infected up to the third node and *F. culmorum* was recovered from one plant at the fourth node (Figures 1 and 2). Out of a total of 286 plants examined, only nine were infected above the second node. Six of these had received fungicide treatment.

Microscopy of fungal colonisation. In stem-base samples (Figures 3–6) taken from plants at GS 33 and 59, all three fungal species colonised the spaces between successive leaf sheaths (Figures 3 and 4). Infection was typically greatest between the outer sheaths at approximately soil level and decreased both above this region and inwards towards the culm. Growth within the tissues occurred from the inner epidermal surface of the outer leaf sheath (Figures 5 and 6). Here hyphae often penetrated and/or emerged along the line of the anticlinal walls between epidermal cells (Figure 5) or entered directly through the epidermal cell wall (Figure 6). Above the stem-base mycelial development between leaf sheaths was sparse with hyphae often closely adpressed in the anticlinal groove between epidermal cells. In one sample at GS 33, in which *F. culmorum* was recovered at the second node, intercellular hyphae were observed both within the tissues of the first and second nodes (Figures 7 and 8) and within the pith parenchyma of the second internode (Figure 9).

At the later growth stages (GS 77–87 and GS 95), each of the fungal species colonised the pith cavity below the first node. There were no obvious differences between species in the morphological features of this colonisation. Typically, hyphae were present on the walls of parenchyma cells lining the cavity (Figures 10–12), and from this mycelium aerial hyphae grew into the cavity space. Often a denser mycelium formed below each node against the lower face of the diaphragm (Figures 10 and 11). There were also intercellular and intracellular hyphae growing within the parenchyma tissue of both the culm and the nodes (Figures 12–14). At GS 95, intercellular hyphae were sometimes observed growing within vascular bundles of the culm, typically associated with xylem vessels (Figures 15–17). At these later growth stages an increasing proportion of plants showed discolouration towards the base of the culm. Colonisation of the pith cavity appeared to be associated with plants showing these macroscopic symptoms. At GS 95, the mean distance to which mycelium within the culm or pith cavity extended above discolouration was similar for each fungal species (Table 2).



Figures 3–9. Colonisation of tissues of wheat culm by *Fusarium culmorum*, GS 33. (3) Stem base with outer leaf sheath removed showing a tiller bud surrounded with mycelium. The left hand margin of the picture is approximately at soil level. Scale bar = 100 μm. (4) Hyphae between consecutive leaf sheaths at stem base. Scale bar = 50 μm. (5–6) Inner epidermis of outermost leaf sheath in a region between stem base and first node. (5) Hypha penetrating/emerging along the line of the anticlinal walls between adjacent epidermal cells. Scale bar = 5 μm. (6) Hyphae (arrows) penetrating/emerging directly through epidermal cell wall. Note hyphal tip on left appears to be below the cuticle. Scale bar = 5 μm. (7) Intercellular hyphae within second node. Scale bar = 20 μm. (8) Intercellular hyphae within first node. Note short side branches on hyphae (arrows). Scale bar = 10 μm. (9) Intercellular hyphae growing between cells of pith parenchyma just below second node. Scale bar = 10 μm.



Figures 10–17. Colonisation of tissues of wheat culm by *Fusarium culmorum*, GS 95. (10–11) Portion of bisected culm at base of second node. (10) Hyphae within the pith cavity. Scale bar = 500 μm. (11) Dense mycelium formed against the base of the second node. Scale bar = 100 μm. (12–13) Area of bisected culm between first and second node. Scale bars = 100 μm. (12) Hyphae growing on the wall of pith cavity and within cells of the cortex. (13) Hyphae within the cells of inner cortex but no colonisation of the outer cortex or epidermis. Note stomata in epidermis of culm. (14) Intracellular hyphae in cortex parenchyma. Between first and second node. Scale bar = 25 μm. (15–17) Systemic colonisation of senescent vascular tissue. Hyphae in xylem vessels. (15) Longitudinally bisected vessel in area between first and second node. Scale bar = 50 μm. (16) Hypha in xylem. Scale bar = 5 μm. (17) Transverse section xylem vessel containing hyphae. Scale bar = 10 μm.

Table 2. Extent of fungal growth above discolouration of the culm

Fungal species	Mean distance (mm)	Standard error	Number of culms
Fusarium culmorum	33.7	5.8	28
Fusarium graminearum	25.2	5.1	31
Microdochium nivale	27.9	5.5	19

Discussion

Results from the present study suggest that each of the species are capable of upward growth from the stem-base of some, apparently symptomless, plants. At each growth stage, *F. culmorum* was the most aggressive pathogen while colonisation by *M. nivale* was typically least extensive. In most cases, during the vegetative phase of plant development, this fungal growth did not appear to be systemic but was rather a colonisation of protected exterior plant surfaces. In young, fast growing healthy plants, these areas may present a niche in which sufficient water and other compounds are available to support a restricted latent endophytic infestation. These findings are in marked contrast to those reported by Hutcheon and Jordan (1992), who at GS 37 recovered the three species used in this trial and *F. avenaceum* from each internode and from the developing ear of plants previously inoculated with mycelial plugs at GS 21. Further, they found that the incidence of ear infection was highest in plants inoculated with *M. nivale* and least in those inoculated with *F. culmorum*. Under our conditions, the success with which each of these species colonised the host was reversed. To demonstrate that ears may become infected through internal systemic colonisation, Hutcheon and Jordan (1992) enclosed each shoot apex in a polyethylene sleeve from GS 37. Whilst such a procedure may have protected ears from inoculation by externally transmitted spores or mycelial fragments, it seems likely that it would also modify both transpiration and photosynthesis of the protected spike. Such modifications could induce physiological stress in the host which might favour the pathogen. This could in part explain some of the contradictions between our results and those reported by these workers.

In the present study, the lack of symptoms during vegetative growth of the host suggests that the limited fungal development may not have been strictly pathogenic. In an experiment using wheat seed artificially inoculated with *F. culmorum* conidia, more seedlings emerged, and a greater proportion of these had symptomless infections, when seed was grown in 'cool wet', rather than 'warm dry', soil conditions (Parry et al., 1994). It seems probable that symptomless infections may develop more rapidly or become pathogenic in those plants which are subject to environmental stress. Drought stress in particular has been shown to promote development of *Fusarium* foot rot (Papendick and Cook, 1974; Cook, 1980). In addition, Cook and Papendick (1972) demonstrated that high nitrogen applications could, by producing low osmotic potentials *in planta*, trigger the sudden development of disease symptoms in wheat infected with *F. culmorum*, even when soil water was not limiting. This may suggest that colonisation of the pith cavity, which was rare in young plants in our experiments, might be more common among plants grown in sub-optimal field conditions. Snijders (1990) found that in both wounded and soil-inoculated wheat plants, colonisation by *F. culmorum* at the stem-base could lead to infection higher in the stem by GS 55–65. He reported development of stem-base infection in almost all plants and noted that fungal growth within the pith cavity did not appear to be restricted by the barrier of tissues at each node. The present results confirm these findings, although the development of appreciable mycelial pads against the lower surface of nodes suggests that fungal growth may be temporarily arrested by these barriers. As plants mature, especially post-anthesis, translocation of assimilates and increased physiological stress during grain development could favour the saprophytic colonisation of senescing tissues. The increasing proportion of plants colonised above stem-base or showing stem-base browning after GS 77-87 supports this view.

The lack of any fungicide control was unexpected since the activity of prochloraz has been demonstrated previously against all three fungi (Parry et al., 1994). Successful application does, however, rely on foliar wetting and run-off which transfers the active compound to the stem-base. Because plants received no foliar watering, it is probable that only a small proportion of the applied fungicide would have penetrated to the lower infected portion of the culm.

True systemic growth within the vascular tissues was only evident in senescent plants at harvest. In these plants, fungal growth within either the culm tissues or the pith cavity extended only a short distance above discolouration of the stem. This, together with the lack of any colonisation of the ear or upper node, strongly suggest that systemic growth is unlikely to contribute to development of Fusarium ear blight of winter wheat.

Acknowledgements

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