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Short communication

Analysis of head and leaf reaction towards *Microdochium nivale*

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Abstract

This research examined the variation in the response of eight commercial wheat cultivars to *Microdochium nivale* isolates using both *in vivo* FHB tests (AUDPC and RHW measurements) and *in vitro* detached leaf assays (LGR). Irrespective of fungal variety, the two Italian cvs Fortore and Norba exhibited the greatest amount of visual disease symptoms (mean AUDPC = 2.2 and 2.3, respectively), being significantly more susceptible than the other six cultivars (AUDPC $\leq$ 1.24) ($P < 0.05$). Irrespective of fungal variety, the Italian cv. Norba and the Irish cv. Falstaff were more susceptible than the other cultivars (except Fatima 2) in terms of RHW ($P < 0.05$), while the cvs Fortore, GK Othalom and Consort were more resistant than the other five cultivars ($P < 0.05$). In the detached leaf assay, the Hungarian cv. GK Othalom and the Italian cv. Norba were more susceptible (mean LGR = 0.79 and 0.81 mm day^{-1}, respectively) to *M. nivale* than the other six cultivars (mean LGR = 0.51–0.72) ($P < 0.05$). Analysis of the relationship between head and leaf reaction to *M. nivale* infection revealed no significant correlation.

Abbreviations: AUDPC – Area Under the Disease Progress Curve; FHB – Fusarium Head Blight; GS – Growth Stage; LGR – Lesion Growth Rate; RHW – Relative Head Weight.

Microdochium nivale (*Monographella nivalis*), formerly known as *Fusarium nivale* is one of the predominant causal organisms associated with Fusarium Head Blight (FHB) in cooler maritime regions of northwest Europe (Parry et al., 1995). *M. nivale* isolates have been classified as either var. *nivale* or var. *majus* according to conidial size and number of septa, biochemistry, pathogenicity, host preference and species-specific PCR analysis (Maurin et al., 1992; Nicholson et al., 1996; Simpson et al., 2000). *M. nivale* head blight disease generally does not reflect the effect of the other FHB pathogens on the head. In contrast to other FHB pathogens, *M. nivale* does not have any great effect on grain quality; however significant reductions have been reported in plant emergence and grain yield after sowing highly infected seed (Humphreys et al., 1995). Little is known about the secondary metabolities produced by *M. nivale*, though it is thought that *M. nivale* does not produce mycotoxins (Chelkowski and Manka, 1984). The objective of this research was to assess the reaction of wheat leaves and heads towards *M. nivale* isolates using both *in vivo* FHB tests (AUDPC and RHW measurements) and *in vitro* detached leaf assays (LGR). No attempt was made to measure other components of partial disease resistance (incubation period and latent period). These tests were conducted using eight commercial wheat cultivars and the relationship between head and leaf reaction to *M. nivale* infection was investigated.

The eight commercial wheat cultivars used originated from Ireland (cvs Madrigal and Falstaff), the UK (cvs Consort and Claire), Hungary

(cvs GK Othalom and Fatima 2) and Italy (cvs Norba and Fortore). The six *M. nivale* isolates used were selected based on the results of the *in vitro* temperature sensitivity experiment (Brennan et al., 2003), and included slow-growing isolates (075 and SO48/1/N), isolates of average growth rate (825 and M7B) and fast-growing isolates (44/S/N and S048/7/M). The six isolates were obtained from three EU countries: Ireland, Italy and the UK. Isolates were stored and conidial inoculum (1×10^5 ml^{-1} Tween 20) produced as described by Doohan et al. (1998).

In the glasshouse experiment, at GS 65 (mid-anthesis) (Zadoks et al., 1974), each isolate was inoculated against each cultivar (48 combinations) (two heads per plant) with 4 ml of conidial suspension (1×10^5 ml^{-1}) using a hand-held nozzle sprayer. Controls (two heads per plant) were sprayed with 0.2% (v v^{-1}) Tween 20 solution. Treatments were replicated five times for each isolate/cultivar combination and the experiment was conducted twice. Following inoculation, the 48 treatments were randomly arranged in the glasshouse. Visual assessment of disease levels was carried out on treated heads at GS 70, 80 and 90 (the percentage of infected spikelets per head) and AUDPC was calculated (Shanner and Finney, 1977). Heads were harvested when ripe; two heads per plant were bulked together and RHW calculated (weight of inoculated relative to uninoculated heads).

The reaction of detached wheat leaves of the eight cultivars towards *M. nivale* isolates was assessed using a modified method of Diamond and Cooke (1997) which used LGR mm day^{-1} as the disease parameter. The 48 treatments were replicated three times and the experiment was conducted twice. The LGR (mm day^{-1}) was calculated from lesion diameters recorded 3, 5, 7 and 10 days post-inoculation.

Normal data distribution (AUDPC, RHW and LGR) was confirmed using Minitab (Minitab release 13©, 1994 Minitab incorporated, USA) (Ryan Joiner test) (Snedecor and Cochran, 1980). The correlation coefficients between replicate experiments were determined using the Pearson Product Moment Correlation (Snedecor and Cochran, 1980). Analysis of variance incorporating Tukey's pairwise comparison at the 5% level of significance was performed using SPSS (SPSS Inc. Chicago, v8.02, 1989–1997).

Visual disease symptoms of wheat heads are expressed as AUDPC values. Irrespective of fungal variety, the two Italian cvs Fortore and Norba exhibited the greatest amount of visual disease symptoms (mean AUDPC = 2.2 and 2.3), being more susceptible than the other six cultivars (AUDPC $\leq$ 1.24) ($P < 0.05$). Of the eight cultivars, only Madrigal showed a significant difference in susceptibility to fungal variety, being more susceptible to var. *nivale* than var. *majus* (AUDPC = 1.3 and 0.9, respectively, $P < 0.05$) (Figure 1A). In general, the levels of *M. nivale*

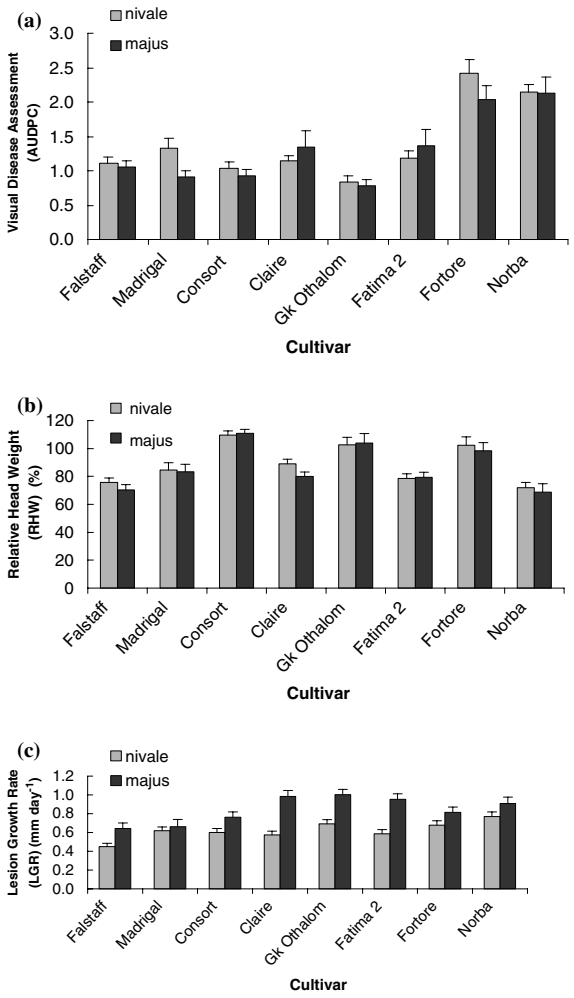

Figure 1. Response of wheat cultivars to *Microdochium* var. *nivale* and var. *majus* isolates in the *in vivo* glasshouse experiment and the *in vitro* detached leaf assay. (A) visual FHB symptoms of wheat heads (AUDPC); (B) head weight of inoculated, relative to uninoculated heads (RHW) (%); (C) *in vitro* detached leaf assay as assessed by LGR (mm day^{-1}). Bars indicate SEM.

FHB visual disease symptoms observed were low (mean% diseased spikelets at GS 90 was 6–36; results not shown). Maurin et al. (1996) reported higher disease levels under both field and controlled environment conditions (43% and 49%, respectively). Also, Diamond and Cooke (1999) reported high disease severities on wheat plants after inoculation with a 1:1 mixture of var. *nivale* and var. *majus* isolates 25 days post-inoculation, depending on the cultivars infected. Overall, *M. nivale* appears generally to be less pathogenic on wheat heads, as assessed by visual symptoms, than other *Fusarium* species (Stack and McMullen, 1985). Perhaps a combined (synergistic) effect of var. *nivale* and var. *majus* on wheat heads would explain the high disease severity achieved by Diamond and Cooke (1999).

Although RHW is not the most accurate method of yield estimation it gives an indication of any yield loss. Irrespective of fungal variety, the Italian cv. Norba and the Irish cv. Falstaff were significantly more susceptible than all other cultivars except Fatima 2 in terms of RHW ($P < 0.05$), while the cvs Fortore, GK Othalom and Consort were significantly more resistant than the other five cultivars ($P < 0.05$ (Figure 1B). The RHW of cv. Falstaff was in fact, significantly reduced by four of the six isolates (*M. nivale* var. *nivale* isolates S048/1/N, 44/2/N and var. *majus* isolates M7B, S048/7/M) ($P < 0.05$). *M. nivale* var. *nivale* and var. *majus* differ from many of the casual agents of FHB in that they have a less significant affect on yield (Wong et al., 1992). But grain infected with *M. nivale* can produce plants negatively affected in both growth and yield (Humphreys et al., 1995). The fact that visual disease scores and yield (RHW) differed significantly for the cultivars suggests that that both Types I and II resistance (resistance to initial infection and resistance to spread) (Schroeder and Christensen, 1963) and type IV resistance (resistance to yield loss or tolerance) (Mesterhazy, 1995) are not similar across all eight cultivars under glasshouse conditions.

In the detached leaf assay, the Hungarian cv. GK Othalom and the Italian cv. Norba were more susceptible (mean LGR = 0.79 and 0.81 mm day^{-1}, respectively) to *M. nivale* than the other six cultivars (mean LGR = 0.51–0.72) ($P < 0.05$). In terms of fungal variety, *M. nivale* var. *majus* was more pathogenic than var. *nivale* towards the Irish cv. Falstaff, the UK cvs Consort and Claire and the Hungarian cvs GK Othalom and Fatima 2 (Figure 1C). Cultivar Falstaff was the most disease resistant against both var. *majus* and var. *nivale* inoculations (mean LGR = 0.64 and 0.45 mm day^{-1}, respectively) (Figure 1C). Acknowledging the low number of isolates, the current work showed that, while there was no significant difference between var. *nivale* and var. *majus* pathogenicity in the FHB glasshouse experiment, var. *majus* was significantly more pathogenic in the detached leaf assay on four of the eight cultivars. These results were in agreement with those of Diamond and Cooke (1997, 1999) who found that seven Irish var. *majus* isolates were more pathogenic towards detached leaves of eight wheat cultivars than seven Irish var. *nivale* isolates. Browne and Cooke (2004) also showed isolates of *M. nivale* var. *majus* were more pathogenic than var. *nivale* isolates on wheat leaves. Differences between var. *majus* and var. *nivale* in terms of host preference has been reported (Diamond and Cooke, 1997; Simpson et al., 2000). Differences in pathogenicity may therefore be associated with host type. Few studies have directly compared the two fungal varieties and no conclusions were drawn on differences in pathogenicity or whether each variety might occupy a different environmental niche under field conditions (Simpson et al., 2000).

The relationship between the response of the eight cultivars to *M. nivale* isolates as assessed by AUDPC and RHW and detached leaves (LGR) was analysed using the Pearson Product Moment Correlation Coefficient (Snedecor and Cochran, 1980). No significant correlations were found. There was a negative correlation found between LGR and AUDPC for cv. Fortore ($r = -0.84$, $P < 0.05$) and RHW for cv. Falstaff ($r = -0.91$, $P < 0.05$) (results not shown). There were positive correlations between LGR and AUDPC for isolates O75 (Italian) and SO48/1/N (UK) ($r = 0.77$ and 0.80, respectively; $P < 0.05$), but not between LGR and RHW (results not shown). These results suggest that in some cultivars host response to disease is organ-specific or that different resistances are operating in mature plant heads compared to seedling leaves. In the *in vivo* FHB experiment, Type I resistance (resistance to initial infection) and Type II resistance (resistance to

spreading) (Schroeder and Christensen, 1963) were assessed while the type(s) of resistance(s) present in detached leaves have not been confirmed; perhaps these could involve restriction of entry and spread of the fungus within the leaf in a manner similar to that which occurs in wheat heads where Type I and Type II resistances may be present. Similarly, Browne and Cooke (2004) reported that of the components of partial disease resistance assessed, lesion length showed the weakest relationship with FHB severity of 19 CIMMYT entries. The sole use of the parameter LGR to assess leaf reaction and isolate pathogenicity must therefore be examined further. Mesterhazy (2002) reported *in vitro* resistance of wheat to *Fusarium* at the seedling blight stage; crown rot resistance and leaf resistance showed no correlation with FHB resistance. Similarly, Miedaner et al. (1997) found no significant correlation between foot rot and FHB resistance in rye, and suggested that this was due to the existence of different mechanisms of resistance towards foot rot and head blight. However, Diamond and Cooke (1999) found significant correlations between *in vivo* FHB reactions and results from the *in vitro* detached leaf assay. The lack of correlation between AUDPC and RHW in the present work could be attributed to yield or physiological host compensation, although disease levels were low.

The lack of correlation between AUDPC, RHW and LGR may also be related to temperature and inoculation technique. The lower constant temperature (20 °C) under which the *in vitro* assay was carried out may have been more suitable for the isolates than the warmer fluctuating glasshouse temperatures (20–30 °C). In the *in vivo* FHB experiment, spray inoculation was used, while in the detached leaf assay inoculation droplets were used. Using ten wheat cultivars, Tamburic-Ilincic et al. (2002) found FHB severity after point and spray inoculation *in vivo* did not correlate well when comparing % diseased spikelets. However, Gilbert (1998) found that spray inoculation in the field correlated well with spray and single floret (point) inoculation in controlled environment cabinets. Results from the current work therefore suggest that further investigation of the detached leaf assay (leaf reaction) is required using a much wider range of isolates and cultivars in order to confirm its relationship with FHB.

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