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Towards the development of a novel *in vitro* strategy for early screening of *Fusarium* ear blight resistance in adult winter wheat plants

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Abstract

A novel *in vitro* bioassay is described for screening *Fusarium* ear blight (FEB) resistance in adult winter wheat plants. Seven winter wheat cultivars were assessed for components of partial disease resistance as 28 day-old detached leaf segments in the laboratory using isolates of *Microdochium nivale* var. *nivale* and *M. nivale* var. *majus*. Results were compared with disease data obtained at anthesis using the same cultivars as whole plants and the same isolates under glasshouse conditions. Significant cultivar differences were observed using detached leaves, with cv. Avalon (a *Fusarium culmorum* ear susceptible cultivar) having the shortest leaf incubation period, greatest leaf lesion development and shortest leaf latent period compared to cv. Spark (a *Fusarium culmorum* ear resistant cultivar), which had the longest leaf incubation period, least leaf lesion development and longest leaf latent period. Using whole plants, cv. Avalon had the shortest ear incubation period and greatest ear disease severity, whilst cv. Spark had the longest incubation period and least ear disease severity. Overall, cultivars of intermediate *F. culmorum* ear resistance expressed intermediate responses to *M. nivale* isolates, using both detached leaves and whole plants. Significant correlations were found with ear disease severity and ear incubation period in whole plants and components of partial disease resistance in detached leaves, with significant correlations obtained between leaf incubation period and ear disease parameters using the *M. nivale* var. *nivale* isolate. In addition, leaf latent period and leaf lesion size showed significant correlations with whole plant reactions using *M. nivale* var. *nivale* and var. *majus* isolates. The *in vitro* screening of cultivars as detached leaves using *M. nivale* isolates may offer a real possibility of a rapid bioassay for the early screening of FEB resistance in wheat and other cereals.

Introduction

The loss of grain yield and quality in wheat from *Fusarium* ear blight (FEB), a disease also known as scab, is one of the most important issues in agriculture at the present time. The disease can be associated with up to seventeen *Fusarium* spp. (Parry et al., 1995) although it is most commonly caused by *F. graminearum* Schwabe (*Gibberella zeae* (Schw.) Petch), *F. culmorum* (WG Smith) Sacc., *F. avenaceum* (Corda ex Fr.) Sacc. (*Gibberella avenacea* Cook), *F. poae* (Peck) Wollenweber and *Microdochium nivale* (Fries) Samuels and Hallet (*Monographella nivalis* (Schnaffit) E. Müller), formerly (prior to 1980) classified as *Fusarium nivale* Ces. ex. Berlese and Vogl. FEB occurs throughout all the major wheat growing areas of the world (Wilcoxson et al., 1992), and is responsible for serious yield losses of up to 70% (Tusa et al., 1981) and the production of a number of different mycotoxins (Eriksen and Alexander, 1998). Epidemics of FEB occur sporadically, especially in years with prolonged periods of precipitation during and after anthesis (Sutton, 1982; Tuite et al., 1990). At present, there is no durable resistance to the disease available to

farmers or breeders and FEB management relies on an integrated combination of cultural management practices, chemical control and the use of the least susceptible or tolerant cultivars available. In view of increasing public concern over high chemical inputs into agricultural systems, the development of more economical and effective but less environmentally-polluting control methods needs to be pursued. There is an increasing interest in the development of cultivars with improved resistance to FEB (Dill-Macky, 1996). Resistance to *Fusarium* spp. has a high priority in cereal breeding in the Northern Hemisphere. Wheat genotypes vary widely in partial disease resistance. Resistant germplasm has been identified in spring wheat from China, Japan, Brazil and in winter wheats from Eastern Europe. Genotypes resistant to *F. graminearum* are also resistant to *F. culmorum* (Snijders, 1990; Mesterhazy, 1987). Scott and Benedikz (1986) found that *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. nivale* and *F. poae* used to inoculate eight winter wheat cultivars, all ranked the cultivars in the same order of resistance. Certain cultivars avoid FEB by flowering early, flowering in boot, or flowering without exposing the anthers (Cook, 1981). As yet, however, no wheat germplasm has been found to be immune to FEB (Chen et al., 1996).

Breeding for resistance to FEB is expensive and time-consuming and it is generally accepted that assessment of resistant cultivars should be undertaken over several years (Bruehl, 1967). Hanson et al. (1950) suggested non-resistant cultivars could be excluded from screening programmes after 1–2 years, but recommended that promising lines should be screened for a minimum of 3 years to ensure that they possessed genuine genetic resistance and were not 'escapes'. In addition, some workers argue that lines should be tested in multiple environments and over different seasons (Cook, 1981). Considering the difficulties in screening for disease resistance, the FEB system might benefit from novel approaches to studies of the host–pathogen interaction, with the aim of the ultimate control of the disease in the field. Buerstmayr et al. (1996) developed an assay using the toxic *Fusarium* metabolite deoxynivalenol (DON), which inhibits eukaryotic protein synthesis, as a selection agent for FEB resistance in wheat. High correlations between *in vitro* tests and FEB resistance have previously been reported by Wang and Miller (1988), Wakulinski (1989) and Lijuan et al. (1991). *In vitro* markers for FEB resistance offer a promising tool for more efficient breeding, and may reduce the need for tedious field screening. Detached leaf segments, instead of whole plants, are frequently employed in disease tests for some pathogens, for example *Erysiphe graminis* DC. f.sp. *avenae* (Roderick and Clifford, 1995), *Stagonospora nodorum* (Berk.) Castellani and Germano (Benedikz et al., 1981), *Septoria tritici* (Arraiano et al., 1998), *Drechslera teres* (Sacc.) Shoemaker (Deadman and Cooke, 1986; Jalli, 1992) and *M. nivale* (Hömmö, 1994; Diamond and Cooke, 1997a). Recent studies (Diamond et al., 1995) have established the importance of foliar lesions on oats caused by *M. nivale* in the epidemiology of the disease under field conditions, and cryoscanning electron microscopy has recently been used to establish the mode of leaf infection by *M. nivale* on wheat, oat and barley leaves (Diamond and Cooke, 1997b).

At present there is no strong evidence for race-specific resistance in wheat to any of the *Fusarium* species causing FEB (Parry et al., 1995). The aim of the present study was to investigate in more detail whether variation in FEB resistance in whole plants grown under glasshouse conditions can be identified by the quantitative assessment of partial disease resistance components using detached leaves infected with *M. nivale*, and whether such assessments could be of importance in breeding programmes for the early identification of new sources of FEB resistant germplasm.

Materials and methods

Origin and maintenance of fungal cultures

Isolates of *M. nivale* from wheat seed were obtained throughout Ireland from the 1996 harvest. Cultures were grown on potato dextrose agar (PDA) (Difco) plates. To prepare single-spore cultures, sporodochia were removed, streaked onto PDA plates and incubated at 18 °C overnight; single germinated conidia were then transferred to new PDA plates and incubated at 18 °C. Eight isolates were used for the detached leaf study, four each of *M. nivale* var. *nivale* (mn1, mn2, mn3, mn4) and of *M. nivale* var. *majus* (mn5, mn6, mn7, mn8). Two isolates of *M. nivale* were used for the glasshouse study (mn1 and mn5), one from each fungal variety.

Detached leaf experiment

Aggressiveness in the *in vitro* study was assessed using a detached leaf method (Diamond and Cooke, 1997a). Wheat cultivars used with different National Institute of Agricultural Botany, Cambridge (NIAB) resistance ratings to FEB were as follows: Spark (highly resistant, NIAB rating 8); Hussar (resistant, NIAB rating 6); Brigadier (resistant, NIAB rating 6); Riband (moderately susceptible, NIAB rating 5); Rialto (moderately susceptible, NIAB rating 5); Reaper (moderately susceptible, NIAB rating 5) and Avalon (highly susceptible, NIAB rating 4). Cultivars were sown in John Innes Compost (JIC) No. 2 (7 : 3 : 2 Soil : Sand : Peat) using 10 seeds 10 cm pot^{-1} in an unheated Isolation Plant Propagator (IPP) unit (Burkard Manufacturing Company Ltd., UK) using a 16 h daylength. After 28 days, the seedlings were harvested, and segments 5 cm in length were cut from the second seedling leaf and placed on the surface (two segments $dish^{-1}$) of 0.5% water agar containing 10 mg l^{-1} kinetin as a senescence retarder. Leaf segments were inoculated with a 10 µl droplet of each *M. nivale* spore suspension respectively (1×10^6 conidia ml^{-1}) at the centre of the adaxial surface and incubated at 20 °C under continuous white light. Control leaves were inoculated with sterile distilled water only. Treatments were replicated three times. The components of partial disease resistance measured as indicators of pathogen aggressiveness were: incubation period (days from inoculation to symptom production), lesion size (mm^2) 7 and 14 days post-inoculation, and latent period (days from inoculation to conidial production).

Glasshouse experiment

Wheat cultivars used in the detached leaf experiment were sown in JIC No. 2 in an unheated glasshouse using 10 seeds 7 cm pot^{-1}. Plants were inoculated at anthesis using a 1×10^6 conidia ml^{-1} 1 : 1 spore suspension mixture of *M. nivale* var. *nivale* isolate mn1, and *M. nivale* var. *majus* isolate mn5, both of which were used in the detached leaf experiment. Control treatments were sprayed with sterile distilled water only. All plants were bagged for 48 h using clear polythene to maintain humidity and encourage disease development. Treatments were replicated four times; ear incubation period (days) and % ear disease severity 10 and 25 days post-inoculation were measured as indicators of pathogen aggressiveness.

Statistical analysis

Data from the glasshouse and the detached leaf experiments were subjected to ANOVA and subsequently compared using Fisher's least significant difference tests. Linear correlation coefficients were computed to determine the strength of the relationship between detached leaf and glasshouse whole plant data.

Results

Detached leaf experiment

Incubation period. All isolates of *M. nivale* used were pathogenic to all wheat cultivars inoculated, but the degree of pathogenicity varied between the isolate–cultivar combinations used. All leaves showed symptoms within 12 days of inoculation. There was an overall shorter incubation period on cv. Avalon than on all other cultivars ($P < 0.001$); cv. Spark had the longest overall incubation period (Figure 1). On all cultivars, the var. *majus* isolates generally had a shorter incubation period than var. *nivale* isolates ($P < 0.05$). No symptoms developed on control leaves.

Lesion size. Figure 2 shows mean lesion sizes on all cultivars after 7 days when inoculated with four *M. nivale* var. *nivale* isolates and four *M. nivale* var. *majus* isolates. On cv. Avalon there was significantly more disease than on any of the other cultivars ($P < 0.001$), and there was significantly less disease overall on cv. Spark ($P < 0.001$). Mean lesion size on cv. Hussar was not significantly different to cvs Reaper, Rialto, Riband or Brigadier. Overall, the var. *majus* isolates were more pathogenic on all cultivars compared to the var. *nivale* isolates. After 14 days (Figure 3) cultivar differences were more evident. Cultivar Avalon had significantly more disease than any of the other cultivars ($P < 0.001$) and cv. Spark had significantly less disease than any of the other cultivars ($P < 0.001$). Cultivars Brigadier and Hussar were not significantly different from each other but had less disease overall ($P < 0.05$) than cvs Reaper, Rialto and Riband. The var. *majus* isolates were again more pathogenic than those of var. *nivale* although significant differences were found between some of the isolates.

Latent period. Leaves of all cultivars had sporulated 22 days post-inoculation (Figure 4). Cultivar Avalon

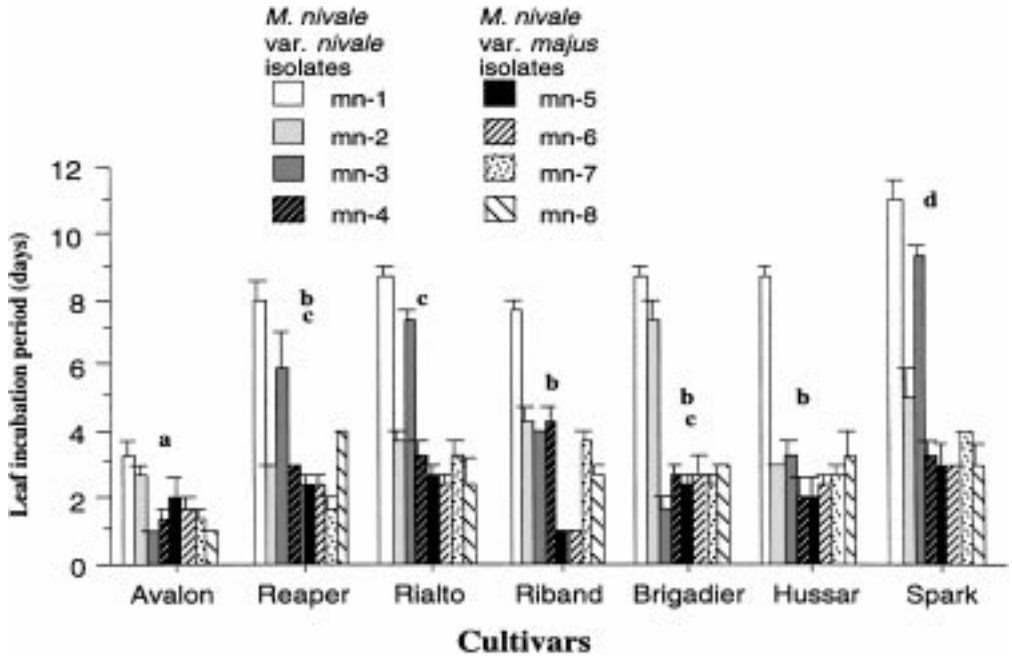

Figure 1. Incubation periods on seven winter wheat cultivars inoculated with four *M. nivale* var. *nivale* and four *M. nivale* var. *majus* isolates on detached leaves. Values are the mean of 3 replicates; each replicate was two leaf segments Petri dish^{-1}. Bars represent standard errors of mean. Different letters indicate statistically significant differences on cultivar effect based on Fisher's protected LSD.

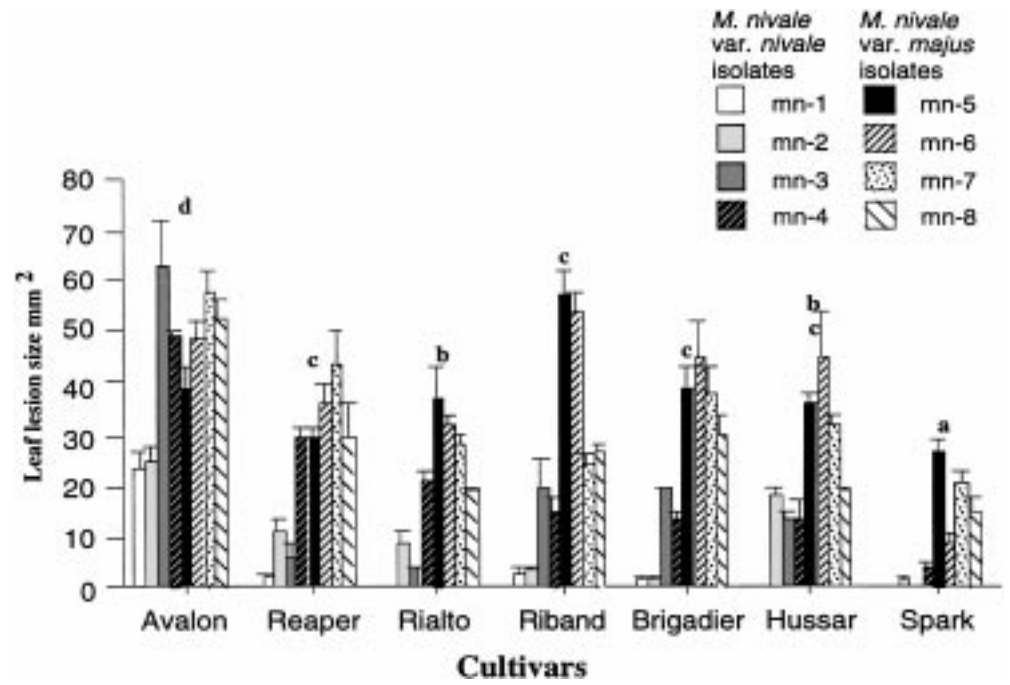

Figure 2. Lesion sizes after 7 days on seven winter wheat cultivars inoculated with four *M. nivale* var. *nivale* and four *M. nivale* var. *majus* isolates on detached leaves. Values are the mean of 3 replicates; each replicate was two leaf segments Petri dish^{-1}. Bars represent standard errors of mean. Different letters indicate statistically significant differences on cultivar effect based on Fisher's protected LSD.

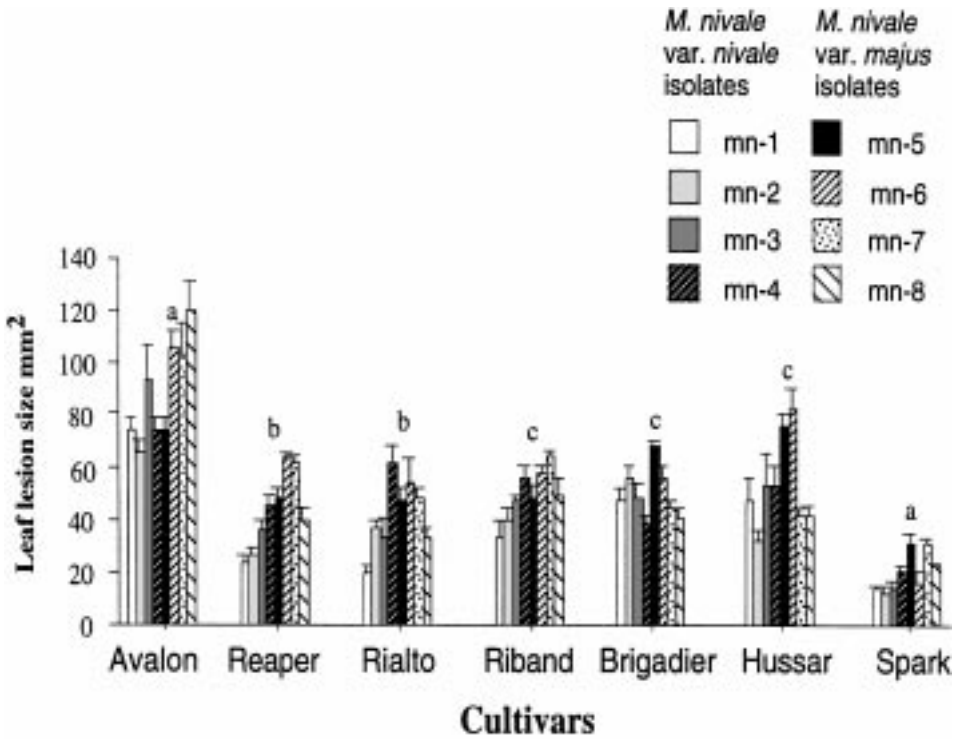

Figure 3. Lesion sizes after 14 days on seven winter wheat cultivars inoculated with four *M. nivale* var. *nivale* isolates and four *M. nivale* var. *majus* isolates on detached leaves. Values are the mean of 3 replicates; each replicate was two leaf segments Petri dish^{-1}. Bars represent standard errors of mean. Different letters indicate statistically significant differences on cultivar effect based on Fisher's protected LSD.

had the shortest latent period whereas cv. Spark had the longest latent period overall ($P < 0.001$). Differences between the other cultivars were less defined; cvs Reaper and Riband were not significantly different to each other, but cv. Brigadier had an overall longer latent period than cvs Reaper, Rialto and Riband ($P < 0.05$) and an overall significantly shorter latent period than cv. Hussar. All cultivars generally showed shorter latent periods with var. *majus* isolates than with var. *nivale* isolates.

Glasshouse experiment

Ear incubation period. All cultivars showed symptoms of disease between 5 and 14 days post-inoculation (Figure 5). Cultivar Avalon had the shortest incubation period ($P < 0.05$) of any of the cultivars, whilst cv. Spark had the longest incubation period ($P < 0.05$). The incubation periods of cvs Reaper, Rialto, Riband and Brigadier were not significantly different from each other and cv. Hussar had a longer incubation period than all other cultivars, except for cv. Spark ($P < 0.05$).

Ear disease severity. There were significant differences in the levels of FEB disease severity between cultivars on both of the recording dates (Figure 6). Overall, cv. Avalon had significantly more disease than any other cultivar ($P < 0.05$) and cv. Spark showed significantly less disease except for cv. Hussar 10 days post-inoculation. Cultivars Reaper, Rialto and Brigadier were not significantly different from each other 10 days post-inoculation, but these cultivars had significantly less disease than cv. Riband ($P < 0.05$), and were significantly different to cv. Hussar. After 25 days (Figure 6), disease levels on cvs Reaper, Riband and Brigadier were not significantly different but all had significantly more disease than cvs Hussar and Rialto ($P < 0.05$). Overall, cv. Avalon remained the most susceptible and cv. Spark the most resistant cultivars.

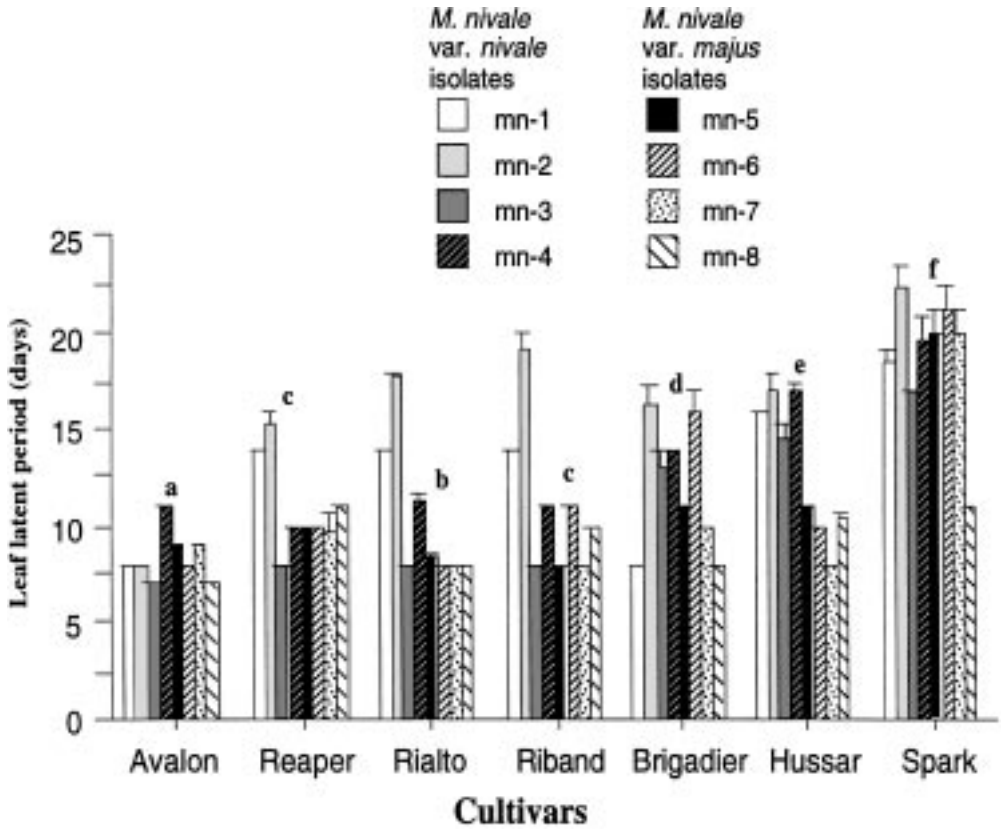

Figure 4. Latent periods on seven winter wheat cultivars inoculated with four *M. nivale* var. *nivale* and four *M. nivale* var. *majus* isolates on detached leaves. Values are the mean of 3 replicates; each replicate was two leaf segments Petri dish^{-1}. Bars represent standard errors of mean. Different letters indicate statistically significant differences on cultivar effect based on Fisher's protected LSD.

Correlation between experiments

There was a strong significant positive correlation between ear incubation period and detached leaf incubation period with the *M. nivale* var. *nivale* isolate (Table 1); however, there were no significant correlations between these two parameters with the *M. nivale* var. *majus* isolate. There were strong significant positive correlations between the ear incubation period and leaf lesion size after 14 days for both the var. *nivale* and the var. *majus* isolate; however, there were no significant correlations between ear incubation period and leaf lesion size after 7 days with either of the fungal varieties. Ear incubation period and var. *nivale* and var. *majus* latent periods on detached leaves were significantly correlated.

When percentage ear disease severity was correlated with detached leaf parameters, there were strong significant negative correlations between leaf incubation periods using the var. *nivale* isolate and ear disease after 10 and 25 days, but there were no significant correlations with any ear disease measurements and the var. *majus* isolate incubation period. Disease severity on the ears was also significantly correlated with detached leaf lesion measurements after 7 and 14 days using the var. *nivale* isolate. There were significant correlations for the var. *majus* isolate between ear and detached leaf disease levels after 10 days on the ears and after 14 days on the leaves; there were, however, no significant correlations between ear and leaf disease levels, using the var. *majus* isolate, after 7 days on the leaves. Ear disease levels after 25 days and the leaf latent period were significantly negatively correlated for var. *nivale* only.

Discussion

The requirement by plant breeders worldwide to provide wheat cultivars with effective and durable disease

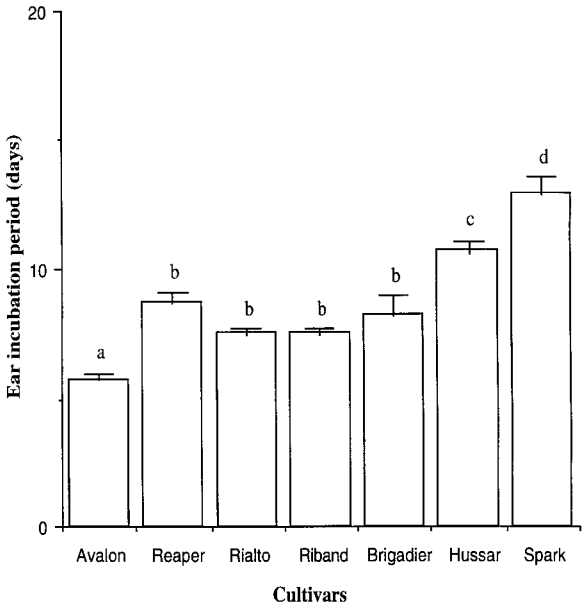

Figure 5. Ear incubation periods on seven winter wheat cultivars inoculated with a 1 : 1 *M. nivale* var. *nivale* (mn1) and *M. nivale* var. *majus* (mn5) isolate mixture on adult plants. Values are the mean of 4 replicates. Bars represent standard errors of mean. Different letters indicate statistically significant differences based on Fisher's protected LSD.

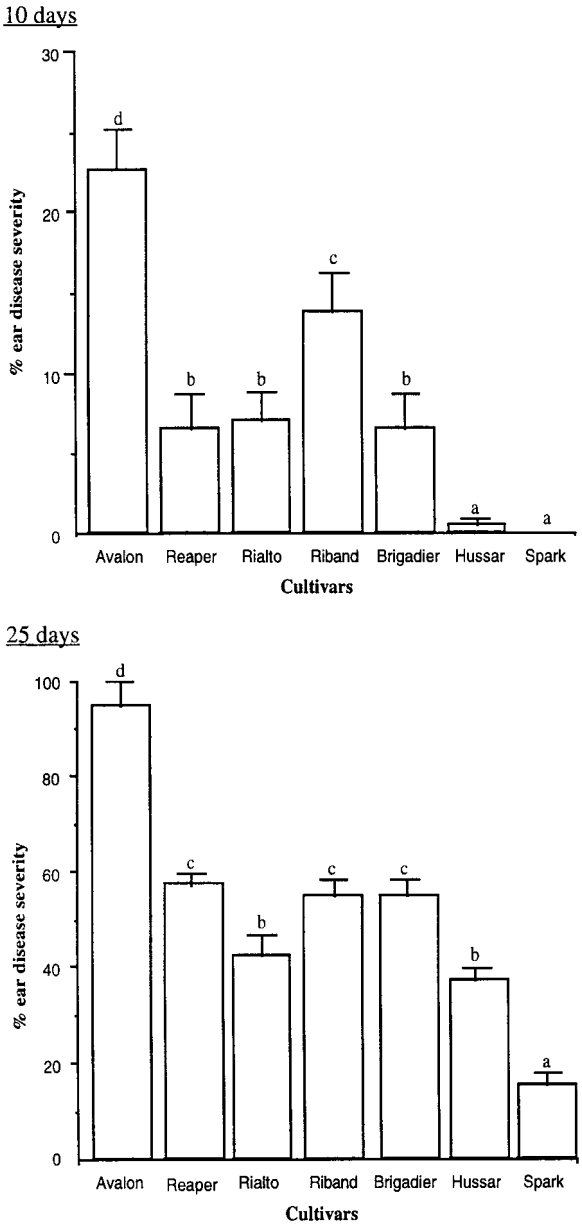

Figure 6. Percentage ear disease severity after 10 and 25 days on seven winter wheat cultivars inoculated with a 1 : 1 *M. nivale* var. *nivale* (mn1) and *M. nivale* var. *majus* (mn5) isolate mixture on adult plants. Values are the mean of 4 replicates. Bars represent standard errors of mean. Different letters indicate statistically significant differences based on Fisher's protected LSD.

resistance to FEB is generally recognised as an important criterion in many cereal breeding programmes. Field trials are the most commonly used method in screening for FEB resistance, yet the interpretation of field data is difficult because the expression of the disease is almost certainly affected by climatic and other environmental conditions, and several years are needed to confidently evaluate FEB resistance in wheat cultivars (Bruehl, 1967). Conclusions from field data are further complicated by the large number of mechanisms that control genetically controlled resistance in the wheat plant. Individually, such mechanisms confine the progression of the pathogen at various stages of pathogenesis and secure plant resistance at particular stages of ear development. Moreover, the expression of plant resistance mechanisms depends on the conditions and timings of plant inoculations and the aggressiveness of the pathogen. These factors almost certainly contribute to the experimental differences observed under field conditions in the performance of a wheat cultivar, both within and between years (Ablova and Slusarenko, 1996). Because of these difficulties, plant breeders need the development of rapid and accurate procedures to support them in their cultivar screening work, and more sensitive methods of disease scoring might enable the host–pathogen interaction to be more clearly observed.

Table 1. Significant correlation coefficients (r) between % ear disease severity and components of partial disease resistance in whole plants and detached leaves

Whole plant measurement	Detached leaf measurement	Correlation coefficient (r)	Probability (P)
Ear incubation period	mn1 incubation period	0.820	0.0206*
Ear incubation period	mn1 lesion size 14 days	−0.884	0.0054**
Ear incubation period	mn5 lesion size 14 days	−0.890	0.0045**
Ear incubation period	mn1 latent period	0.793	0.0310*
Ear incubation period	mn5 latent period	0.866	0.0085**
Ear disease-10 days	mn1 incubation period	−0.922	0.0014**
Ear disease-10 days	mn1 lesion size 7 days	0.872	0.0072**
Ear disease-10 days	mn1 lesion size 14 days	0.913	0.0020**
Ear disease-10 days	mn5 lesion size 14 days	0.793	0.0031**
Ear disease-25 days	mn1 incubation period	−0.971	<0.0001***
Ear disease-25 days	mn1 lesion size 7 days	0.853	0.0112*
Ear disease-25 days	mn1 lesion size 14 days	0.867	0.0083**
Ear disease-25 days	mn1 latent period	−0.814	0.0226*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
mn1 = *M. nivale* var. *nivale* isolate used in the glasshouse experiment (as a 1 : 1 mixture with mn5) and individually in detached leaf work.
mn5 = *M. nivale* var. *majus* isolate used in the glasshouse experiment (as a 1 : 1 mixture with mn1) and individually in detached leaf work.

The use of genetic resistance to diseases such as FEB offers the most promising tool for control of mycotoxin contamination of cereal grains (D'Mello et al., 1996). It has been reported that cereal cultivars tolerant to *Fusarium* mycotoxin production such as deoxynivalenol (DON) generally have an increased resistance to FEB; it is believed that the toxins play some part in the aggressiveness of the pathogen and interfere with the plant's defence response which requires protein synthesis (Snijders and Krechting, 1992; Buerstmayr et al., 1996). Snijders and Perkowski (1990) demonstrated that in wheat cultivars resistant to FEB caused by *F. culmorum*, DON concentrations in the harvested grain were low compared to susceptible genotypes which yielded high DON concentrations. These results are fully supported by Wong et al. (1995) in their work with FEB-resistant Chinese wheat genotypes and FEB-susceptible Canadian genotypes. Therefore the development and the rapid screening of FEB-resistant cultivars offers the chance to reduce the risk of mycotoxin-contaminated grain entering the food chain.

The wheat cultivars in the current study were chosen on the basis of their NIAB ratings to FEB disease, which are determined using artificial inoculations with *F. culmorum* only (John Clarkson, NIAB Cambridge, personal communication). Detached leaf and glasshouse tests using *M. nivale* inoculations clearly confirmed that cv. Spark was the most resistant to ear disease and cv. Avalon was the most susceptible. The responses of the other cultivars were less well defined than either cv. Avalon or cv. Spark; these other cultivars are rated as being intermediate in their resistance to FEB by NIAB. Thus it would appear that leaf and ear resistances to *M. nivale* are strongly related to ear resistance against *F. culmorum*. Snijders (1990) and Mesterhazy (1987) found resistance to *F. graminearum* was also related to that for *F. culmorum*, and Scott and Benedikz (1986) established evidence for common resistance to the five most prevalent components of the FEB complex. It would seem that resistances to all FEB-causing species may well share a common genetic background (Mesterhazy, personal communication).

In this study, there were many significant correlations between detached leaf and whole plant ear responses, particularly when whole plant data were correlated with the var. *nivale* isolate measurements on detached leaves. The relatively low non-significant correlation coefficient (+0.488) for the ear incubation period with the var. *majus* isolate leaf incubation period could have been affected by the short incubation period of the isolate observed on detached leaves at 20 °C, this resulting in the poor separation of cultivar scores. A lower test temperature may therefore be required for this isolate. Furthermore, it would be highly desirable

to relate detached leaf data to FEB scores using individual isolates of *M. nivale* var. *nivale* and var. *majus* in ear inoculations rather than the isolate mixture used here. There are many instances in which a detached leaf method has been shown to accurately predict whole plant disease responses to pathogens, for example *Stagonospora nodorum* (Baker and Smith, 1978; Benedikz et al., 1981), *Septoria tritici* (Arraiano et al., 1998) and *Drechslera teres* (Deadman and Cooke, 1986). The results from the present study support such work, as disease scores of wheat cultivars used in the detached leaf tests significantly correlated with several measurements from the glasshouse whole plant study. Data obtained in the current work also indicate that leaf and ear resistances in wheat against *M. nivale* are correlated. Eyal et al. (1973) reported wheat cultivars resistant to *S. tritici* at the seedling stage were also resistant at the adult plant (booting) stage. Certain wheat cultivars, however, may fail to show correlations between detached leaf or seedling resistance and field resistance because of an adult plant component to the resistance (Arraiano et al., 1998). Fried and Meister (1987) showed that in the *Stagonospora* (*Septoria*) *nodorum* wheat pathosystem, there was independent segregation of genes controlling head and leaf resistance which are thus independently inherited; field screening for ear glume blotch cannot therefore be replaced by seedling or detached leaf tests.

The potential for screening for FEB resistance *in vitro* was particularly indicated by the incubation period of the var. *nivale* isolate on detached leaves strongly correlating with the response of the adult plants to the pathogen, suggesting that this leaf parameter could be used as a pre-screening criterion. However significant correlations were also obtained between ear disease reactions and leaf lesion size and latent period. Niks and Skinnes (1998) state that latent period is probably the best predictor of the level of partial disease resistance in the field in most plant pathosystems. In practical terms, ear disease severity is the only criterion easily measurable under field conditions in a *Fusarium* breeding programme, and therefore correlations obtained in the current work with ear disease severity are probably of most practical importance. Such an *in vitro* system would enable the seedling to be grown, harvested and tested, and the results obtained within 28 days. If this rapid technique were to be used as part of a breeding programme, only those cultivars found to show resistance traits would then be screened under field conditions. This would result in considerable savings in field and glasshouse space, and would remove the necessity for time-consuming inoculations of mature plants.

The practical feasibility of the leaf *in vitro* system described here has yet to be fully evaluated for FEB; further work is required to develop the bioassay in which more *M. nivale* isolates of differing aggressiveness would be tested using a greater range of wheat cultivars under different *in vitro* conditions of temperature, inoculum load and other variables. The feasibility of using the bioassay for barley and oats in which FEB symptoms are less defined also needs to be determined. Work is also required to establish the relationship of data obtained using the bioassay with field data, particularly ear disease severity, grain yield and mycotoxin content of harvested grain. However, as *M. nivale* is the only member of the FEB complex with the ability to rapidly cause leaf lesions, this pathogen remains the major tool for the *in vitro* evaluation of ear resistance to other members of the FEB complex. Caution is required when selecting wheat cultivars with resistance ratings based exclusively on the results of the detached leaf tests described here; however, testing cultivars as detached leaves with *M. nivale* would seem to offer a real possibility of a rapid bioassay in screening for resistance to FEB of wheat and other cereals.

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