Eur J Plant Pathol (2010) 126:203–216
DOI 10.1007/s10658-009-9533-0

Evaluation of pathogenicity and aggressiveness of *F. langsethiae* on oat and wheat seedlings relative to known seedling blight pathogens

Samuel M. Imathiu · Martin C. Hare · Rumiana V. Ray · Matthew Back · Simon G. Edwards

Received: 7 February 2009 / Accepted: 14 September 2009 / Published online: 1 October 2009
© KNPV 2009

Abstract *Fusarium* and *Microdochium* species are causal agents of seedling blight of small-grain cereal crops where they may contribute to a significant reduction in crop establishment and final yield. Two experiments were carried out to investigate the potential pathogenicity and aggressiveness of *F. langsethiae*, a recently identified fungus linked with the contamination of cereals with high levels of the trichothecene mycotoxins, HT-2 and T-2. An artificial seed inoculation method involving conidial suspensions was used and the experiments conducted in a growth cabinet set at either 5 or 15°C with a 12 h photoperiod. Known seedling blight pathogens of the genus *Fusarium* and *Microdochium* were used for comparison. At 15ºC, *F. culmorum*, *M. nivale* and *M. majus* caused seedling blight of oats and wheat with *F. culmorum*, on average being the most aggressive than the latter two. At 5ºC, only *F. culmorum* and *M. nivale* caused seedling blight of oats and wheat. Under the experimental conditions employed, *F. langsethiae* and *F. poae* failed to produce seedling blight disease indicating that these two species are not pathogenic to oat and wheat cultivars, Gerald and Claire respectively, at the seedling stage of development. They are therefore unlikely to affect crop establishment and other yield components such as tiller number, grain yield per head as well as grain weight if there is no subsequent foot-rot and/or head blight where infected seeds are sown.

Keywords Cereals · Fusarium seedling blight · *Fusarium* · *Microdochium*

S. M. Imathiu · M. C. Hare · R. V. Ray · M. Back · S. G. Edwards (✉)
Crop and Environment Research Centre,
Harper Adams University College,
Newport, Shropshire TF10 8NB, UK
e-mail: sedwards@harper-adams.ac.uk

Present Address:
R. V. Ray
School of Biosciences, University of Nottingham,
Sutton Bonington LE12 5RD, UK

Introduction

Fusarium seedling blight (FSB) is a cereal disease whose symptoms and severity vary depending on the causal pathogen, cereal cultivar and the prevailing environmental conditions (Parry et al. 1995; Doohan et al. 2003). Symptoms on cereals range from pre-emergence death, post-emergence death and lesions on stems and leaves (Millar and Colhoun 1969; Wisnieska and Busko 2005). Infected seedlings surviving the disease have been reported to lack vigour, tiller poorly and frequently send up a single stem on which a small head develops (Parry et al. 1995; Haigh et al. 2009).

A large number of fungi have been implicated in seedling blight of cereals worldwide (Grey and Mathre 1988), but the disease is predominantly

caused by species of the *Fusarium* and *Microdochium* genera. Species within these two genera reported to cause severe seedling blight are *F. culmorum, F. avenaceum, F. graminearum*, *M. nivale* and *M. majus* (previously *M. nivale* var. *nivale* and *M. nivale* var. *majus* (Glynn et al. 2005)) (Colhoun 1970; Parry et al. 1995). *Fusarium culmorum* has been reported to be most destructive to older seedlings while *F. avenaceum* and *F. graminearum* are more damaging to young seedlings (Wisnieska and Busko 2005). FSB can result in poor crop establishment as a result of thin stands resulting from pre- and post-emergence death which may significantly reduce crop yields (Jones 1999) as a result of reduced grain-bearing heads. In a survey conducted in Ireland (1991–1992), infection on winter wheat by *Microdochium* species resulted in 50% reduction in emergence (Humphreys et al. 1995). According to Johnson (1914), the greatest damage was reported for oats compared with wheat and barley; he reported that a 10 acre field of oats in Ohio, USA was destroyed by seedling blight and foot-rot. Simmonds (1928) reported that under greenhouse conditions, seedling blight of oats caused by *F. culmorum* may cause a loss of 75–100% in yield. The author, however, noted that such percentages seldom prevailed in the field although serious losses may occur. De Tempe (1968) reported that *F. graminearum* was an oat seedling pathogen which caused 31% seedling death, 18% severely diseased seedlings and 32% slightly diseased seedlings.

The primary source of inoculum for FSB may differ for different pathogens. The inoculum can originate from infected soil, crop debris or seed and there is conflicting reports as to which is most important. Pereyra et al. (2004) demonstrated that survival and inoculum production by *F. graminearum* was related to the rate of crop debris decomposition. They reported that the survival of *F. graminearum* was inversely related to the wheat debris decomposition rate and that it could be detected even after 2 years in buried residue. Other literature cites contaminated seed or soil or both as the main source of seedling blight inoculum. Jones (1999) reported infected seed as the most important source of seedling blight inoculum for *Fusarium* species causing the disease in wheat. This observation is supported by Hare and Parry (1996) and Hare et al. (1999) who highlighted the importance of infected seed as a source of inoculum for *F. culmorum* and *M. nivale* seedling blight in wheat. The authors reported that seeds infected by either *F. culmorum* or *M. nivale* resulted in reduced germination and emergence. Reports of both infected seed and infected soil acting as a source of inoculum for the disease include those of Doohan et al. (2003), Grey and Mathre (1988), Colhoun (1970), Fernandez and Chen (2005), and Wisnieska and Busko (2005).

Among the abiotic factors determining the incidence and severity of FSB in susceptible cereals, soil temperature and moisture have been reported as the most important (Colhoun et al. 1968; Colhoun 1970; Holmes and Channon 1975). *Microdochium* species are reported to be devastating seedling blight pathogens of cereals at low temperatures as they can cause severe damage to seedlings even in freezing conditions (Mahuku et al. 1998). Millar and Colhoun (1969) reported that *Microdochium* species seedling blight incidence was greatest in cold and dry soil conditions. These species caused greatest seedling blight at 6.1°C compared with 10.4, 13.4 and 16.4°C with the disease decreasing with the increase in temperature. Other studies supporting these findings include those of Colhoun and Park (1964), Colhoun et al. (1968) and Colhoun (1970). Seedling blight as a result of *Fusarium* species has been demonstrated to be severe at higher temperatures considered non-optimal for *Microdochium* species. Johnston and Greaney (1941) studied the influence of soil temperature on pathogenicity of several isolates of *F. culmorum*, *F. oxysporum*, *F. avenaceum* and *F. equiseti* on wheat seedlings and observed the rate of disease development increasing with the increase in soil temperature from 10 to 25ºC. Colhoun (1970) and Colhoun and Park (1964) have reported reduced ability of *F. culmorum* to produce severe seedling blight of wheat at temperatures <10°C and increased severity at higher temperature up to around 24°C. Chongo et al. (2001) reported the inability of *F. graminearum* to affect emergence and seedling infection of oats, barley, wheat and rye at 10°C. However, increasing temperature from 10 to 30°C dramatically reduced seedling emergence and increased seedling disease severity.

Fusarium langsethiae is a toxigenic species mainly found in oats, wheat and barley in Central and Northern European countries (Torp and Adler 2004;

Torp and Nirenberg 2004). It had been reported to be a potential producer of HT-2 and T-2 toxins in Norwegian cereals (Bottalico and Perrone 2002). More recently, it has been implicated in the production of high HT-2 and T-2 in cereal crops across Europe (Edwards et al. 2009). The known distribution of *F. langsethiae* may be limited due to the fact that it has only been recently identified. In previous field and glasshouse inoculated experiments, *F. langsethiae* failed to produce any disease symptoms on oats and wheat heads but contaminated the grain with HT-2 and T-2 (Imathiu 2008; Imathiu et al. 2009). Although pathologic lesions apparently caused by *F. langsethiae* have been reported in Austrian wheat (Torp and Adler 2004), it is not clear whether this was caused by *F. langsethiae* or other *Fusarium* species within the infected 'glume spots' reported. *Fusarium langsethiae* infection of durum wheat in Italy has recently been reported (Infantino et al. 2007) but the authors did not report whether the fungus caused any disease symptoms on the crop. In this regard, the epidemiology of *F. langsethiae* is not well understood.

Currently, there are very few specific reports of damage to the oat crop by seedling blight. In particular, there is no published work on seedling blight caused by *F. langsethiae* on any crop. This study aimed to investigate the potential pathogenicity and aggressiveness of *F. langsethiae* on oats and wheat at the seedling stage of development relative to known FSB pathogens of the genus *Fusarium* and *Microdochium*. The objectives of the study were: to investigate whether *F. langsethiae* was pathogenic to oats and wheat seedlings, to investigate whether isolates of *F. langsethiae* from oats and wheat differed in their pathogenicity towards wheat and oat seedlings, and to determine whether *F. langsethiae* pathogenicity in oat and wheat differed from that of other FSB pathogens.

Materials and methods

Two experiments were carried out. The first experiment was performed at 15ºC while the second experiment was carried out at 5ºC. Both experiments were carried out using winter oat cv. Gerald and winter wheat cv. Claire as they were the most popular cultivars in the UK at the time of the study.

Fungal species and conidial production

All fungal isolates used in this study are listed in Table 1. They were all isolated from either oat or wheat grain in the UK. Conidial suspensions were obtained by cultivating single-spore isolates on potato dextrose agar (PDA, Merck, Germany). The cultures were incubated at room temperature (*ca.* 22ºC) under natural light for 10 days. Conidia were dislodged and harvested by flooding the individual cultures with 5 ml of sterile distilled water (SDW) and colony surfaces gently agitated with a sterilised L-shaped glass rod. The conidia obtained were filtered through two layers of sterile muslin cloth to remove mycelia. The concentration of conidia was determined using a haemocytometer (Weber Scientific International, UK) and adjusted to a concentration of 5×10^5 conidia ml^{-1} before combining to form a composite inoculum of three isolates (except for *M. nivale* where one isolate failed to sporulate) of the same concentration for each species (Table 1). *Fusarium langsethiae* isolates were divided into two populations based on the host from which they were isolated (wheat or oats).

Table 1 Fungal isolates used in the study of oat and wheat seedling blight caused by *Fusarium* and *Microdochium* spp

Isolate	Species	Host
FC/X/W/006	*F. culmorum*	Wheat
FC/X/W/002	*F. culmorum*	Wheat
FC/95/W/005	*F. culmorum*	Wheat
Fp/01/W/006	*F. poae*	Wheat
Fp/01/W/004	*F. poae*	Wheat
Fp/W/005	*F. poae*	Wheat
MM/X/XW/010	*M. majus*	Wheat
MM/X/W/006	*M. majus*	Wheat
MM/X/XW/011	*M. majus*	Wheat
MN/X/XW/007	*M. nivale*	Wheat
MN/X/W/001	*M. nivale*	Wheat
Fl 041/11	*F. langsethiae*	Oat
Fl 2004/59	*F. langsethiae*	Oat
Fl 077/3	*F. langsethiae*	Oat
FlW 2004/171(a)	*F. langsethiae*	Wheat
FlW 2001/69(a)	*F. langsethiae*	Wheat
FlW 2004/170	*F. langsethiae*	Wheat

Seed material

Oat cv. Gerald and wheat cv. Claire seed samples were evaluated for natural contamination by FSB pathogens according to the following plating technique. Grain samples were thoroughly mixed before representative sub-samples were taken. Twenty gram samples were scooped into 50 ml sterile universal tubes and soaked in sodium hypochlorite (1.2% available chlorine) with added Tween 20 (0.05%) for 3 min to surface-sterilise. They were then rinsed three times with SDW before drying in Petri dishes in a laminar air flow cabinet. Grains were plated (5 grains per plate) on PDA amended with streptomycin sulphate (130 µg ml^{-1}) to inhibit bacterial growth and incubated at room temperature (*ca.* 22ºC) under natural light. After 7 days of incubation, fungal colonies present were identified from their colony and conidial morphology (Summerell et al. 2003; Leslie and Summerell 2006).

Seed inoculation

Twenty-five grams of surface-sterilised dry seeds prepared as detailed above were inoculated with 1 ml of 5×10^5 conidia ml^{-1} suspension of the composite inoculum (Table 1). The controls were treated with an equal volume of SDW. Inoculated seeds and non-inoculated controls were shaken by hand in sterile conical flasks for 2 min and transferred into sterile Petri dishes before sowing.

Seeding medium and sowing

John Innes Seed and Cutting Compost (Gem Gardening, UK) was passed through an 8 mm sieve to remove stones and other debris. The compost was then autoclaved for 1 h at 121ºC and allowed to cool to room temperature. Inoculated seeds were sown in sterile 300 ml glass jars (10 seeds per jar which constituted an experimental unit) after adding 40 g compost (32% moisture content). Seeds were placed on the surface of the compost and then covered by more compost to a depth of approximately 2 cm. Screw tops were placed on the jars which were then arranged in an incubator (Sanyo Versatile Environmental Test Chamber, Japan) according to a randomised block design with each treatment replicated eight times. The incubator was set at either 5 or 15ºC with an alternating 12/12 h light/dark cycle. At sowing, 100 inoculated seeds from each treatment were placed on fresh PDA plates (5 seeds per plate) to evaluate the success of the inoculation method.

Seedling blight disease assessment and rating

Seedlings were assessed for the disease at growth stage (GS) 12 (Zadoks et al. 1974) according to the scale detailed in Table 2 and percentage disease index (% DI) for each treatment was calculated using the equation:

$$\%\mathrm{DI} = \frac{\left(\frac{(\mathrm{N}\times\mathrm{Healthy})+(\mathrm{N}\times\mathrm{Slight})+(\mathrm{N}\times\mathrm{Moderate})+(\mathrm{N}\times\mathrm{Severe})+(\mathrm{N}\times\mathrm{Pre-emergence\ death})}{\sum \mathrm{N}}\right)}{4}\times 100$$

Where N is the number of seedlings in that scale category (Table 2).

Re-isolation from diseased seedling tissues

To confirm that the disease symptoms observed on seedlings were as a result of the inoculum applied, five 1 cm segments of diseased seedling stem-bases (after disease assessment) were plated on PDA after surface-sterilisation using sodium hypochlorite (1.2% available chlorine) for 1 min, followed by rinsing with SDW and blot-drying. They were incubated at 22°C for 4 days after which fungal colonies growing from them were transferred onto fresh PDA and identified as described previously.

Results

Results obtained from plating out oat and wheat seeds to check on possible natural contamination before commencement of the experiments revealed that 8% of wheat grains were infected by *F. culmorum* while 3% of oat grains were infected by

Table 2 Oat and wheat seedling blight disease rating scale (0–4) at Zadoks GS 12

Scale	Description
0	No disease symptoms
1	Slight disease. Stem-base browning present but does not girdle stem-base. Up to 25% area with lesions
2	Moderate disease. Stem-base browning present which completely girdles stem base. 25–50% of area with lesions
3	Severe disease. Stem-base browning completely girdles the stem-base, dark brown in colour and/or extends up the stem base to above ground level parts of the seedling. More than 50% of area with lesions
4	Pre-emergence death. Seedlings germinated but dead before emerging on the soil surface

Source: Modified from Glynn (2002).

M. nivale. The inoculation method was successful as the recovery of the fungal species used was 100% from all treatments. From the diseased cereal tissues plated, it was possible to re-isolate *F. culmorum*, *M. nivale* and *M. majus* from oats and wheat segments from seedlings treated with those pathogens. This fulfilled Koch Postulates 3 and 4 (cultures of the suspected causal organism must reproduce at least some of the symptoms of the disease, and the suspected causal organism must be re-isolated from the plant and shown to be identical with the organism originally isolated respectively) for these three species.

Oat and wheat *F. langsethiae*-inoculated seeds gave rise to seedlings that were healthy-looking, growing with vigour and that did not differ visually from those of uninoculated controls at both 5 and 15°C (Fig. 1). Symptoms of the disease observed caused by the pathogenic fungi were pre-emergence death, post-emergence death and development of lesions along seedling mesocotyl and coleoptile regions which appeared to differ greatly with the fungal species in question. Lesions caused by *M. nivale* and *M. majus* mainly appeared well above the seed while those caused by *F. culmorum* appeared from the point where the coleoptile joined the seed. Lesions caused by *M. nivale* were more conspicuous at 5ºC than those observed at 15ºC. On the other hand, lesions resulting from *F. culmorum* infection were more obvious at 15°C than at 5ºC. Seedlings inoculated with *F. culmorum* appeared to be weaker and stunted compared with seedlings inoculated with *M. nivale*. Post- and pre-emergence death on oat and wheat seedlings was observed with *F. culmorum* only. For some seedlings the coleoptile did not emerge above the soil surface before being killed, whilst other seedlings looked healthy above the soil surface but with a diseased, almost rotten mesocotyl. *Microdochium nivale* mycelia were observed to be growing on the soil surface and along healthy-looking seedling stems at 5ºC, an attribute not observed at 15ºC or with other species at either temperature. No lesions were observed on roots of any seedlings; the roots were well developed even when the coleoptile and mesocotyl were covered with lesions.

Experiment 1 (15°C)

(a) Effect of inoculation on overall % disease index (% DI) The fungus/cereal (host) interaction was not significant ($P>0.05$). There was a significant difference ($P=0.038$) for the % DI between oat and wheat seedlings (Table 3). Oat seedlings developed more disease than wheat seedlings although the difference was small. Highly significant differences for % DI between fungi ($P<0.001$) were observed. A Student-Newman-Keuls multiple comparison of means test revealed that *F. culmorum*, *M. nivale* and *M. majus* were the only fungal species pathogenic and causing seedling blight of oats and wheat when compared with uninoculated controls. Although the three fungal species resulted in statistically similar disease levels, on average, *F. culmorum* was found to cause the greatest disease; *F. culmorum* caused 1.5 times more seedling blight than *M. nivale* and 7 times more disease than *M. majus* (Table 3). Oat and wheat isolates of *F. langsethiae,* as well as *F. poae* did not cause any significant seedling blight disease symptoms compared with the uninoculated controls. The controls were not entirely clean of infection as a low number of diseased seedlings occurred for the uninoculated wheat and oats. This was to be expected

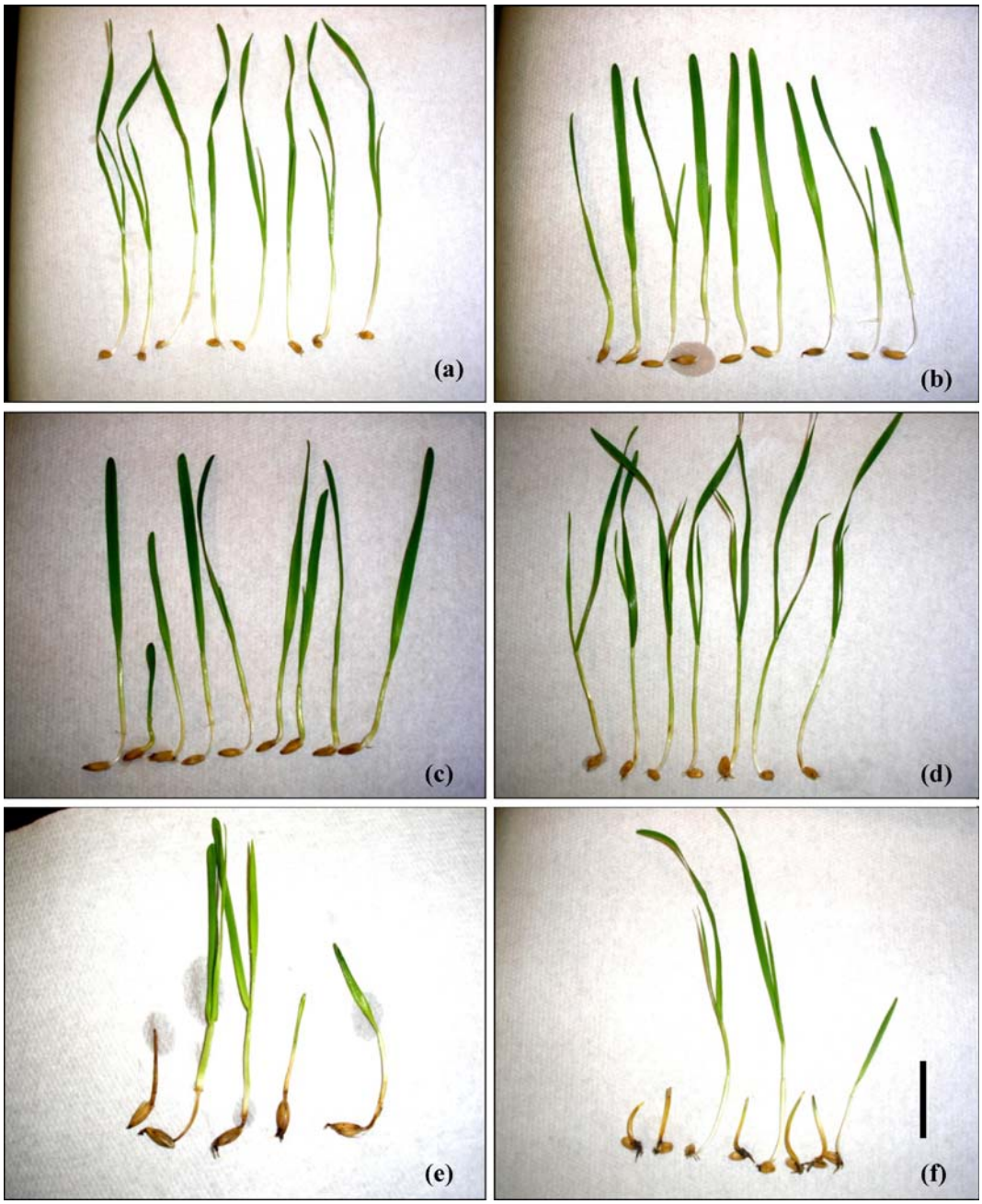

Fig. 1 Seedling blight disease caused by *Fusarium* and *Microdochium* spp. on oat and wheat seedlings grown at 15ºC and assessed at Zadoks GS 12. **a** and **b** oat and wheat seedlings respectively resulting from *F. langsethiae*-inoculated seeds (no symptoms), **c** and **d** oat and wheat seedlings respectively resulting from *M. nivale*-inoculated seeds, **e** and **f** oat and wheat seedlings respectively resulting from *F. culmorum*-inoculated seeds. Bar=40 mm

as both seed lots contained a low background level of *Fusarium* or *Microdochium* infection

(b) Effect of inoculation on lesion development and pre-emergence death Lesions were observed on a number of seedlings from all the treatments including the uninoculated controls. *Microdochium nivale* resulted in the greatest number of seedlings with lesions for both oats and wheat followed by *F. culmorum* and *M. majus* (Table 4). These three

Table 3 Effect of artificial seed inoculation with isolates of *Fusarium* and *Microdochium* species on seedling blight disease of oats and wheat at 15ºC. Values are % DI of 8 replicates and those with the same superscript letter are not statistically different based on the Student-Newman-Keuls test ($P<0.05$)

Fungus	Cereal		Overall mean (Fungus)
	Oats	Wheat	
Uninoculated (control)	1.48	2.26	1.87[a]
Fusarium culmorum	69.83	63.44	66.63[b]
Fusarium langsethiae (oat isolates)	7.34	1.56	4.45[a]
Fusarium langsethiae (wheat isolates)	3.73	1.87	2.80[a]
Fusarium poae	5.16	3.47	4.31[a]
Microdochium nivale	46.58	43.85	45.22[b]
Microdochium majus	7.43	10.47	8.95[b]
Overall mean (cereal)	20.22	18.13	
	Cereal		Fungus
P-value	0.038		< 0.001
SEM (*df*=95)	0.700		1.310

species caused lesions on a slightly greater number of wheat than oat seedlings. Oat and wheat isolates of *F. langsethiae*, the uninoculated controls and to a lesser extent *F. poae* resulted in a similar number of seedlings with lesions. With the exception of *F. culmorum*, none of the other species caused pre-emergence death of seedlings. *Fusarium culmorum* resulted in a similar number of pre-emergence seedling deaths for both oats and wheat.

(c) Effect of inoculation on seedling emergence The interaction between cereal and fungus was not significant ($P>0.05$). A highly significant difference ($P<0.001$) in the number of emerged seedlings was observed between oats and wheat; 11% more wheat than oat seedlings had emerged at GS 12 (Table 5). A highly significant difference ($P<0.001$) for the percentage seedling emergence between the fungal species was also observed. Only *F. culmorum* resulted in a significant reduction in seedling emergence (45%) when compared with the controls at 15°C. Oat and wheat isolates of *F. langsethiae*, *F. poae*, *M. nivale* and *M. majus* did not produce a significant reduction in seedling emergence when compared with the controls.

Table 4 Effect of artificial seed inoculation with isolates of *Fusarium* and *Microdochium* species on the percentage of oats and wheat seedlings developing lesions and pre-emergence death at 15°C

Fungus	Cereal			
	Oats		Wheat	
	% number of seedlings with lesions	% pre-emergence death	% number of seedlings with lesions	% pre-emergence death
Uninoculated (control)	11	0	9	0
Fusarium culmorum	55	25	60	24
Fusarium langsethiae (oat isolates)	15	0	6	0
Fusarium langsethiae (wheat isolates)	12	0	7	0
Fusarium poae	18	0	10	0
Microdochium nivale	85	0	95	0
Microdochium majus	24	0	34	0

Table 5 Effect of artificial seed inoculation with isolates of *Fusarium* and *Microdochium* species on oat and wheat seedling emergence at 15ºC. Values are of 8 replicates and those with the same superscript letter are not statistically different based on the Student-Newman-Keuls test ($P<0.05$)

Fungus	Cereal emergence (%)		Overall mean (Fungus)
	Oats	Wheat	
Uninoculated (control)	84	99	91[b]
Fusarium culmorum	54	57	55[a]
Fusarium langsethiae (oat isolates)	92	100	96[b]
Fusarium langsethiae (wheat isolates)	84	99	91[b]
Fusarium poae	80	97	89[b]
Microdochium nivale	82	97	90[b]
Microdochium majus	90	97	94[b]
Overall mean (cereal)	81	92	
	Cereal		Fungus
P-value	< 0.001		< 0.001
SEM (*df*=95)	14		25

Experiment 2 (5°C)

(a) Effect of inoculation on overall % disease index (% DI) The interaction between cereal and fungus was not significant ($P>0.05$). The % DI between oat and wheat seedlings did not differ significantly from each other (Table 6) as opposed to the experiment carried out at 15ºC (Table 3). Highly significant differences for % DI between fungi ($P<0.001$) were observed. Only *F. culmorum* and *M. nivale* caused significant disease when compared with the controls and at the same magnitude at this temperature. Oat and wheat isolates of *F. langsethiae*, *F. poae* and *M. majus* did not result in any significant seedling blight disease of these two cereals when compared with the uninoculated controls (Table 6). Although *F. culmorum* and *M. nivale* statistically caused disease of the same severity, it was interesting to note that the latter resulted in 1.3 times more disease than the former at 5ºC. It was evident that all fungi were more aggressive at a higher temperature of 15ºC than that at 5ºC (Tables 3 and 6).

(b) Effect of inoculation on lesion development and pre-emergence death The number of seedlings developing lesions at 5°C was found to be lower than that observed at 15°C. The same trend was observed with *M. nivale*, *F. culmorum* and *M. majus* as that reported at 15°C; *M. nivale* caused the greatest number of seedlings with lesions followed by *F. culmorum* and then *M. majus* (Table 7). As reported at 15°C (Table 4), *M.*

Table 6 Effect of artificial seed inoculation with isolates of *Fusarium* and *Microdochium* species on seedling blight disease of oats and wheat at 5ºC. Values are % DI of 8 replicates and those with the same superscript letter are not statistically different based on the Student-Newman-Keuls test ($P<0.05$)

Fungus	Cereal		Overall mean (Fungus)
	Oats	Wheat	
Uninoculated (control)	0.0	0.0	0.0[a]
Fusarium culmorum	24.2	32.0	28.1[b]
Fusarium langsethiae (oat isolates)	2.0	0.7	1.3[a]
Fusarium langsethiae (wheat isolates)	0.0	0.3	0.2[a]
Fusarium poae	0.0	0.0	0.0[a]
Microdochium nivale	38.0	37.0	37.5[b]
Microdochium majus	10.6	1.9	6.2[a]
Overall mean (cereal)	10.7	10.3	
	Cereal		Fungus
P-value	0.849		< 0.001
SEM (*df*=95) -	–		3.93

Table 7 Effect of artificial seed inoculation with isolates of *Fusarium* and *Microdochium* species on the percentage of oat and wheat seedlings developing lesions and pre-emergence death at 5°C

Fungus	Cereal			
	Oats		Wheat	
	% number of seedlings with lesions	% pre-emergence death	% number of seedlings with lesions	% pre-emergence death
Uninoculated (control)	0	0	1	0
Fusarium culmorum	31	1	40	2
Fusarium langsethiae (oat isolates)	5	0	3	0
Fusarium langsethiae (wheat isolates)	0	0	1	0
Fusarium poae	0	0	0	0
Microdochium nivale	60	0	84	0
Microdochium majus	17	0	8	0

nivale and *F. culmorum* resulted in a greater number of wheat seedlings with lesions than oat seedlings. Wheat isolates of *F. langsethiae*, uninoculated controls, *F. poae* and to a lesser extent oat isolates of *F. langsethiae* resulted in the least number of seedlings with lesions. *Fusarium culmorum* was the only species that caused pre-emergence death of seedlings but to a much lower extent than that reported at 15°C.

(c) Effect of inoculation on seedling emergence There was no significant interaction between cereal and fungus at 5°C ($P>0.05$). A highly significant difference ($P<0.001$) in seedling emergence between oats and wheat was observed with 32% more emerged wheat than oat seedlings (Table 8). There were no significant differences in seedling emergence as a result of all the fungal treatments. On average, fewer seedlings emerged at 5°C than at 15°C with the exception of seedlings resulting from *F. culmorum*-inoculated seeds.

Discussion

Reaction of cereal cultivars to seedling blight caused by fungi of the genera *Fusarium* and *Microdochium* was evaluated using various parameters such as seedling emergence, symptom expression and the effect on the final yield such as grain weight. Of these parameters, the earliest and most commonly employed predictors are seedling emergence and

Table 8 Effect of artificial seed inoculation with isolates of *Fusarium* and *Microdochium* species on oat and wheat seedling emergence at 5ºC. Values are of 8 replicates

Fungus	Cereal emergence (%)		Overall mean (Fungus)
	Oats	Wheat	
Uninoculated (control)	51	96	74
Fusarium culmorum	76	96	86
Fusarium langsethiae (oat isolates)	62	96	79
Fusarium langsethiae (wheat isolates)	59	95	77
Fusarium poae	67	96	81
Microdochium nivale	65	99	82
Microdochium majus	65	96	81
Overall mean (cereal)	64	96	
	Cereal		Fungus
P-value	< 0.001		< 0.153
SEM (*df*=95)	17		–

symptom development along the seedling coleoptile and mesocotyl regions. Based on these two parameters, *F. langsethiae* appears not to be a seedling pathogen of oats and wheat as there was no significant seedling blight disease compared with uninoculated controls. Further evidence supporting this result was obtained from the number of seedlings with lesions from each treatment, seedling emergence and pre-emergence death data at both 5 and 15°C where only *F. culmorum* was found to significantly reduce emergence and cause pre-emergence death. This result may have been influenced by other factors such as the concentration and form of the inoculum applied. For example, increased inoculum is expected to result in increased disease severity. However, results from an experiment carried out in the glasshouse where oat seeds placed on *F. langsethiae*-colonised agar and sown in sterilised soil resulted in seedlings growing normally (results not shown) would suggest high inoculum loads with *F. langsethiae* would still not produce seedling blight in wheat and oats. Lower spore loads of less than 5×10^5 conidia ml^{-1} which was used in this study have been demonstrated to cause severe seedling blight by some fungi. Millar and Colhoun (1969) and Colhoun (1970) observed seedling blight of wheat when seeds were inoculated with 5×10^4 and 10^4 conidia ml^{-1} of *M. nivale* and *F. culmorum* respectively. Glynn (2002) showed that *Microdochium* species can cause seedling blight of wheat at conidial concentrations as low as 10^3conidia ml^{-1} while Hare (1997) demonstrated that *Microdochium* species can produce seedling blight symptoms on wheat when 4×10^3 conidia ml^{-1} were inoculated. All these previous studies support the argument that *F. langsethiae* is either non-pathogenic or a weak pathogen of these cereals at the seedling stage of development. This non-pathogenic nature of *F. langsethiae* on oat and wheat seedlings is not surprising as it also failed to produce FHB symptoms on the same hosts in previous inoculated glasshouse and field experiments (Imathiu 2008; Imathiu et al. 2009).

Pathogenicity of *F. langsethiae* on oat and wheat seedlings may be affected by the degree of plant resistance. It is possible that the cultivars of oat and wheat used in this study were naturally resistant to *F. langsethiae* infection at the seedling stage of development. Timmermans and Osman (2007) have recently reported that spring wheat cultivars differ in their sensitivity to seedling blight. The authors also reported that cultivars with higher early growth rates appeared to be less sensitive to seedling blight. They were investigating the differences between six spring wheat cultivars for emergence and early development after seed infection by *F. culmorum*. Unlike FHB, limited information is available in the scientific literature concerning cereal varietal resistance to FSB. The only information available from the UK HGCA list of recommended cultivars of cereals used in this study is that of cv. Clare whose degree of resistance to FHB is a score of 7 (on a scale of 1–9, 9 being resistant) (Anon. 2007). In the UK, there is currently no information on the degree of resistance of cereals to seedling blight and it cannot be assumed that resistance of a cereal to FHB would predict a seedling blight result. For example, Browne and Cooke (2005) reported wheat cv. Claire considered to have relatively good resistance to FHB to be quite susceptible to seedling pathogens in a seed germination assay. They also reported cv. Xi 19 which has poor FHB resistance exhibiting the highest resistance in the seed germination assay. Lack of correlation between seedling blight and FHB has been reported elsewhere in the literature. Arseniuk et al. (1993) reported lack of significant correlation between seedling and head infection with *Fusarium* species for winter wheat and triticale. This is in agreement with the study of Grey and Mathre (1988) who reported a lack of correlation between seedling disease and FHB of spring barley caused by *F. culmorum*. To confirm the non-pathogenic results obtained for *F. langsethiae*, further studies are required using a wide range of oat and wheat varieties with varying resistance to FHB.

Another explanation for the lack of disease symptoms is that *F. langsethiae* might have caused infection of the seedlings with no symptoms apparent, thus resulting in a symptomless, latent infection. This hypothesis is supported by findings published by Fitzgerald and Cooke (1983) who reported successful re-isolation of *S. nodorum* from symptomless coleoptiles of barley in a study investigating seedling blight of barley caused by *Septoria nodorum*. In another similar observation, Clement and Parry (1998) reported the recovery of *F. culmorum*, *F. graminearum* and *M. nivale* from stem tissues of winter wheat in apparently symptomless plants. Although the seedlings in this study may have been infected by *F.*

langsethiae, on the basis of lack of symptoms which constitutes presence of a disease, one may infer that this fungus is not a pathogen of the two hosts at the seedling stage. If *F. langsethiae* was a symptomless pathogen, this could present problems later in the plant development as a potential reservoir which can act as an inoculum source for ear or panicle infection later in the season.

It was observed that both oat and wheat isolates of *F. langsethiae* did not result in any seedling blight disease of either host. No previous studies investigating the potential pathogenicity of *F. langsethiae* on seedlings have been published for comparison with these findings. Similarly to *F. langsethiae*, *F. poae* seedling blight disease index did not differ significantly from the uninoculated controls. These two fungi have some attributes in common; their spore morphology is similar and their distribution has been found to be statistically correlated implying similar environmental requirements (Kosiak et al. 2003) and possibly similar host preference and pathogenicity. Unlike *F. langsethiae*, the pathogenicity of *F. poae* on seedlings has been studied, though the literature is limited. Results obtained for *F. poae* in this study are in agreement with those of Colhoun and Park (1964) who found that *F. poae* was not a seedling pathogen of wheat in a study investigating the effect of soil moisture and temperature on seedling infection. They are also supported by Bolton and Nuttal (1967) who reported that *F. poae* cultures isolated from oats, wheat and barley, and tested on the same host seedlings did not cause seedling blight.

In a previous detached leaf assay, oat and wheat isolates of *F. langsethiae* were shown to be pathogenic to both hosts but were more aggressive towards oats (Imathiu et al. 2009). The difference in pathogenicity as determined by a detached leaf assay and a seedling blight assay may be due to the use of different plant parts and growth stages which may differ in the form of resistance(s) inherent in each of them. Miedaner (1997) reported that most resistances are influenced by interactions between cereal cultivar, plant part and growth stages which may be caused by different resistance mechanisms. Browne and Cooke (2005) reported a lack of correlation between the resistance detected in a seed germination assay and the partial disease resistance components (lesion length, incubation period and latent period) detected using a detached leaf assay. The authors concluded that resistances detected in seed germination and detached leaf assays are independent of each other.

Various *Fusarium* species have been reported to cause root rot of cereals including *F. graminearum, F. culmorum, F. avenaceum, F. equiseti* and *F. acuminatum* (Parry et al. 1995). The ability and extent to which they are able to do so, however, depends on many factors including cereal degree of resistance, species or isolate pathogenicity and aggressiveness, and the prevailing environmental conditions during plant growth (Gonzalez and Trevathan 2000). Lack of any disease symptoms on the roots of all seedlings including those exhibiting seedling blight symptoms on stem-bases may be due to the inability of the isolates to cause root disease or environmental conditions were not conducive. As detailed previously there may be some resistance differences between different target tissue and/or growth stage to infection by the same fungal species.

Similar small disease scores on oat and wheat seedlings were recorded for *F. langsethiae*, *F. poae* and the uninoculated control group at each of the two temperatures employed which did not differ significantly from each other. This can be explained by the presence of *F. culmorum* and *M. nivale* in the original seed material. Despite the surface-sterilisation of the seeds before sowing, a carry-over of natural field infection occurred probably as a result of internal seed-borne contamination. After surface-sterilisation of seeds, it was determined that 8% of wheat seeds were infected with *F. culmorum* while 3% of oat seeds were infected by *M. nivale*.

The effects of soil temperature and moisture on cereal seedling blight have been well studied and generally cited as the most important abiotic factors determining the severity of seedling blight on cereals (Dickson et al. 1923). In the whole of this study, moisture content was not varied; therefore, differences in aggressiveness of the same fungal species on each host can only be attributed to temperature differences under which the artificially inoculated seeds were grown. In this study temperature seemed to be a critical factor in determining the pathogenicity and extent of disease development by *F. culmorum*, *M. nivale* and *M. majus*. At 15ºC, *F. culmorum*, *M. nivale* and *M. majus* caused seedling blight of oats and wheat. On average, *F. culmorum* was found to be the most aggressive and *M. majus* the weakest pathogen of the three fungi. At 5ºC, only *F. culmorum*

and *M. nivale* caused seedling blight where they produced disease of similar severity. Results from this study would indicate that *M. majus* ceases to be a seedling blight pathogen of oat and wheat at 5ºC and that it is a weak pathogen of both hosts at 15ºC. Although *F. culmorum* and *M. nivale* seem to be pathogens at these two temperatures, their degree of aggressiveness differed quite markedly at both temperatures. *Fusarium culmorum* caused more disease at 15ºC than 5ºC. It was also found to cause more pre- and post-emergence death of seedlings at the higher temperature meaning that this species requires warmer soil temperatures to cause severe disease as reported by Colhoun and Park (1964) and Colhoun (1970). *Microdochium nivale* caused almost similar percentage disease index at both 5°C and 15ºC although its symptoms were more conspicuous on infected seedlings at 5°C than at 15ºC. *Microdochium nivale* was also found to produce the greatest number of seedlings with lesions at the two temperatures but unlike those caused by *F. culmorum*, these lesions were less severe. Due to its more obvious symptoms at the lowest temperature, it appears therefore that *M. nivale* given more time may cause more severe disease at 5°C than at 15ºC. This conclusion is supported by the fact that mycelium of this fungus was observed growing on the soil surface and along seedling stems at 5°C but not at 15ºC, and this is probably why it was previously called a snow mould fungus. This is further supported by the fact that warmer climatic conditions have been shown to favour seedling blight disease development by causal species other than *M. nivale* which requires cooler conditions (Doohan et al. 2003), and that *M. nivale* has been shown to grow relatively fast at 5ºC (Pettitt et al. 1996). Other reports supporting pathogenicity and aggressiveness of *M. nivale* at low temperatures include work carried out by Doohan et al. (2003), Parry et al. (1995), Mahuku et al. (1998) and Millar and Colhoun (1969).

So far, seedling blight results obtained for *M. nivale* and *M. majus* seem to be contradictory. Results obtained from this study are supported by those of Simpson et al. (2000; 2004) who reported that *M. nivale* caused more severe stem-base disease symptoms than *M. majus* in oats, wheat and rye. However, these findings disagree with those of Glynn (2002) and Hare (1997) who reported that isolates of *M. majus* were more pathogenic than those of *M. nivale* on wheat seedlings. The reason for these differences is not clear, but it may be that there is variability in pathogenicity and aggressiveness within isolates of each species. Mesterhazy (1978) reported the aggressiveness of individual *Fusarium* isolates varied greatly from each other. It may also have been due to the conditions under which each experiment was carried out, for example, Glynn's (2002) experiments were carried out under fixed humidity of 80% which was not a factor under investigation in this study.

This study has demonstrated that *F. langsethiae* and *F. poae* may not be important seedling blight disease-causing fungi of oats and wheat. They are unlikely to lead to reduced crop stand in the field, and therefore, yield if there is no subsequent foot-rot and/or head blight where infected seeds are sown. The results have also demonstrated that seedling blight may not be a part of the disease cycle for *F. langsethiae* and *F. poae*. The immediate major concern for *F. langsethiae* in small-grain cereal crops is the contamination of grain with mycotoxins HT-2 and T-2 and more research is required to identify the source of the inoculum for wheat ears and oat panicles at later growth stages of cereal development. Studies on systemic or asymptomatic infection by *F. langsethiae* in cereals are warranted to determine whether they are important for infection by this important mycotoxin-producing pathogen of cereals.

Acknowledgements Authors wish to thank Quaker Oats Ltd, Home-Grown Cereals Authority and Harper Adams University College for funding the study.

References

Anon. (2007). *Recommended lists 2007/08 for cereals and oilseeds*. London: HGCA.

Arseniuk, E., Goral, T., & Czembor, H. J. (1993). Reaction of triticale, wheat and rye accessions to graminaceous *Fusarium* spp. infection at seedling and adult plant growth stages. *Euphytica, 70*, 175–183.

Bolton, A. T., & Nuttal, V. W. (1967). Pathogenicity studies with *Fusarium poae*. *Canadian Journal of Plant Science, 48*, 161–166.

Bottalico, A., & Perrone, G. (2002). Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. *European Journal of Plant Pathology, 108*, 611–624.

Browne, R. A., & Cooke, B. M. (2005). Resistance of wheat to *Fusarium* spp. in an *in vitro* seed germination assay and

preliminary investigations into the relationship with FHB resistance. *Euphytica, 141*, 23–32.

Chongo, G., Gossen, B. D., Kutcher, H. R., Gilbert, J., Turkington, T. K., Fernandez, M. R., et al. (2001). Reaction of seedling roots of 14 crop species to *Fusarium graminearum* from wheat heads. *Canadian Journal of Plant Pathology, 23*, 132–137.

Clement, J. A., & Parry, D. W. (1998). Stem-base disease and fungal colonisation of winter wheat grown in compost inoculated with *Fusarium culmorum*. F. graminearum and Microdochium nivale. *European Journal of Plant Pathology, 104*, 323–330.

Colhoun, J. (1970). Epidemiology of seed-borne *Fusarium* diseases of cereals. *Annales Academiae Scientiarum Fennicae Series A IV, Biologica, 168*, 31–36.

Colhoun, J. A., & Park, D. (1964). *Fusarium* diseases of cereals I. Infection of wheat plants, with particular reference to the effects of soil moisture and temperature on seedling infection. *Transactions of the British Mycological Society, 47*, 559–5720.

Colhoun, J., Taylor, G. S., & Tomlinson, R. (1968). *Fusarium* diseases of cereals II. Infection of seedlings by F. culmorum and F. avenaceum in relation to environmental factors. *Transactions of the British Mycological Society, 51*, 397–404.

De Tempe, J. (1968). Testing oat seed for *Fusarium* spp. and *Helminthosporium avenae*. *Proceedings of the International Seed Testing Association, 33*, 589–602.

Dickson, J. G., Eckerson, S. H., & Link, K. P. (1923). The nature of resistance to seedling blight of cereals. *Pathology, 9*, 434–439.

Doohan, F. M., Brennan, J., & Cooke, B. M. (2003). Influence of climatic factors on *Fusarium* species pathogenic to cereals. *Plant Pathology, 109*, 755–768.

Edwards, S. G., Barrier-Guillot, B., Clasen, P., Hietaniemi, V., & Pettersson, H. (2009). Emerging issues of HT-2 and T-2 toxins in European cereal production. *World Mycotoxin Journal, 2*, 173–179.

Fernandez, M. R., & Chen, Y. (2005). Pathogenicity of *Fusarium* species on different plant parts of spring wheat under controlled conditions. *Plant Disease, 89*, 164–169.

Fitzgerald, W., & Cooke, B. M. (1983). A seedling blight of barley caused by *Septoria nodorum*. *Plant Pathology, 32*, 217–219.

Glynn, N. C. (2002). *Studies on the epidemiology and chemical control of fusarium seedling blight of wheat using molecular techniques*. Open University, UK. PhD Thesis.

Glynn, N., Hare, M. C., Parry, D. W., & Edwards, S. G. (2005). Phylogenetic analysis of EF-1 alpha gene sequences from isolates of *Microdochium nivale* leads to elevation of varieties *majus* and *nivale* to species status. *Mycological Research, 109*, 872–880.

Gonzalez, M. S., & Trevathan, L. E. (2000). Identity and pathogenicity of fungi associated with root and crown rot of soft red winter wheat grown on the upper coastal plain land resource area of Mississippi. *Journal of Phytopathology, 148*, 77–85.

Grey, W. E., & Mathre, D. E. (1988). Evaluation of spring barleys for reaction of *F. culmorum* seedling blight and root rot. *Canadian Journal of Plant Science, 68*, 23–30.

Haigh, I. M., Jenkinson, P., & Hare, M. C. (2009). The effect of a mixture of seed-borne *Microdichium nivale* var. *majus* and *Microdochium nivale* var. *nivale* infection on fusarium seedling blight severity and subsequent stem colonisation and growth of winter wheat in pot experiments. *European Journal of Plant Pathology, 124*, 65–73.

Hare, M. C. (1997). Epidemiology and chemical control of fusarium seedling blight of winter wheat (*Triticum aestivum* L.). Open University, UK. PhD Thesis.

Hare, M. C., & Parry, D. W. (1996). Observations on the maintenance and measurement of water in simple pot experiments and its effect on seed-borne *Fusarium culmorum* seedling blight of winter wheat. *Annals of Applied Biology, 129*, 227–235.

Hare, M. C., Parry, D. W., & Baker, M. D. (1999). The relationship between wheat seed weight, infection by *Fusarium culmorum* or *Microdochium nivale*, germination and seedling disease. *European Journal of Plant Pathology, 105*, 859–866.

Holmes, J. I., & Channon, A. G. (1975). Glasshouse studies on the effect of low temperature on infection of perennial ryegrass seedlings by *Fusarium nivale*. *Annals of Applied Biology, 79*, 43–48.

Humphreys, J., Cooke, B. M., & Storey, T. (1995). Effects of seed-borne *Microdochium nivale* on establishment and grain yield of winter-sown wheat. *Plant Varieties and Seeds, 8*, 107–117.

Imathiu, S. M. (2008). *Fusarium langsethiae* infection and mycotoxin production in oats. Harper Adams University College, UK. PhD Thesis.

Imathiu, S. M., Ray, R. V., Back, M., Hare, M. C., & Edwards, S. G. (2009). *Fusarium langsethiae* pathogenicity and aggressiveness towards oats and wheat in wounded and unwounded *in vitro* detached leaf assays. *European Journal of Plant Pathology, 124*, 117–126.

Infantino, A., Pucci, N., Conca, G., & Santori, A. (2007). First report of *Fusarium langsethiae* on durum wheat kernels in Italy. *Plant Disease, 91*, 1362.

Johnson, E. C. (1914). A study of some imperfect fungi isolated from wheat, oats and barley. *Journal of Agricultural Research, 1*, 475–487.

Johnston, C. L., & Greaney, F. J. (1941). Studies on the pathogenicity of *Fusarium* species associated with root rot of wheat. *Phytopathology, 32*, 670–684.

Jones, R. K. (1999). Seedling blight development and control in spring wheat damaged by *Fusarium graminearum* Group 2. *Plant Disease, 83*, 1013–1018.

Kosiak, B., Torp, M., Skjerve, M., & Thrane, U. (2003). The prevalence and distribution of *Fusarium* species in Norwegian cereals: a survey. *Acta Agriculturæ Scandinavica, Section B, Soil and Plant Science, 53*, 168–176.

Leslie, J. F., & Summerell, B. A. (2006). *The Fusarium laboratory manual*. USA: Blackwell.

Mahuku, G. S., Hsiang, T., & Yang, L. (1998). Genetic diversity of *Microdochium nivale* isolates from turf grass. *Mycological Research, 102*, 559–567.

Mesterhazy, A. (1978). Comparative analysis of artificial inoculation methods with *Fusarium* spp. on winter wheat varieties. *Phytopathologische Zeitschrift, 93*, 12–25.

Miedaner, T. (1997). Breeding wheat and rye for resistance to *Fusarium* diseases-a review. *Plant Breeding, 116*, 201–220.

Millar, C. S., & Colhoun, J. (1969). *Fusarium* diseases of cereals VI. Epidemiology of Fusarium nivale on wheat. *Transactions of the British Mycological Society, 52*, 195–204.

Parry, D. W., Jenkinson, P., & McLeod, L. (1995). Fusarium ear blight (scab) in small grain cereals-a review. *Plant Pathology, 44*, 207–238.

Pereyra, S. A., Dill-Macky, R., & Sims, A. L. (2004). Survival and inoculum production of *Gibberella zeae* in wheat residue. *Plant Disease, 88*, 724–730.

Pettitt, T. R., Parry, D. W., & Polley, R. W. (1996). Effect of temperature on the incidence of nodal foot rot symptoms in winter wheat crops in England and Wales caused by *Fusarium culmorum* and *Microdochium nivale*. *Agricultural and Forest Meteorology, 79*, 233–243.

Simmonds, P. M. (1928). Studies in cereal diseases III. Seedling blight and foot-rots of oats caused by *Fusarium culmorum* (W.G.Sm.) Sacc. Dominion of Canada Department of Agriculture. Bulletin 105.

Simpson, D. R., Rezanoor, H. N., Parry, D. W., & Nicholson, P. (2000). Evidence for differential host preference in *Microdochium nivale* var. *majus* and *Microdochium nivale* var. *nivale*. *Plant Pathology, 49*, 261–268.

Simpson, D. R., Thomsett, M. A., & Nicholson, P. (2004). Competitive interactions between *Microdochium nivale* var. *majus*, *M. nivale* var. *nivale* and *Fusarium culmorum* in *planta* and *in vitro*. *Environmental Microbiology, 6*, 79–87.

Summerell, B. A., Sallech, B., & Leslie, J. F. (2003). A utilitarian approach to *Fusarium* identification. *Plant Disease, 87*, 117–128.

Timmermans, B. G. & Osman, A. M. (2007). Differences between spring wheat cultivars for emergence and early development after seed infection with *Fusarium culmorum*. 3 rd QLIF Congress, Hohenheim, Germany, March 20-23, 2007. Archived at: http://orgprints.org/9751/01/timmermans-osman-2007-spring-wheat.pdf (Accessed on 2nd December, 2008).

Torp, M., & Adler, A. (2004). The European *Sporotrichiella* Project: a polyphasic approach to the biology of new *Fusarium* species. *International Journal of Food Microbiology, 95*, 241–245.

Torp, M., & Nirenberg, H. I. (2004). *Fusarium langsethiae* sp. nov. on cereals in Europe. *International Journal of Food Microbiology, 95*, 247–256.

Wisnieska, H., & Busko, M. (2005). Evaluation of spring wheat resistance to fusarium seedling blight and head blight. *Biologia (Bratislava), 60*, 287–293.

Zadoks, J. C., Chang, T. T., & Konzak, C. F. (1974). Decimal code for growth stages of cereals. *Weed Research, 15*, 415–421.