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Fusarium langsethiae pathogenicity and aggressiveness towards oats and wheat in wounded and unwounded *in vitro* detached leaf assays

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Abstract *In vitro* detached leaf assays involving artificial inoculation of wounded and unwounded oat and wheat leaves were used to investigate the potential pathogenicity and aggressiveness of *F. langsethiae*, which was linked recently to the production of type A trichothecenes, HT-2 and T-2 in cereals in Europe. In the first two experiments, two assays compared disease development by *F. langsethiae* with known fusarium head blight pathogen species each used as a composited inoculum (mixture of isolates) at 10°C and 20°C and found all fungal species to be pathogenic to oat and wheat leaves in the wounded leaf assay. In the unwounded leaf assay, *F. langsethiae* was not pathogenic to wheat leaves. Furthermore, there were highly significant differences in the aggressiveness of pathogens as measured by lesion length ($P<0.001$). In the second two experiments, pathogenicity of individual *F. langsethiae* isolates previously used in the composite inoculum was investigated on three oat and three wheat varieties. The wounded leaf assay showed that all isolates were pathogenic to all oat and wheat varieties but only pathogenic towards oat varieties in the unwounded assay. Highly significant differences ($P<0.001$) in lesion length were found between cereal varieties as well as between isolates in the wounded assay. Significant differences in lesion lengths ($P=0.014$) were also observed between isolates in the unwounded assay. Results from the detached leaf assays suggest that *F. langsethiae* is a pathogen of wheat and oats and may have developed some host preference towards oats.

Keywords Fusarium head blight · Lesion length · Mycotoxins · HT-2 and T-2

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Introduction

Fusarium head blight (FHB; also scab, fusarium ear blight, head fusariosis) is a wide-spread destructive disease of small-grain cereal crops caused by a number of *Fusarium* species including *F. culmorum*, *F. avenaceum*, *F. graminearum*, *F. poae*, *M. nivale* and *M. majus* (Xu et al. 2005; Glynn et al. 2005). In contrast to wheat and barley, where damage to spikes by FHB pathogens is normally distinct and can be quantified, this disease is rarely recognisable in a standing oat crop (Diamond and Cooke 1999; Gilbert and Tekauz 2000). Lower levels of *Fusarium* infection or infection by less pathogenic *Fusarium* species has been reported to not result in typical head blight symptoms in oats (Henriksen and Elen 2005). Head blight disease severity of cereals has been found to

vary greatly in different years and locations, being dependent on the environmental conditions (Rossi et al. 2001).

FHB has been reported to reduce oat yield by 12–48% (Perkowski and Kiecana 1997) and wheat yield by up to 70% (Tusa et al. 1981). In addition, FHB-causing pathogens can potentially reduce the quality of grains for their intended end use. Presence of *Fusarium*-damaged grains has resulted in downgrading of the produce, making growers lose quality premiums leading to reduced domestic and export markets. With the exception of *Microdochium* species (Xu et al. 2005), other fungal pathogens involved in FHB are potential producers of mycotoxins, which are of concern because they can affect food safety as many of them have been associated with chronic and acute mycotoxicoses in humans and livestock.

Recently, a newly identified toxigenic *Fusarium* species, *Fusarium langsethiae*, has been isolated in infected oats, wheat and barley in central and northern Europe (Torp and Adler 2004; Torp and Nirenberg 2004). This species has been implicated in the production of high levels of HT-2 and T-2 mycotoxins in cereals in Norway (Langseth and Rundberget 1999; Torp and Langseth 1999) and in oats in the UK (Edwards 2007a). HT-2 and T-2 mycotoxins are currently being considered for legislation by the European Commission. Disease symptoms by *F. langsethiae* are rarely observed in small-grain cereals. Although a small percentage of wheat ears with infected 'glume spots' thought to have been caused by *F. langsethiae* in the field have been observed in Austria (Adler and Torp 2004), no disease symptoms have been observed on oats and wheat during inoculated glasshouse and field studies (unpublished work). *Fusarium langsethiae* can readily be isolated from symptomless oat and wheat grains but currently, no evidence of its pathogenicity on these cereals has been demonstrated.

To date, there are no varieties of cereals available which are completely resistant to *Fusarium*. Use of partially resistant varieties is a major component of an integrated control programme to reduce FHB and/or mycotoxin contamination of harvested grain. This can be more readily realised if fast and less costly methods of evaluation are used. One such novel development has been the use of detached leaf assays to study diseases for some pathogens (Deadman and Cooke 1986; Diamond et al. 1995; Roderick and Clifford 1995; Diamond and Cooke 1999). Detached leaf assays have commonly been used in experiments investigating FHB resistance of wheat, using components of partial disease resistance (PDR) and their relationship to FHB (Diamond and Cooke 1999; Browne and Cooke 2004, 2005; Browne et al. 2005, 2006). Lesion length is one of the components of PDR measured as an indicator of fungal pathogenicity and aggressiveness. These two terms, 'pathogenicity' and 'aggressiveness' are sometimes used interchangeably in fungal plant pathology though they have distinct meanings. According to Schafer (1994), pathogenicity is the capacity of a fungus to cause disease, thus a qualitative measure, while according to Shaner et al. (1992), aggressiveness is a description of the rate at which an amount of disease is reached with more aggressive pathogens reaching this amount faster, thus a quantitative measure. Diamond and Cooke (1999) reported a high correlation between lesion length and FHB severity. According to Browne and Cooke (2004), the *Microdochium* genus is used to study PDR because compared with other FHB complex pathogens, it provides more distinct and observable symptoms on detached unwounded leaves. *Microdochium majus* has been found to be more pathogenic to wheat than *M. nivale* in detached leaf assays (Diamond and Cooke 1999; Browne and Cooke 2004).

The epidemiology of *F. langsethiae* is not understood and host plant preference is unknown as isolates have been recovered from oat, barley and wheat grains. The aim of this study was to assess the potential of oat and wheat isolates of *F. langsethiae* to cause disease on wounded and unwounded detached oat and wheat leaves relative to other *Fusarium* and *Microdochium* species.

Materials and methods

Fungal species and spore production

All fungal isolates used in this study were recovered from wheat or oat grains from the UK (Table 1). Single-spore isolates were cultured on potato dextrose agar (Merck, Germany) at 20°C for 10 days under natural light. Conidia were dislodged and harvested by flooding the cultures with 5 ml of sterile distilled water (SDW) and the suspensions passed through four

Table 1 Fungal isolates used in the oat and wheat detached leaf assays

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*	Oats
Fl 2004/59	*F. langsethiae*	Oats
Fl 077/3	*F. langsethiae*	Oats
Fl 2004/171	*F. langsethiae*	Wheat
Fl 2001/69	*F. langsethiae*	Wheat
Fl 2004/170	*F. langsethiae*	Wheat

layers of sterile muslin cloth to remove mycelia. Concentrations of spore suspensions were determined using a haemocytometer (Weber Scientific International, UK) and adjusted to a working concentration of 5×10^5 conidia ml^{-1}.

Production of leaf material

Surface-sterilised oat and wheat seeds were sown in sterilised John Innes Compost No. 2 and placed in a growth cabinet (Sanyo Versatile Environmental Test Chamber, Japan) at 20°C with a 12 h photoperiod. Seedlings were harvested after 14 days and 4 cm length segments were cut from the tip of the primary seedling leaf for use in the experiments.

Experiment 1: Pathogenicity and aggressiveness using a composite inoculum of each *Fusarium* and *Microdochium* species on wounded detached leaves

A composite inoculum of each fungal species consisting of three isolates (except *M. nivale* which consisted only of two isolates as one isolate failed to sporulate) for this experiment was used (5×10^5 spores ml^{-1}). Harvested leaf segments of oats cv. Gerald and wheat cv. Claire were injured at the centre of the adaxial surface using a sterile 10 μl micropipette tip and placed (adaxial surface facing up) on the surface of 0.5% water agar amended with kinetin (10 mg l^{-1}). Four leaf segments were placed in each Petri dish which constituted one replicate. A 5 μl conidial suspension was placed on each wound. Control leaves were treated with 5 μl SDW. Each treatment was replicated four times and the Petri dishes arranged in a randomised block design in a growth cabinet (Sanyo Versatile Environmental Test Chamber, Japan) at either 10°C or 20°C with a 12 h photoperiod.

Experiment 2: Pathogenicity and aggressiveness using a composite inoculum of each *Fusarium* and *Microdochium* species on unwounded detached leaves

Experiment two was carried out as detailed for experiment 1 except that leaves were not injured and a 10 μl conidial suspension (10 μl SDW for controls) was used as inoculum.

Experiment 3: Pathogenicity and aggressiveness of oat- and wheat-derived separate single-spore isolates of *F. langsethiae* on wounded detached leaves

Experiment 3 was carried out as detailed for experiment 1 but single-spore isolates of *F. langsethiae* were used instead of a composite inoculum on three oat cultivars (Gerald, Kinross and Millennium) and three wheat cultivars (Claire, Ambrosia and Malacca). This experiment was performed at 20°C with each treatment replicated twice.

Experiment 4: Pathogenicity and aggressiveness of oat- and wheat-derived separate single-spore isolates of *F. langsethiae* on unwounded detached leaves

Experiment 4 was carried out as detailed for experiment 2 using oat and wheat isolates of *F. langsethiae* singly instead of a composite inoculum on three oat and three wheat cultivars as detailed for experiment 3. This experiment was performed at 20°C with each treatment replicated twice.

Disease assessment

Disease development and fungal species aggressiveness on injured and uninjured leaf segments was measured by taking lesion lengths (mm) 6 and 7 days post-inoculation respectively for wounded and un-

wounded detached leaf assays. Lesion length was measured as a water-soaked necrotic and/or chlorotic area clearly visible by placing the Petri dishes over a light box.

Statistical analysis

Lesion length data from the experiments was subjected to analysis of variance (ANOVA) using GenStat (Release 8.1, Rothamsted Experimental Station, UK). Experiments 2 and 4 (unwounded detached leaves) lesion length data were transformed ($\log_{10}(x+1)$) to stabilise the variance. For multiple mean comparisons, Student–Newman–Keuls' tests were performed.

Results

Experiment 1: Pathogenicity and aggressiveness on wounded detached leaves using a composite inoculum of each *Fusarium* and *Microdochium* species

All fungi caused lesions on detached wounded leaves of oats and wheat by the sixth day post-inoculation at 10°C and 20°C. No lesions were observed on control leaves. Lesions, almost oval in shape, appeared as dark-brownish water-soaked patches characterised by necrosis and chlorosis with more necrosis on oat leaves and more chlorosis on wheat leaves at both 10°C and 20°C. Generally, lesion lengths were greater at 20°C than 10°C. Representative lesions observed at 20°C are shown in Fig. 1. Oat and wheat isolates of *F. langsethiae* appeared to cause a substantial amount of necrosis compared to the other fungi, especially on oats (Fig. 1). They produced similar disease symptoms on both oat and wheat leaves at each of the two temperatures. Their lesion characteristics were distinct from those of other pathogens on both oat and wheat in that they caused a well defined dark necrotic ring around the wound which had more defined edges on oat leaves than wheat leaves.

The fungus/host interaction for lesion length at 10°C was significant ($P=0.002$; Table 2) and highly significant at 20°C ($P<0.001$; Table 3). There was no significant difference in lesion length caused by composite inoculum of wheat and oat isolates of *F. langsethiae* on either host or at either temperature. *Fusarium langsethiae* isolates resulted in the longest lesions on oat leaves and these lesions were significantly longer than those caused by this species on wheat leaves at 10°C and 20°C (Tables 2 and 3). Other fungi were equally aggressive to oat and wheat leaves at 10°C. At 20°C, *M. nivale* caused the longest lesions on wheat leaves and these were significantly longer than the lesions caused by the same species on oat leaves (Table 3). Overall, *M. majus* was the least aggressive.

Experiment 2: Pathogenicity and aggressiveness on unwounded detached leaves using a composite inoculum of each *Fusarium* and *Microdochium* species

No disease developed on control leaves treated with SDW. It was also observed that both oat and

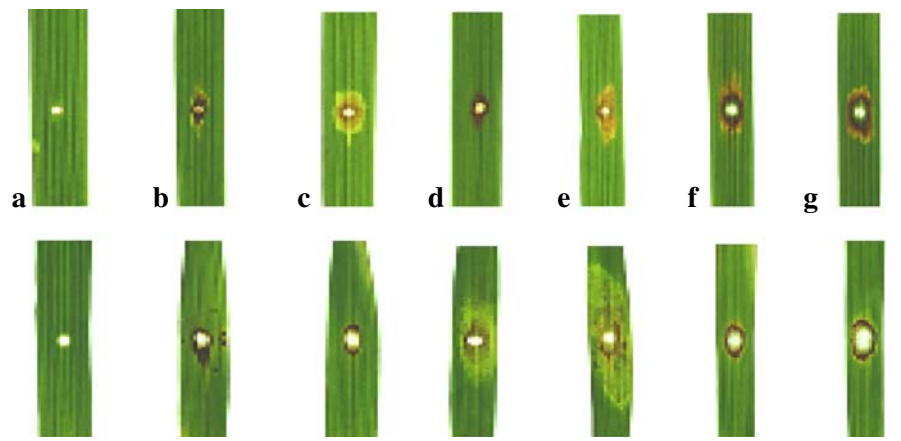

Fig. 1 Wounded detached leaves 6 days post-inoculation at 20°C; *a*, *b*, *c*, *d*, *e*, *f* and *g* are oat leaves treated with SDW, *F. culmorum*, *F. poae*, *M. majus*, *M. nivale*, *F. langsethiae* (oat isolates) and *F. langsethiae* (wheat isolates), respectively. Corresponding wheat leaves are below. Bar = 20 mm

Table 2 Lesion length (mm) on detached oat and wheat leaves artificially inoculated on an inflicted wound with *Fusarium* and *Microdochium* spp. and incubated at 10°C for 6 days

Fungus	Cereal	
	Oats	Wheat
Fusarium culmorum	1.66a	2.34ab
Fusarium poae	3.16b	2.50ab
Microdochium majus	1.63a	1.44a
Microdochium nivale	2.41ab	2.84ab
Fusarium langsethiae (oat isolates)	4.44c	2.81ab
Fusarium langsethiae (wheat isolates)	4.16c	2.50ab
P-value	0.002	
SEM (*df*=33)	0.323	
%CV	24	

Values are means of four replicates. Values with the same letter are not statistically different based on the Student–Newman–Keuls test ($P<0.05$)

wheat isolates of *F. langsethiae* did not cause any visible symptoms on wheat leaves at 10°C and 20°C. Lesion characteristics for each fungus on each host were similar at 10°C and 20°C although they were more distinct at the higher temperature. Representative lesions observed at 20°C are shown in Fig. 2. Lesions were characterised by necrosis and chlorosis. More necrosis than chlorosis was observed on oat leaves than wheat leaves for all fungi causing lesions in the detached leaf assay. Oat and wheat isolates of *F. langsethiae* produced almost entirely necrotic lesions on oat leaves while *M. nivale* produced extensive chlorotic lesions on wheat leaves. A non-extensive tuft of mycelia produced by *M. nivale* was visible on oat leaves.

The interaction between fungus and host for lesion length was highly significant ($P<0.001$) at 10°C and 20°C (Tables 4 and 5). In general, lesion lengths were greater at 20°C compared with 10°C. All fungi caused similar disease in terms of lesion length on oat leaves at 10°C and 20°C. There was no significant difference between lesion lengths caused by composite inoculum of wheat and oat isolates of *F. langsethiae* on either host or at either temperature. No isolates of *F. langsethiae* produced visible lesions on wheat leaves at 10°C or 20°C. *Microdochium nivale* was the most aggressive pathogen, producing the longest lesions, on wheat leaves at both temperatures.

Experiment 3: Pathogenicity and aggressiveness of separate single-spore isolates of *F. langsethiae* on wounded detached leaf assay

No lesions developed on the control leaf segments inoculated with SDW. All oat and wheat isolates of *F. langsethiae* caused lesions on wounded detached leaves of all oat and wheat cultivars. The lesion characteristics developed by individual *F. langsethiae* isolates were similar to those resulting from the composite inoculum used in experiment 1 (Fig. 1).

The cereal cultivar/fungal isolate interaction was not significant ($P=0.873$). A highly significant ($P<0.001$) difference between *F. langsethiae* isolates was observed. Two oat and one wheat isolate (041/11, 077/3, 2004/170) caused lesions of similar length statistically but significantly ($P<0.05$) longer than those caused by the other isolates (2004/171, 2001/69, 2004/59), which produced lesions of similar length (Table 6). A highly significant ($P<0.001$) difference between cereal cultivars was also evident. Among the six cereal cultivars (three wheat and three oat), wheat cv. Malacca developed the shortest lesions while oat cv. Gerald developed significantly longer lesions than the other cultivars. All wheat cultivars differed significantly in lesion length from each other. Lesion lengths among the wheat cultivars decreased in the following order; Ambrosia, Claire and Malacca. Among the oat cultivars, Kinross and Millennium developed lesions of similar length which were

Table 3 Lesion length (mm) on detached oat and wheat leaves artificially inoculated on an inflicted wound with *Fusarium* and *Microdochium* spp. and incubated at 20°C for 6 days

Fungus	Cereal	
	Oats	Wheat
Fusarium culmorum	4.59b	5.03b
Fusarium poae	4.84b	3.31ab
Microdochium majus	2.00a	3.88b
Microdochium nivale	4.72b	8.47d
Fusarium langsethiae (oat isolates)	7.69cd	3.66b
Fusarium langsethiae (wheat isolates)	6.59c	4.34b
P-value	<0.001	
SEM (*df*=33)	0.459	
%CV	19	

Values are means of four replicates. Values with the same letter are not statistically different based on the Student-Newman-Keuls test ($P<0.05$).

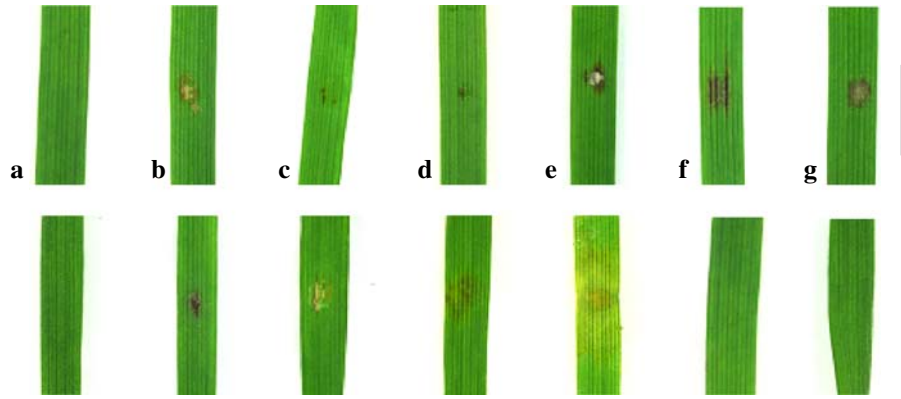

Fig. 2 Unwounded detached leaves 7 days post-inoculation at 20°C; *a*, *b*, *c*, *d*, *e*, *f* and *g* are oat leaves treated with SDW, *F. culmorum*, *F. poae*, *M. majus*, *M. nivale*, *F. langsethiae* (oat isolates) and *F. langsethiae* (wheat isolates), respectively. Corresponding wheat leaves are below. Bar = 20 mm

significantly shorter than those which developed on Gerald.

Experiment 4: Pathogenicity and aggressiveness of separate single-spore isolates of *F. langsethiae* on unwounded detached leaf assay

As observed in the previous three experiments reported in this study, no lesions developed on the control leaves treated with SDW. In addition to this observation, all isolates of *F. langsethiae* did not cause any lesions on unwounded detached leaves of any wheat cultivar but they were found to cause lesions on all oat cultivars tested. Lesions caused by all isolates on all oat leaves were similar in characteristics. They resembled those reported in experiment 2 where these isolates were used as composite inoculum (Fig. 2).

Due to the absence of lesions on unwounded wheat leaves only oat cultivars were included in the ANOVA. There was no significant variety/isolate interaction (P=0.38). There was a significant difference (P=0.014) between lesion lengths caused by each isolate (Table 7). One oat isolate (014/11) produced significantly (P<0.05) longer lesions than

Table 4 Transformed lesion length ($\log_{10}$ (x+1)mm)) on detached oat and wheat leaves artificially inoculated with *Fusarium* and *Microdochium* spp. and incubated at 10°C for 7 days

Fungus	Cereal	
	Oats	Wheat
Fusarium culmorum	0.42 (1.62)bc	0.31 (1.03)b
Fusarium poae	0.56 (2.64)c	0.37 (1.36)b
Microdochium majus	0.34 (1.20)b	0.34 (1.18)b
Microdochium nivale	0.59 (2.86)c	0.94 (7.79)d
Fusarium langsethiae (oat isolates)	0.48 (2.04)bc	0.00 (0.00)a
Fusarium langsethiae (wheat isolates)	0.60 (3.03)c	0.00 (0.00)a
P-value	<0.001	
SEM (*df*=33)	0.049	
%CV	24	

Values are means of four replicates (back-transformed values in parenthesis). Values with the same letter are not statistically different based on the Student–Newman–Keuls test (P<0.05)

Table 5 Transformed lesion length ($\log_{10}$ (x+1)mm)) on detached oat and wheat leaves artificially inoculated with *Fusarium* and *Microdochium* spp. and incubated at 20°C for 7 days

Fungus	Cereal	
	Oats	Wheat
Fusarium culmorum	0.56 (2.64)b	0.27 (0.85)a
Fusarium poae	0.52 (2.30)b	0.21 (0.62)a
Microdochium majus	0.58 (2.77)b	0.61 (3.12)b
Microdochium nivale	0.71 (4.08)b	1.21 (15.40)c
Fusarium langsethiae (oat isolates)	0.78 (5.02)b	0.00 (0.00)a
Fusarium langsethiae (wheat isolates)	0.70 (4.01)b	0.00 (0.00)a
P-value	<0.001	
SEM (*df*=33)	0.070	
%CV	28	

Values are means of four replicates (back-transformed values in parenthesis). Values with the same letter are not statistically different based on the Student–Newman–Keuls test (P<0.05)

Table 6 Lesion lengths (mm) caused by *F. langsethiae* isolates on wounded detached leaves of three oat and three wheat cultivars incubated at 20°C for 6 days

Cereal cultivar[a]	*F. langsethiae* isolate[b]						Factor mean
	041/11 (o)	077/3 (o)	2004/59 (o)	2004/171 (w)	2001/69 (w)	2004/170 (w)	
Ambrosia (W)	3.00	3.25	2.50	2.12	2.50	2.94	2.72cd
Claire (W)	2.56	2.87	2.00	1.25	1.31	2.75	2.12b
Malacca (W)	2.00	2.25	1.12	1.00	1.00	2.00	1.56a
Gerald (O)	3.37	4.12	2.56	2.69	2.50	3.25	3.08d
Kinross (O)	3.00	3.12	2.25	2.00	2.12	2.87	2.56c
Millennium (O)	2.87	3.00	2.00	1.81	2.12	2.50	2.38bc
Factor mean	2.80b	3.10b	2.07a	1.81a	1.93a	2.72b	

P-value: cereal cultivar <0.001, isolate <0.001; SEM (*df*=35): cereal cultivar 0.095, isolate 0.095; %CV=14; Factor means with the same letter are not statistically different based on the Student–Newman–Keuls test ($P<0.05$)

[a] Cereal: (W), wheat; (O), oats

[b] Isolated from: (w), wheat; (o), oats

two wheat isolates (2004/171, 2001/69). There were no significant differences between oat cultivars ($P=0.07$) although the trend was the same as in experiment 3 with average lesion length for each cultivar decreasing in the following order; Gerald, Kinross and Millennium.

Discussion

In experiments 1 and 3 of this study, wounds were inflicted on detached leaves; this would have allowed fungi unable to penetrate the cuticle during natural infection to overcome this barrier. Successful infection and disease development by pathogenic fungi with or without wounding is manifested through appearance of symptoms such as discoloured, malformed, necrotic or chlorotic areas on the affected plant part.

All fungal isolates used as composite inoculum or singly in experiments 1 and 3 of this study, where leaves were wounded, were pathogenic but differed from one another in their aggressiveness (measured by lesion lengths at fixed times) towards detached oat and wheat leaves. However, in experiment 2 where leaves were unwounded, oat and wheat isolates of *F. langsethiae* used as composite inoculum were only pathogenic to oat leaves whereas all other fungi were pathogenic to both hosts. The degree of host damage, as in the first and third experiments, varied between fungal species or isolates. Experiments 3 and 4 successfully demonstrated that all *F. langsethiae* isolates used as composite inoculum in the previous two experiments, when used singly, were indeed pathogenic to wounded oats and wheat leaves and only on oat leaves in the unwounded assay. These two experiments also showed that variability in aggressiveness between isolates of *F. langsethiae* does exist

Table 7 Transformed lesion lengths ($\log_{10}(x+1)$ mm)) caused by *F. langsethiae* isolates on unwounded detached leaves of three oat cultivars incubated at 20°C for 7 days (back-transformed values in parenthesis)

Oat cultivar	*F. langsethiae* isolate[a]						Factor mean
	041/11 (o)	077/3 (o)	2004/59 (o)	2004/171 (w)	2001/69 (w)	2004/170 (w)	
Gerald	0.92 (7.3)	0.69 (3.9)	0.80 (5.3)	0.56 (2.6)	0.61 (3.1)	0.75 (4.6)	0.72 (4.3)
Kinross	0.76 (4.8)	0.74 (4.5)	0.73 (4.3)	0.71 (4.1)	0.53 (2.4)	0.66 (3.6)	0.69 (3.9)
Millennium	0.74 (4.4)	0.65 (3.5)	0.57 (2.7)	0.55 (2.5)	0.63 (3.2)	0.62 (3.2)	0.63 (3.2)
Factor mean	0.80 (5.4)b	0.69 (3.9)ab	0.70 (4.0)ab	0.60 (3.0)a	0.59 (2.9)a	0.68 (3.8)ab	

P-value: cereal cultivar 0.070, isolate 0.014; SEM (*df*=17): cereal cultivar 0.039; %CV=14; Factor means with the same letter are not statistically different based on the Student–Newman–Keuls test ($P<0.05$)

[a] Isolated from (w) wheat, (o) oats

but this does not appear to depend on the host crop (oats and wheat) from which they were originally isolated. There was more necrosis on lesions developed on oat leaves than those developed on wheat leaves, which had more chlorosis in all experiments irrespective of the temperature employed. These results agree with those of Browne and Cooke (2005) who found that *M. majus* caused wheat lesions that differed from oat lesions, with wheat lesions being accompanied by chlorosis of leaf tissue. This may imply that death of cells and living tissue occurs at a faster rate in oat leaves than wheat leaves irrespective of the fungal pathogen involved, as necrosis occurs as a result of cell death. Since *F. langsethiae* infection resulted in more necrosis than any other fungal pathogen in this study, it may be inferred that this pathogen causes more cell death on wounded oat and wheat leaves but only in unwounded oat leaves under the study conditions employed.

A number of abiotic factors including temperature have been found to influence fungal pathogenicity and aggressiveness. In this study, lesion lengths varied from one fungal pathogen to another at all temperatures employed in experiments 1 and 2, being greatest at 20°C for all the pathogens irrespective of the cereal host and whether or not the leaves were damaged. These results agree with those of Browne and Cooke (2004) who demonstrated that *M. nivale* isolates caused greatest lesion length at 20°C compared with 10°C, 15°C and 25°C. Only one temperature (20°C) was employed in experiments 3 and 4 as the earlier experiments had shown that lesion characteristics, where they developed, were the same for each species at both 10°C and 20°C but only differed in size. In experiment 1, *F. langsethiae* was the most aggressive on oats and moderately aggressive on wheat. There was no significant difference observed in aggressiveness between composite inoculum of *F. langsethiae* isolated from oats and wheat at either temperature in experiments 1 and 2, implying that they are probably from the same population of *F. langsethiae*, but further genetic analysis of a wider range of isolates would be required to demonstrate this with certainty. Experiments 3 and 4 demonstrated that variability in aggressiveness occurred among isolates of *F. langsethiae* although no clear trend was observed in aggressiveness of these isolates depending on the source from which they were isolated (oats or wheat).

Contrary to other research (Diamond and Cooke 1999; Browne and Cooke 2004), *M. nivale* was found to be more aggressive than *M. majus* in experiments 1 and 2. The difference in these results may be due to the contrasting isolates and host cultivars used in this study and previous work.

Many *Fusarium* species and strains have been reported to produce different phytotoxic secondary metabolites including the mycotoxins HT-2 and T-2, which have been found to cause among other symptoms, necrosis and chlorosis in plant tissues (Zonno and Vurro 2002). HT-2 and T-2 have been shown to be phytotoxic to wheat seedlings where they have been demonstrated to retard coleoptile elongation which was correlated with head blight susceptibility in wheat (Eudes et al. 1998). The same mycotoxins have been shown to reduce seed germination of branched broomrape (*Orobanche ramosa*) by up to 100% (Zonno and Vurro 2002), cause death of protoplasts in tomato (Paciolla et al. 2004) and reduce mitotic activity in onion (Rahman et al. 1993). This may explain why *F. langsethiae* caused more necrosis than any other pathogen in this study as it is known to be a potent producer of HT-2 and T-2 mycotoxins (Torp and Nirenberg 2004). Snijders and Krechting (1992) have reported that fungal toxins can play some part in the aggressiveness of the fungal pathogen interfering with plant defence mechanisms. Therefore, greater lesion lengths and the necrosis, particularly on oat leaves, by *F. langsethiae* may partly or wholly have been due to these mycotoxins.

In the first two experiments Gerald and Claire were used as these were the two most popular winter oat and wheat cultivars in the UK at the time of the study. When these experiments highlighted differences between these two cereals it was necessary to determine if this difference was consistent across a number of wheat and oat cultivars. The three wheat cultivars used in subsequent experiments were selected based on the range of head blight resistance available in UK cultivars. All UK cultivars are screened for head blight resistance using *F. graminearum* and *F. culmorum*-inoculated experiments. Claire, Malacca and Ambrosia have a resistance rating of 7, 5 and 3 respectively on a scale of 1–9, 9 being most resistant (Anon 2008). There are currently no head blight resistance ratings for oat cultivars although HT-2 and T-2 content has been quantified from winter oat cultivar trials. Gerald, Kinross and Millennium were

selected as they represented cultivars with high, moderate and low HT-2 and T-2 concentrations, respectively, within these trials (Edwards 2007a). Lesion sizes on wheat leaves were not consistent with wheat cultivar resistance scores in the wounded detached leaf assay, in that Claire with a FHB resistance rating of 7 developed longer lesions than Malacca, which has a resistance rating of 5. However, the trends in lesion lengths obtained from this study for the three oat cultivars were consistent with their reported differences in HT-2 and T-2 accumulation (Edwards 2007a).

Screening for disease resistance in the field has been found to be time-consuming and is influenced by climatic conditions making interpretation of data difficult (Bruehl 1967). These assays, which require only 21 days from seed sowing to obtaining results, could be of potential use in evaluating the susceptibility of different oat cultivars to *F. langsethiae.* Previous studies have shown detached leaf assays can predict whole plant disease responses to pathogens (Deadman and Cooke 1986; Arraiano et al. 2001). *Fusarium langsethiae* may not cause any disease symptoms on infected oats panicles and wheat ears, as observed during glasshouse and field inoculation studies (unpublished work); therefore, these assays could be useful in predicting the susceptibility of oat cultivars to infection by this fungal species.

The results from the detached leaf assays suggest that *F. langsethiae* is a pathogen of oats and wheat. Results from experiment 2 and 4 would indicate that *F. langsethiae* could be a greater problem on oats than on wheat which agrees with HT-2 and T-2 concentrations found in commercial crops of wheat and oats in the UK (Edwards 2007a, b). However, it must be remembered that other plant characteristics, such as the large morphological differences in oat and wheat head structure, will also have a major impact on their susceptibility to head blight pathogens.

The lack of symptoms on heads of cereals would indicate that either head blight resistance results in superficial saprophytic growth of *F. langsethiae* or that symptom expression does not occur, resulting in cryptic infection in the field. Clear symptoms in the detached leaf assay may correlate to cultivar partial resistance in oats to *F. langsethiae*. This would allow a rapid screening of potential breeding material and progeny of oats which would reduce the incidence of mycotoxins in raw and processed oats. More studies with increased number of oat cultivars are required to confirm that cultivar PDR of oats to *F. langsethiae* is correlated to lesion size in an *in vitro* assay. Development of a quantitative PCR assay for *F. langsethiae* would facilitate further studies on the infection of cereals by this species. Further work investigating the role of HT-2 and T-2 during *F. langsethiae* infection is required as trichothecenes have been reported as virulence factors in the development of FHB by other *Fusarium* species.

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