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# A comparative assessment of potential components of partial disease resistance to *Fusarium* head blight using a detached leaf assay of wheat, barley and oats

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**Abstract**

The relative resistance of 15 winter barley, three winter wheat and three winter oat cultivars on the UK recommended list 2003 and two spring wheat cultivars on the Irish 2003 recommended list were evaluated using *Microdochium nivale* in detached leaf assays to further understand components of partial disease resistance (PDR) and *Fusarium* head blight (FHB) resistance across cereal species. Barley cultivars showed incubation periods comparable to, and latent periods longer than the most FHB resistant Irish and UK wheat cultivars evaluated. In addition, lesions on barley differed from those on wheat as they were not visibly chlorotic when placed over a light box until sporulation occurred, in contrast to wheat cultivars where chlorosis of the infected area occurred when lesions first developed. The pattern of delayed chlorosis of the infected leaf tissue and longer latent periods indicate that resistances are expressed in barley after the incubation period is observed, and that these temporarily arrest the development of mycelium and sporulation. Incubation periods were longer for oats compared to barley or wheat cultivars. However, oat cultivars differed from both wheat and barley in that mycelial growth was observed before obvious tissue damage was detected under macroscopic examination, indicating tolerance of infection rather than inhibition of pathogen development, and morphology of sporodochia differed, appearing less well developed and being much less abundant. Longer latent periods have previously been related to greater FHB resistance in wheat. The present results suggest the longer latent periods of barley and oat cultivars, than wheat, are likely to play a role in overall FHB resistance if under the same genetic control as PDR components expressed in the head. However the limited range of incubation and latent periods observed within barley and oat cultivars evaluated was in contrast with wheat where incubation and latent periods were shorter and more variable among genotypes. The significance of the various combinations of PDR components detected in the detached leaf assay as components of FHB resistance in each crop requires further investigation, particularly with regard to the apparent tolerance of infection in oats and necrosis in barley, after the incubation period is observed, associated with retardation of mycelial growth and sporulation.

## Introduction

*Fusarium* head blight (FHB) is one of the most serious fungal diseases in cereal production although most research has focused on wheat and barley with oats receiving less attention. There is no complete resistance to FHB although wheat and barley genotypes have been identified with partial resistance. At present there is no strong evidence for species-specific resistance to FHB, associated with at least 17 causal organisms, in wheat (Parry et al., 1995) or barley (Steffenson, 2003). In the cooler maritime regions of northwest Europe *Fusarium culmorum* predominates and *Microdochium nivale* assumes a greater importance in the FHB disease complex. *Microdochium nivale*

is used in the detached leaf assay as it causes distinct leaf symptoms for observing partial disease resistance (PDR) components compared to other fungal species implicated in the FHB complex (Diamond and Cooke, 1999; Browne and Cooke, 2004b). There is no strong evidence for species-specific resistance in wheat to any of the fungal species implicated in the FHB complex, including *M. nivale* (Parry et al., 1995; Diamond and Cooke, 1999). High correlations were found between *Fusarium* spp. and *M. nivale* in germination rate using a seed germination assay, inoculated with conidial suspensions of *F. culmorum*, *F. graminearum*, *F. avenaceum* and *M. nivale* (Browne and Cooke, 2005) and between components of PDR detected in the detached leaf assay in wheat using *M. nivale* and whole plant FHB resistance against *F. culmorum* (Diamond and Cooke, 1999; Browne and Cooke, 2004b) and *Fusarium graminearum* (Browne et al., 2005).

Despite a lack of strong evidence for species-specific resistance, differences in host preference have been reported for *M. nivale* (Diamond and Cooke, 1997a; Simpson et al., 2000), which is differentiated into var. *majus* and var. *nivale* based on conidial morphology (Wollenweber, 1931; Gams and Muller, 1980). The majority of isolates from wheat and barley seed have been found to be *M. nivale* var. *majus* (Parry et al., 1995; Diamond and Cooke, 1997a) although a higher proportion of *M. nivale* var. *nivale* isolates were obtained from barley than from wheat (Diamond and Cooke, 1997a). *Microdochium nivale* var. *nivale* was predominantly isolated from oats (Diamond and Cooke, 1997a). However, *M. nivale* var. *majus* and var. *nivale* are able to cross-infect between different cereal hosts (wheat, barley and oats) irrespective of their original host (Diamond and Cooke, 1997a). It is unclear as to why differences in host preference are found although *M. nivale* var. *majus* is more pathogenic to wheat than var. *nivale* in detached leaf assays (Diamond and Cooke, 1997a, 1999; Browne and Cooke 2004b) and in a seed germination assay (Browne and Cooke, 2005) while *M. nivale* var. *nivale* has been reported to cause greater disease to the stem-base of oat seedlings than var. *majus* (Simpson et al., 2000). Differences among individual isolates of *Fusarium* spp. for pathogenicity to plant growth stages in wheat (crown rot and FHB) has also been found (Akinsanmi et al., 2004).

In European wheat, FHB resistance was most strongly correlated to the PDR component latent period (time from inoculation to sporulation) in the detached leaf assay and to a lesser extent incubation period (time from inoculation to first symptoms of damage to the leaf surface under macroscopic observation) (Diamond and Cooke, 1999; Browne and Cooke 2004b); this pattern of relatively long incubation and latent periods was also found in moderately FHB resistant US cultivars (Browne et al., 2005). However, where exotic sources of resistance have been incorporated, longer incubation periods have been correlated with increased FHB susceptibility (Browne and Cooke, 2004b; Browne et al., 2005). It is hypothesised that the contrasting relationship between FHB resistance and incubation period may reflect the different genetic control of the highly effective FHB resistance found in exotic germplasm and the more moderate FHB resistance in commercially grown cultivars (Browne and Cooke, 2004b; Browne et al., 2005). It is notable that the most resistant line in the US 2002 southern FHB screening nursery had the unusual combination of the shortest incubation period observed in wheat to date and amongst the longest latent period suggesting that such a combination may be desirable for FHB resistance (Browne et al., 2005).

Although PDR components detected in the detached leaf assay identify an important component of the moderate FHB resistance found in commercially grown wheat (Diamond and Cooke, 1999; Browne and Cooke, 2004b; Browne et al., 2005), resistances, of lesser effect, detected in a seed germination assay and independent of the PDR components detected in the detached leaf assay have also been identified (Browne and Cooke, 2005) as have morphological and developmental characteristics related to disease avoidance/escape (Gervais et al., 2003). Much of the highly effective resistances in exotic wheat germplasm do not appear to be detected in the detached leaf (Browne and Cooke, 2004b; Browne et al., 2005) or seed germination assays (Browne and Cooke, 2005).

The relative susceptibility of wheat, barley and oats to FHB is unclear and resistance mechanisms and potential susceptibility factors across these crops are poorly understood. Nevertheless a number of authors have used comparative assessments between cereal crops in order to facilitate

improving the limited understanding of FHB resistance among cereal species (Liu et al., 1997; Langevin et al., 2004). The aims of the research reported here were to comparatively assess the PDR components detectable in a range of commercial cultivars of wheat, barley and oats using the *M. nivale* detached leaf assay.

## Materials and methods

Cultivars of wheat, barley and oats used in this study (Table 1) were selected from those on the 2003 UK recommended list for cereals. In addition, two spring wheat cultivars on the 2003 Irish recommended list, Alexandria and Raffles, and the FHB resistant wheat genotype Frontana with known resistances from the detached leaf assay (Browne and Cooke, 2004b) were included. The cultivars were grown in a controlled environment chamber and the first leaf harvested on day 14; 4 cm sections were placed on the surface of 0.5% water agar (four leaves per Petri dish) containing 10 mg $l^{-1}$ kinetin as a senescence retarder, as described by Browne and Cooke (2004b). Single-spore isolates of *M. nivale* var. *majus* (DoA1/M, DoA2/M, DoA3/M, DoA12/M and Dard1/M) isolated from wheat seed from the Irish 2001 harvest, pre-screened for pathogenicity to detached leaves of wheat, were used in this study. In addition, a further isolate of *M. nivale* var. *majus* (M7B) and three isolates of *M. nivale* var. *nivale* (44/S/N, SO28/2/N and SO48/1/N) from wheat (obtained courtesy of Dr. Josephine Brennan, University College Dublin, Ireland and Dr. Simon Edwards, Harper Adams University College, UK, respectively) were used. Mycelium-free conidial inoculum of *M. nivale* was produced on potato dextrose agar covered in cellophane (CPDA) (Browne and Cooke, 2004a) and incubated on cool plates (Cooke, 1980) for 7 days under a diurnal cycle of near-ultraviolet (NUV) and white light. Leaf segments were inoculated at the centre of the adaxial surface with a 10 $\mu$l droplet of *M. nivale* spore suspension adjusted to $1 \times 10^6$ conidia $ml^{-1}$. The detached leaves were then incubated at either 10 or 15 °C under a 24 h diurnal cycle of NUV and white light (12 h NUV and white light, 12 h dark).

*Table 1.* Commercial cultivars of wheat, barley and oats evaluated in the detached leaf assay in Experiment 1[a], Experiment 2[b] and Experiment 3[c]

| Winter barley | Winter wheat | Winter oats |
|---|---|---|
| Angela[a,b,c] | Biscay[a,b,c] | Gerald[b,c] |
| Antonia[a,b,c] | Claire[a,b,c] | Jalna[b,c] |
| Haka[a,b,c] | Frontana[a,b] | Millenium[b,c] |
| Heligan[a,b] | Solstice[a,b,c] | |
| Jewel[a,b] | | |
| Leonie[a,b] | Spring wheat | |
| Pastoral[a,b] | | |
| Pearl[a,b,c] | Alexandria[1,2] | |
| Pict[a,b] | Raffles[a,b] | |
| Regina[a,b,c] | | |
| Scylla[a,b] | | |
| Siberia[a,b,c] | | |
| Static[a,b] | | |
| Sumo[a,b] | | |
| Vanessa[a,b] | | |

In experiment 1, barley and wheat cultivars (Table 1) were inoculated separately with five wheat isolates of *M. nivale* var. *majus* (DoA1/M, DoA2/M, DoA3/M, DoA12/M and M7B) using two replicates and incubated at 10 °C. In experiment 2, barley, wheat and oat cultivars (Table 1) were inoculated with the *M. nivale* var. *majus* wheat isolate Dard1/M, known to have high pathogenicity to detached wheat leaves, using five replicates and incubated at 10 °C. In experiment 3, barley cvs Angela, Antonia, Haka, Pearl, Regina and Siberia, wheat cvs Biscay and Claire and oat cvs Gerald, Jalna and Millenium (Table 1) were inoculated with *M. nivale* var. *majus* isolate Dard1/M and three isolates of *M. nivale* var. *nivale*, 44/S/N, SO28/2/N and SO48/1/N, using five replicates and incubated at 15 °C. In each experiment, each value was the mean of four observations (one Petri dish × four leaves) for each replicate. Assessments of symptom appearance and sporulation were carried out daily under a compound microscope (magnification ×40). The PDR components measured were: incubation period (days from inoculation to symptom development) and latent period (days from inoculation to sporulation) as described by Browne and Cooke (2004b). Chlorotic lesions were identified by placing the detached leaves over a light box.

### *Statistical analysis*

ANOVA were conducted using Genstat V software. The detached leaf assay experiments were

analysed as a randomised block design, with two blocks in experiment 1 and five blocks in experiments 2 and 3.

## Results

There were significant differences for incubation period among barley and wheat cultivars ($P < 0.001$) in experiment 1 (Figure 1) each bar representing the mean of five isolates. All barley cultivars showed incubation periods comparable to those of cvs Solstice, Biscay and Claire, and the genotype Frontana, and had significantly longer incubation periods than the susceptible wheat cvs Raffles and Alexandria. Variation in incubation periods among barley cultivars was less than that observed among the wheat cultivars. There was no significant isolate × cultivar interaction for incubation period (data not presented). While all isolates sporulated on detached leaves of wheat cultivars within 14 days after inoculation, no sporulation was observed on any of the barley cultivars at this time reflecting the large differences in the PDR component latent period between cereal species.

In experiment 2, (Figure 2) incubation periods were shorter than in experiment 1, consistent with a more pathogenic isolate, Dard1/M. Differences between oat, barley and wheat cultivars were highly significant ($P < 0.001$). Again the incubation periods of all barley cultivars were comparable to those observed for wheat cvs Solstice, Biscay and Claire and the wheat genotype Frontana. The incubation periods on oat cvs Gerald, Jalna and Millenium were significantly longer than those of the barley or wheat cultivars. However in addition to differences in the length of incubation period there were marked differences in the first appearance of damage to the leaf surface, used for defining incubation period, of wheat, barley and oats. On wheat, dull-grey green water-soaked lesions were present often extending outside the area of the initial inoculum droplet (Figure 3a). On barley, symptoms consisted of similar dull grey-green water-soaked lesions, but on leaves where symptoms were first observed, they were less extensive than those on wheat (Figure 3b). Oats differed from both barley and wheat as mycelial growth was observed on the leaf surface outside the inoculum droplet, but without apparent damage to the leaf under macroscopic examination (Figure 3c) indicating a tolerance to growth of the pathogen on the leaf surface rather than inhibition of pathogen development. Again, well before day 14, extensive sporulation had occurred on all wheat cultivars; however only sporadic sporulation occurred on barley cultivars and no sporulation was observed on oats by day 14 reflecting vary marked differences in the PDR component latent period between the three cereal species. Lesions observed on barley differed from those on wheat as although these were necrotic chlorosis of the underlying leaf tissue (visible over a light box), they frequently did not occur until sporulation.

The pathogenic *M. nivale* var. *majus* isolate Dard1/M and three *M. nivale* var. *nivale* isolates

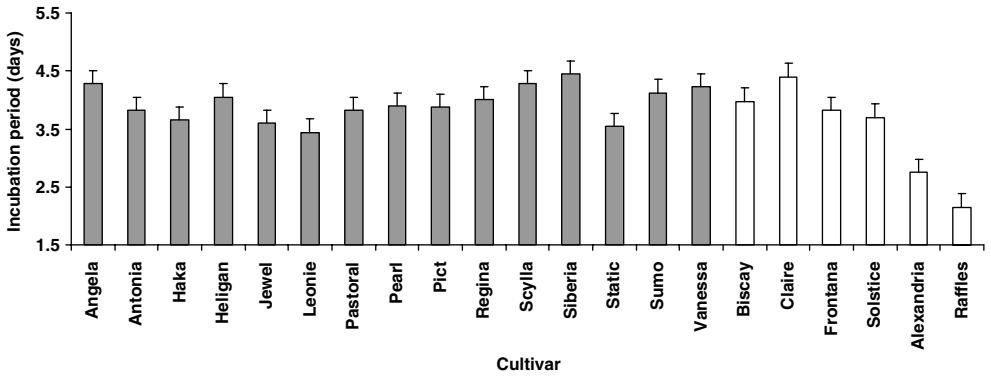


*Figure 1*. Experiment 1. Incubation periods of barley ■ and wheat □ cultivars inoculated with isolates of *M. nivale* var. *majus* on detached leaves incubated at 10 °C. Bars represent standard error of the mean.

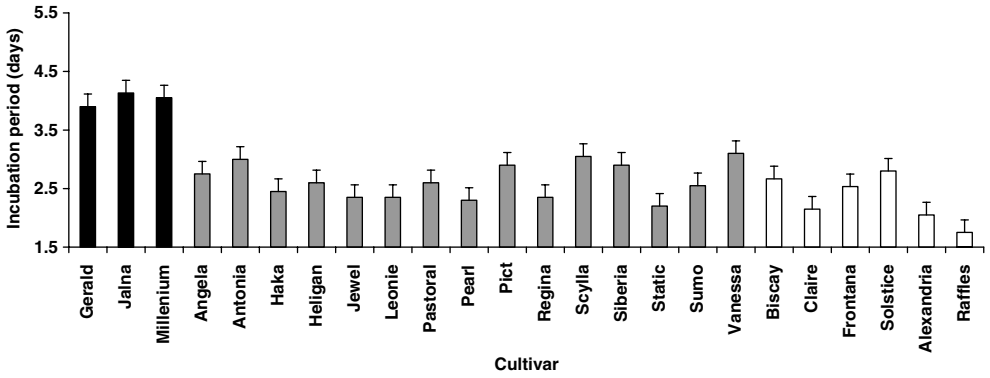


*Figure 2.* Experiment 2. Incubation periods of oat ■, barley ■ and wheat □ cultivars inoculated with *M. nivale* var. *majus* isolate Dard1/M on detached leaves incubated at 10 °C. Bars represent standard error of the mean.

44/S/N, SO28/2/N and SO48/1/N were used at a higher incubation temperature of 15 °C in experiment 3 (Figure 4). Incubation periods were longer for *M. nivale* var. *nivale* isolates than for the var. *majus* isolate across all cereal species ($P < 0.001$). As in experiments 1 and 2 all barley cultivars had incubation periods comparable to or longer than the wheat cvs Biscay and Claire ($P < 0.001$) (Figure 4a); oat cvs Gerald, Jalna and Millenium showed the longest incubation periods. There was a significant isolate × cultivar interaction ($P < 0.001$) with greater differences in incubation periods using the *M. nivale* var. *nivale* isolates than the more pathogenic var. *majus* isolate particularly between wheat cultivars and barley and oat cultivars. The correlation between incubation period for Dard1/M (*M. nivale* var. *majus*) and the three isolates of *M. nivale* var. *nivale* ranged from $r = 0.51$ (NS) to $r = 0.69$ ($P < 0.05$). However, the overall ranking for incubation periods of wheat, barley and oats were consistent across all isolates; therefore only mean effects of all isolates on wheat, barley and oats are presented (Figure 4a).

By day 4 at 15 °C (isolate Dard1/M) sporulation occurred on most wheat leaves with sporodochia forming a pattern along the rows of stomata on the adaxial leaf surface in and around the inoculum droplet and mycelium was observed growing over the leaf surface (Figure 3d). In barley, lesions extended beyond the inoculum droplet but were less extensive than on wheat, and growth of mycelium was not evident (Figure 3e). Symptom expression was not as extensive on oats as in wheat and barley, although as at 10 °C, mycelial growth was observed under macroscopic observation without obvious damage to the underlying leaf tissue (Figure 3f). Wheat differed from barley and oats in that lesions were consistently accompanied by chlorosis of the leaf tissue. This was most readily observed by placing the leaves over a light box and was generally not observed in barley until sporulation occurred. By day 10 after inoculation extensive mycelial growth from the infected leaf over the water agar surface was observed in wheat, incubated at 15 °C (Figure 5a). Mycelial growth was observed less frequently in barley and was less extensive where it did occur, as was leaf chlorosis and necrosis (Figure 5b). In oats necrotic lesions first occurred in sporadic isolated spots which were difficult to detect (Figure 5c) rather than in consolidated lesions, as occurred in wheat and barley, although mycelial growth from the infected leaf was quite extensive.

Differences in latent periods across oat, barley and wheat were highly significant ($P < 0.001$). Again oat cultivars had the longest latent periods, across all isolates, while those of both barley and oats were much longer than those observed on the wheat cultivars. There were significant differences between isolates of *M. nivale* ($P < 0.001$) with a significant cultivar × isolate interaction ($P < 0.001$) (Figure 4b). *Microdochium nivale* var. *nivale* isolates caused longer latent periods (as for incubation period) than var. *majus* on wheat cvs Biscay and Claire. However this pattern was not observed on oats and barley; despite longer incubation periods for *M. nivale* var. *nivale* isolates, latent periods (time to sporulation) were similar for *M. nivale* var. *majus* and var. *nivale* isolates (data not presented).

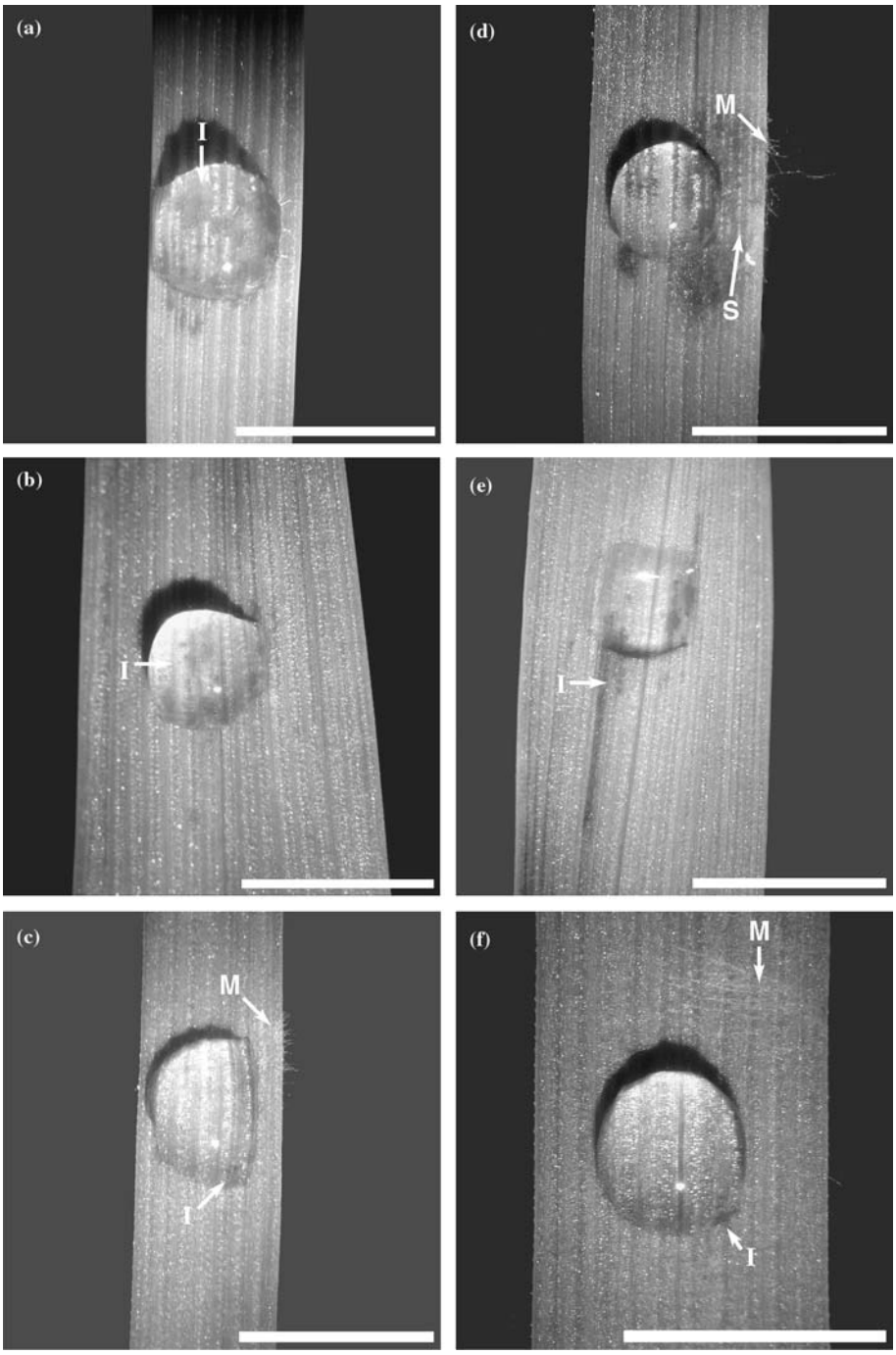


*Figure 3*. Detached leaves 4 days post-inoculation with *M*. *nivale* isolate Dard1/M. Experiment 2: (a) wheat cv. Biscay, (b) barley cv. Angela and (c) oat cv. Gerald incubated at 10 °C and Experiment 3: (d) wheat cv. Biscay, (e) barley cv. Antonia and (f) oat cv. Millenium incubated at 15 °C. Bars = 5 mm. Symptoms of initial infection I, mycelium M and sporodochia S.

Nevertheless the relative rankings of wheat, barley and oats were consistent across all isolates. The correlation between latent period for Dard1/M (*M*. *nivale* var. *majus*) and the three isolates of *M*. *nivale* var. *nivale* ranging from $r = 0.80$ ($P < 0.01$) to $r = 0.88$ ($P < 0.001$) were closer than those

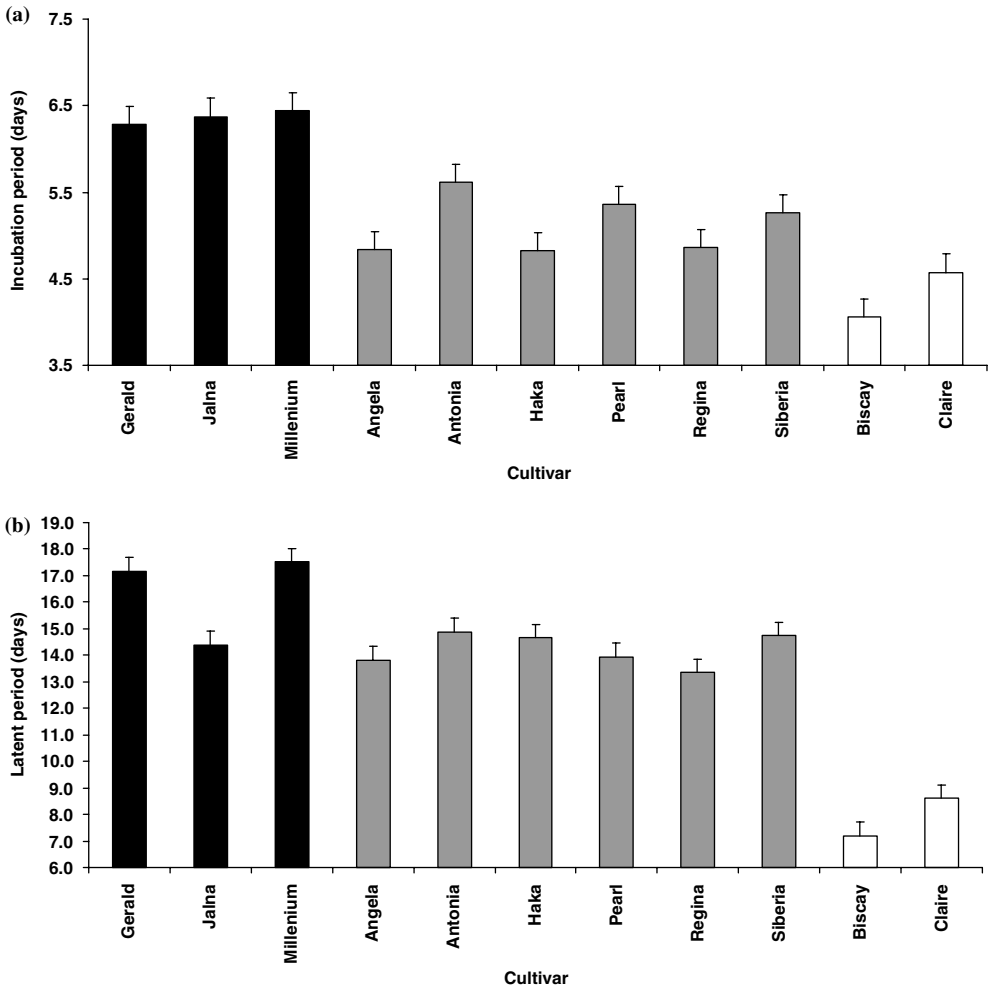


*Figure 4*. Experiment 3. Incubation period (a) and latent period (b), of oat ■, barley ■ and wheat □ cultivars inoculated with *M. nivale* var. *majus* isolate Dard1/M and *M. nivale* var. *nivale* isolates 44/S/N, SO28/2/N and SO48/1/N on detached leaves incubated at 15 °C. Bars represent standard error of the mean.

observed for incubation period. In a small number of detached leaves sporulation did not occur in barley although this was not consistently associated with any particular cultivar; these were excluded from the analysis. In wheat (Figure 5d) as in barley (Figure 5e) sporodochia were observed in lines between the veins above the stomata on the leaf surface. However in oats, the appearance and distribution of sporodochia differed from both wheat and barley; the sporodochia did not form patterns along the leaf surface where stomata were located and were much less abundant after the onset of sporulation (Figure 5f).

The relative latent period and extent of sporulation was also reflected in differences in the location where sporulation first occurred in wheat, barley and oats. On detached wheat leaves sporulation occurred most frequently in close proximity to the initial inoculum droplet, at the mid-point of the leaf (Figure 3d); however, in barley sporulation was first observed most frequently at the cut ends of the detached leaves where necrosis also occurred, although the leaf tissue between the inoculum droplet and the leaf ends appeared relatively healthy (Figure 6a). In oats a more general chlorosis of the leaf was observed where mycelium was present (Figure 6b) and necrosis and sporulation were more restricted to the cut ends of the leaves.

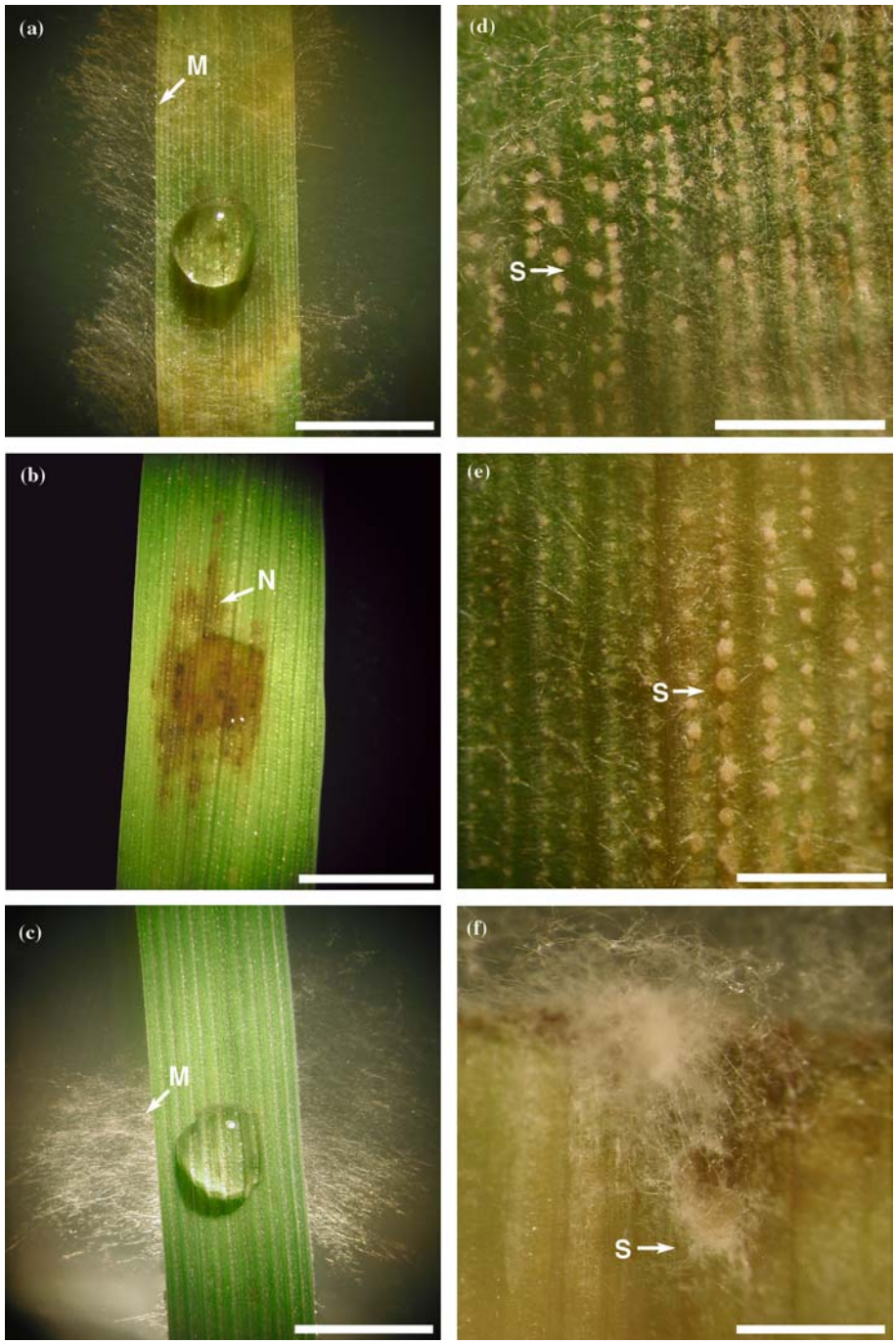


*Figure 5*. Experiment 3. Detached leaves 10 days post-inoculation with *M. nivale* isolate Dard1/M of (a) wheat cv. Biscay, (b) barley cv. Angela and (c) oat cv. Millenium incubated at 15 °C. Bars = 5 mm. Sporodochia on detached leaves 14 days post-inoculation with *M. nivale* isolate Dard1/M on (d) wheat cv. Claire (e) barley cv. Haka and (f) oat cv. Millenium incubated at 15 °C. Bars = 1 mm. Necrosis N, mycelium M and sporodochia S.

## Discussion

Evaluation of PDR components revealed marked differences between cereal species in incubation period, latent period and the symptoms associated with the evaluation of PDR components. The extent of the differences in symptom appearance illustrates that these must also be considered in the comparison of PDR components across cereal and other graminaceae species. Barley showed longer latent periods than the most resistant Irish and UK commercial wheat cultivars with similar or longer incubation periods. In addition, lesions observed on barley differed from those on wheat as chlorosis of the underlying leaf tissue (visible over a light box) frequently did not occur until sporulation; this was in contrast to wheat where necrosis and or chlorosis was observed when lesions were present. This observation suggests that in barley the development of the pathogen was slowed or arrested, due to resistance mechanisms expressed after the incubation period was completed. These findings are consistent with Perry (1986) who reported that in the field the outer leaves of the stem-base in barley were frequently necrotic and brown, and although *M. nivale* could be isolated, the author concluded that there was no evidence that the fungus caused the symptoms but rather persists in the tissue producing sporodochia when senescence occurs. The necrotic lesions in barley may therefore reflect a defence response after initial penetration of the leaf tissue (incubation period) rather than be solely an indicator of susceptibility. The temperature and isolates used were varied between experiments to optimise the assay for detecting individual PDR components, particularly latent period, in barley and oats and to confirm the repeatability of differences in the PDR components between crops across incubation temperatures and isolates.

Oats had longer incubation and latent periods than wheat or barley; however, the reaction of oat cultivars differed from both wheat and barley as quite extensive mycelial growth occurred on the leaf surface using both *M. nivale* var. *majus* and var. *nivale*, before obvious symptoms of infection, indicating tolerance to infection on the leaf rather than inhibition of pathogen development on the leaf surface. The findings of Liu et al. (1997) of infection and mycotoxin contamination of oat grains without visual symptoms of FHB infection and of Diamond et al. (1995) of foliar growth of *M. nivale* on oats in whole plants without extensive damage to the underlying tissue, may also reflect apparently symptomless infection. In what may be a somewhat similar tolerant response on wheat leaves inoculated with *F. graminearum*, Evans and Dill-Mackey (2003) found the mycotoxin DON accumulated on non-symptomatic leaves of the wheat genotype Frontana. In contrast, large lesions but no DON accumulation were found on the wheat cv. Alsen. In barley at the early stages of infection mycelial growth was not obvious even where necrosis was observed well beyond the initial point of inoculation. It is likely that mycelial growth was occurring, as necrosis and sporulation at the cut ends of the leaves were observed, although frequently the leaf tissue between the necrotic leaf ends and the initial point of inoculation appeared relatively healthy, observations supported by Perry (1986) during the isolation of *M. nivale* in barley from apparently healthy tissue. The shortest incubation period to date was found in wheat, entry 28, (Browne et al., 2005) with the unusual combination of a long latent period and was the most resistant genotype in the 2002 US southern soft red wheat FHB screening nursery. This suggests a short incubation period when combined with a long latent period may be a useful combination of PDR components for the inhibition of pathogen development and particularly for initial infection associated with disease incidence.

Latent period provided an indication only of relative resistances and not absolute relativity between the different crops. For example comparing latent period alone in oats to that in wheat and barley would give an underestimate of inhibition of sporulation as latent period does not take into account the very limited extent of sporulation occurring in oats. This is less problematic in wheat and barley where the pattern of sporulation was more comparable. The location of first sporulation reflected the relative latent periods between the three crops. In wheat sporulation occurred initially close to the point of inoculation at the mid-point of each detached leaf. However, onset of sporulation occurred most frequently at the cut ends in barley and almost exclusively at the cut ends in oats, possibly reflecting a greater difficulty in invading host tissue in the undamaged parts of the leaf than in wheat. In addition, sporodochia in oats were poorly formed and less abundant than in wheat and barley

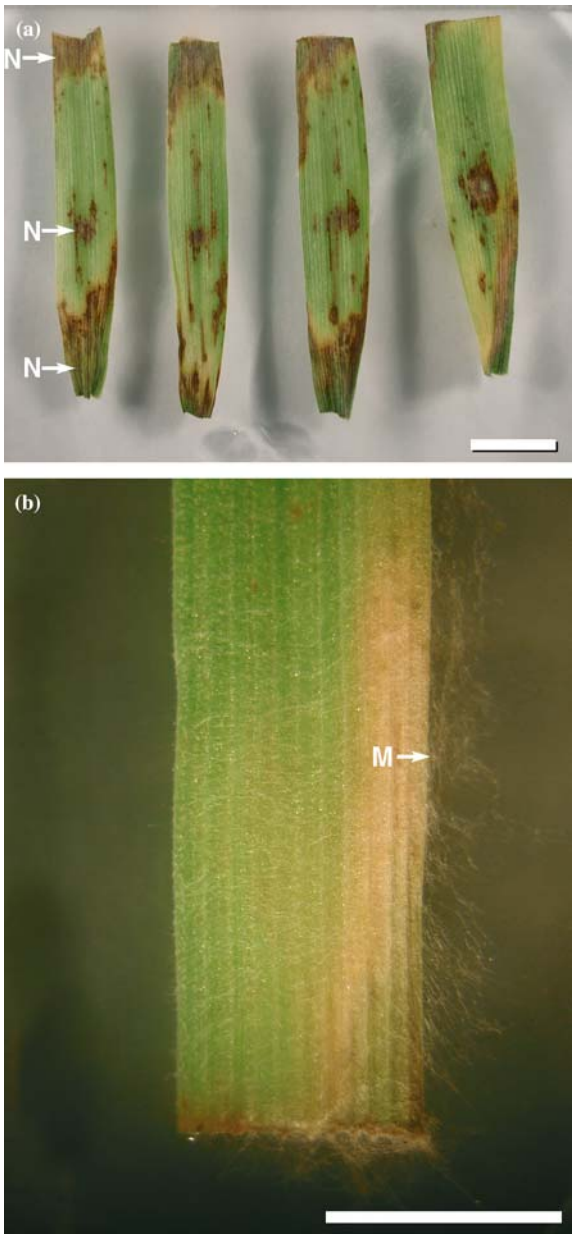


*Figure 6*. Experiment 3. Detached leaves 14 days post-inoculation with *M. nivale* isolate Dard1/M of (a) barley cv. Haka (Bar = 10 mm) and (b) oat cv. Millenium incubated at 15 °C. (Bar = 5 mm). Necrosis N and mycelium M.

where sporodochia were observed in lines between the veins above the stomata on the leaf surface. Diamond and Cooke (1997b), in scanning electron microscope studies, observed that sporodochia on detached leaves of oats had a less regular and compact structure than those of wheat and barley.

The present results comparing infection by both fungal varieties of *M. nivale*, while preliminary, suggest that while *M. nivale* var. *majus* has higher pathogenicity to detached leaves in wheat (Diamond and Cooke, 1997a, 1999; Browne and Cooke, 2004b) this may not be the case in barley and oats where incubation periods were longer for *M. nivale* var. *nivale* but where latent periods occurred at a similar time post-inoculation. The longer incubation periods for *M. nivale* var. *nivale* in barley and oats may be a strategy whereby the fungus is not exposed to resistances expressed after initial infection as rapidly as var. *majus* allowing the pathogen to colonise the leaf surface using extracellular enzymes. The longer latent periods of barley and oats than wheat in the current study may therefore explain the greater frequency of isolation (host preference) of *M. nivale* var. *nivale* than var. *majus* in barley and oats (Diamond and Cooke 1997a). Further investigations into the infection of both *M. nivale* var. *majus* and var. *nivale* in wheat, barley and oats are desirable to further understand possible implications of the host preference of both fungal varieties particularly at the early stages of infection during the incubation period.

The susceptibility of wheat, barley and oats to FHB is also influenced by morphological and developmental traits not detected by the detached leaf assay. Furthermore, symptoms observed in the detached leaf assay will not be identical to symptoms observed in whole plants; however, the relative resistances of wheat and barley in the assays detected by latent period are supported by Perry (1986) who did not observe direct infection by *M. nivale* germ-tubes through the epidermis or stomata of barley coleoptiles, as observed on wheat by Malalasekera et al. (1973). Simpson et al. (2000) found oat seedlings to be less susceptible than wheat when the stem-base of seedlings was inoculated. Langevin et al. (2004), evaluating FHB resistance to point inoculation (termed Type II resistance) across six cereal species, observed that barley showed greater FHB resistance with less disease spread than wheat. In addition these authors observed that *F. graminearum* moved externally from one barley floret to another within the dense spike without penetrating the rachis, consistent with what could be expected from the longer incubation (growth on the leaf surface) and latent periods (inhibition of growth though host tissue). It is notable that longer incubation periods

in the detached leaf assay were related most strongly, in wheat, to increased disease incidence and that longer latent periods were related most strongly to greater Type II resistance (Browne et al., unpublished). In field surveys disease incidence is reported to be higher in barley than in wheat while severity tends to be less (Gilbert et al., 1999; McCallum et al., 1999) with minimal spread on infected glumes following initial infection in barley (Tekauz, 1999). Transgenic approaches to increasing FHB resistance in wheat include the incorporation of a barley chitinase and RIP genes into wheat (Mackintosh et al., 2003). Evaluating PDR components using the detached leaf assay could provide a useful method for studying such transgenic approaches further. It should be noted that further research is necessary across a wider range of genetic backgrounds for barley and oats to ascertain variation in PDR components, particularly incubation period and their potential utility for FHB resistance breeding.

The results here suggest that a shorter incubation period combined with a longer latent period may be a desirable combination of PDR components (retarding pathogen development rather than tolerance of mycelial growth). In wheat the aim might be to reach the level of resistance as detected by latent period in barley or oats and to manipulate resistance and susceptibility factors not detected by the leaf (Browne and Cooke, 2004b) and seed germination assays (Browne and Cooke, 2005) and which remain poorly understood. As high levels of resistance detected by latent period in the detached leaf assay are already present in barley, the use of other resistances will also be important in developing cultivars with greater FHB resistance. This paper provides a basis on which investigations into the relationship between PDR components detected in the detached leaf assay and whole plant resistance in barley and oats can begin.

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