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Development and evaluation of an *in vitro* detached leaf assay for pre-screening resistance to *Fusarium* head blight in wheat

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Abstract

An *in vitro* detached leaf assay, involving the inoculation of detached leaves with *Microdochium nivale*, was further developed and used to compare with whole plant resistance ratings to *Fusarium* head blight (FHB) of 22 commercial cultivars and published information on 21 wheat genotypes, identified as potential sources for FHB resistance. An incubation temperature of 10 °C and isolates of *M. nivale* var. *majus* of intermediate pathogenicity were found to be the most suitable for the differential expression of several components of partial disease resistance (PDR), namely incubation period, latent period and lesion length, in wheat genotypes used in the detached leaf assay. There were highly significant differences ($P < 0.001$) for each component of PDR within commercial cultivars and CIMMYT genotypes. Positive correlations were found between incubation period and latent period ($r = 0.606$; $P < 0.001$ and $r = 0.498$; $P < 0.001$, respectively, for commercial cultivars and CIMMYT genotypes), inverse correlations between incubation period and lesion length ($r = -0.466$; $P < 0.01$ and $r = -0.685$; $P < 0.001$, respectively) and latent period and lesion length ($r = -0.825$; $P < 0.001$ and $r = -0.848$; $P < 0.001$, respectively). Spearman rank correlations between individual PDR components and UK 2003 recommended list ratings were significant for incubation period ($r_s = 0.53$; $P < 0.05$) and latent period ($r_s = 0.70$; $P < 0.01$) but not for lesion length ($r_s = -0.26$). Commercial cultivars identified with high resistances across all three PDR components in the detached leaf assay also had high whole plant FHB resistance ratings, with the exception of cv. Tanker which is more susceptible than the results of the detached leaf assay suggested, indicating an additional susceptibility factor could be present. Agreement between resistances found in the detached leaf assay and resistance to FHB suggests resistances detected in detached leaves are under the same genetic control as much of the resistances expressed in the wheat head of the commercial cultivars evaluated. In contrast, high resistances in each of the PDR components were associated with higher susceptibility across 19 CIMMYT genotypes previously evaluated as potential breeding sources of FHB resistance (incubation period: $r = 0.52$; $P < 0.01$, latent period: $r = 0.53$; $P < 0.01$, lesion length: $r = -0.49$; $P < 0.01$). In particular, the CIMMYT genotypes E2 and E12 together with Summai #3, known to have high levels of whole plant FHB resistance, showed low levels of resistance in each PDR component in the detached leaf assay. Such whole plant resistances, which are highly effective and not detected by the detached leaf assay, do not appear to be present in Irish and UK commercial cultivars. The most resistant Irish and UK commercial cultivars were comparable to the genotype Frontana and the most resistant CIMMYT germplasm evaluated in the leaf assay.

Introduction

The *Fusarium* head blight (FHB) complex has been associated with at least 17 *Fusarium* spp. Three predominate internationally, *F. graminearum*, *F. culmorum* and *F. avenaceum* although in the cooler maritime regions of northwest Europe *F. culmorum* tends to dominate and *F. poae* and *Microdochium nivale* assume a greater importance (Parry et al., 1995). Severe infections of FHB occur sporadically and can cause

significant yield loss and the accumulation of important mycotoxins. There is no complete resistance to FHB, although genetic resistance is considered to offer the most promising tool for the control of mycotoxin contamination of grains. At present, there is no strong evidence for race-specific resistance in wheat to any of the *Fusarium* species (Parry et al., 1995). There are a number of resistance types proposed for FHB in wheat. The main two are Type I, resistance to initial infection, and Type II, resistance against spread of the pathogen within the host (Schroeder and Christensen, 1963). Type I is measured by spray inoculation and Type II by point inoculation of a single floret on the wheat head. In addition, there are a number of novel resistance mechanisms proposed; Type III, the ability to degrade DON (Miller and Arnison, 1986), Type IV, tolerance to DON (Wang and Miller, 1988) and Type V, yield tolerance (Mesterhazy, 1989; 1995; Mesterhazy et al., 1999).

Resistance to FHB in cereal breeding has taken a high priority worldwide. However, the evaluation of resistance has been slow due to the necessity to avoid escapees by evaluating resistance in whole plants over several years and in different environments (Bruehl, 1967). Breeding has also been hampered by the difficulty of incorporating resistance into adapted high-yielding cultivars (Chen et al., 1997; Mesterhazy et al., 1999). There has, therefore, been interest in developing *in vitro* assays to provide methods for prescreening FHB resistance. These include a detached leaf assay (Diamond and Cooke, 1999), seedling resistance (Mesterhazy, 1987; Snijders, 1990) and response to the *Fusarium* mycotoxin deoxynivalenol (DON) (Buerstmayr et al., 1996). Diamond and Cooke (1999) reported a significant relationship between some components of partial disease resistance (PDR) to *M. nivale* in a detached leaf assay and whole plant resistance to *M. nivale* and *F. culmorum* in seven commercially available cultivars of winter wheat. However, Miedaner (1997) summarizing data from a number of studies, suggested strong interactions existed between plant growth stages, host genotypes and plant organs, respectively. Mesterhazy (1987) reported correlations between seedling and FHB resistance, although this was not valid for every genotype. High correlations between DON tolerance and FHB resistance have been reported by Wang and Miller (1988), Wakulinski (1989) and Lijuan et al. (1991); however other authors reported no such correlations (Bruins et al., 1993; Mesterhazy, 2002).

The objectives of the experiments reported in this paper were to develop and further evaluate the use of a rapid detached leaf assay for pre-screening FHB resistance in wheat and to further understand the nature of host resistance to FHB.

Materials and methods

Detached leaf assay

Wheat genotypes with a range of FHB resistance, including commercial cultivars and germplasm selected for FHB resistance, were obtained for this study (Table 1). The 20 commercially available winter wheat cultivars were those listed in the UK recommended list 2002 for which FHB resistance ratings based on visual symptoms of FHB in trials spray-inoculated with *F. culmorum* and conducted over a number of seasons (John Clarkson, NIAB, Cambridge, UK, personal communication) are published (a higher figure indicating greater resistance). The two spring wheat cultivars, Alexandria and Raffles, are in the Irish Recommended List 2002 (Table 1). The apparently resistant germplasm consisted of 19 spring wheat genotypes from the International Maize and Wheat Improvement Centre (CIMMYT), which had been evaluated for FHB resistance by assessing visual symptoms of FHB in spray-inoculated trials with *F. culmorum* over three years at University College Dublin (Dardis and Walsh, 2003). Although all 19 genotypes were classified as resistant by CIMMYT, only some entries were consistently superior to the susceptible checks (Irish commercial spring wheat cvs Alexandria, Baldus and Chablis) under Irish conditions (Dardis and Walsh, 2003) (Table 1). In addition, Frontana and Summai #3, two renowned sources of FHB resistance for breeding, were included.

The wheat genotypes were grown in John Innes No. 2 compost in a controlled environment chamber with a 12 h photoperiod, a RH of 75%, and a day/night temperature of 18 °C/12 °C. After 14 days, 5 cm segments from the mid-section of the first leaf were harvested, and placed adaxial surface uppermost on the surface of 0.5% water agar containing 10 mg l^{-1} kinetin as a senescence retarder. In the preliminary investigations of incubation temperature and isolate, two leaves per Petri dish were used. In all further experiments four were used. The leaf segments were left overnight on the water agar for inoculation the following day.

Single-spore isolates of *M. nivale* var. *majus* were isolated from wheat seed from the Irish 2001 harvest. A further three isolates of *M. nivale* var. *nivale*, 44/S/N,

Table 1. Commercial cultivars and CIMMYT genotypes evaluated in the detached leaf assay

Commercial cultivars	UK recommended list 2003 FHB ratings	CIMMYT entries			
		Pedigree	Entry no.*	CIMMYT entry no. in 1st Yangtze	FHB severity (day 35)*
Winter wheat		Gov/AZ//MUS/3/DODO/4/BOW	1	5	18.5
Access	5	NANJING 8508/3/CHUM18//JUP/BJY	2	241	10.1
Biscay	8	CBRD/BAU	3	61	27.3
Buchan	6	CATBIRD	4	27	29.9
Charger	4	GOV/AZ//MUS/3/DODO/4/BOW	5	4	21.8
Claire	7	SHA3/SERI//SHA4/LIRA	6	105	61.8
Consort	6	XIANG82.2661/2*KAUZ	7	295	30.4
Deben	6	GOV/AZ//MUS/3/DODO/4/BOW	8	3	20.6
Equinox	5	CBRD/KAUZ	9	191	55.1
Hereward	5	CBRD/KAUZ	10	185	49.4
Madrigal	6	CBRD/KAUZ	11	186	61.1
Malacca	6	NANJING8331/3/SJZ10//ALD/PVN	12	211	15.6
Napier	5	ALD/PVN//YMI#6/3/KAUZ/4/NANJING8331	13	350	23.3
Option	6	ZHENGJIANG8709/3/CHUM18//JUP/BJY	14	95	36.9
Riband	5	JB9-38/CBRD	15	81	35.0
Savanagh	6	79.218/CMH84.3379	16	410	45.8
Shamrock	6	R37/GHL121	17	252	21.9
Soissons	7	SHA7//PRL/VEE#6/3/FASAN	18	68	42.5
Solstice	7	XIANG82.2661/2*KAUZ	19	292	26.9
Tanker	5				
Xi 19	5	Check mean (Alexandria, Baldus and Chablis)			37.1
Spring wheat					
Alexandria					
Raffles					

*(Dardis and Walsh, 2003).

SO48/1/N and SO28/2/N, and two isolates of var. *majus*, 44/3/M and 2/2/M were obtained from Paul Nicholson, John Innes Centre, Norwich, UK originally isolated from wheat seed by Simon Edwards, Harper Adams University College, Newport, UK. Conidial inoculum of *M. nivale* was produced on potato dextrose agar (PDA) covered in 'cellophane' (Browne and Cooke, 2003) and incubated on coolplates (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 µl droplet of *M. nivale* spore suspension adjusted to 1×10^6 conidia ml^{-1}. Control leaf segments were inoculated with distilled water only containing one drop of Tween 20 l^{-1}. The leaf segments were incubated under a 24 l^{-1} diurnal cycle of NUV and white light.

In the investigation of the effect of temperature on the detached leaf assay, 10 wheat genotypes, E1, E2, E3, E9, E10, E11, Frontana, Summai #3, Alexandria and Raffles were inoculated with one isolate of *M. nivale* var. *majus* and one of *M. nivale* var. *nivale* and incubated at 10, 15, 20 and 25 °C using three replicates. All further experiments were carried out at 10 °C. In the investigation of the effect of isolate on the detached leaf assay, the 10 wheat genotypes were inoculated with 10 different isolates, seven *M. nivale* var. *majus* isolates: DoA1/M, DoA3/M, DoA8/M, DoA17/M, OP1A/M, OP2A/M and 44/3/M and three *M. nivale* var. *nivale* isolates: 44/S/N, SO48/1/N and SO28/2/N, using three replicates.

Once the appropriate incubation temperature of 10 °C and the *M. nivale* var. *majus* isolates with intermediate pathogenicity had been selected, the wheat genotypes were divided into two groups, one comprised mainly of commercial cultivars and the second of the CIMMYT genotypes. These were tested in two separate experiments (1 and 2 respectively). The genotypes Frontana and Summai #3, the commercial cvs Biscay, Soissons, Alexandria and Raffles and the CIMMYT genotypes E3 and E1 were included in both groups to allow comparison between each experiment. In experiments 1 and 2, each wheat genotype value for

each component of PDR was the mean of four Petri dishes for each of five isolates of *M. nivale* var. *majus*, so that each value was the mean of 80 observations (20 Petri dishes × four leaf segments) with three replications and blocking separated by time. The isolates used, which varied between blocks, included DoA1/M, DoA2/M, DoA3/M and 44/3/M (Browne and Cooke, 2003), DoA17 and the isolate DoA12 with similar pathogenicity to isolate DoA2/M, and 2/2/M with lower pathogenicity than the isolate 44/3/M (data not shown). Assessments of symptom appearance and sporulation were carried out daily under a compound microscope (magnification 40×). The components of PDR measured were: incubation period (days from inoculation to first appearance of symptoms which varied at different incubation temperatures and are described in the results section), latent period (days from inoculation to first appearance of sporodochia on the leaf surface, occurring both within and outside the original inoculum droplet and at first translucent and later turning a salmon pink) and lesion length (measured on day 7 as the necrotic area visible by placing the Petri dishes over a light box).

Statistical analysis

ANOVA were conducted using Genstat V software. All experiments were analysed as a randomised block design, with three blocks. ANOVA for the incubation temperature experiment included incubation temperature, wheat genotypes and incubation temperature × wheat genotypes interaction, whilst the isolate experiment included isolate, wheat genotypes and isolate × wheat genotypes interaction. ANOVA for the detached leaf assays contained 26 mainly commercial wheat cultivars and 25 mainly CIMMYT genotypes, respectively. Correlations between PDR components and the semi-quantitative UK recommended list FHB ratings were conducted using Spearman rank correlation on Statview. All other correlation analyses were conducted using Microsoft Excel.

Results

Detached leaf assay: incubation temperature and isolate

Both isolates of *M. nivale*, Dard1/M and 44/S/N, were most pathogenic at 20 °C ($P < 0.001$) causing

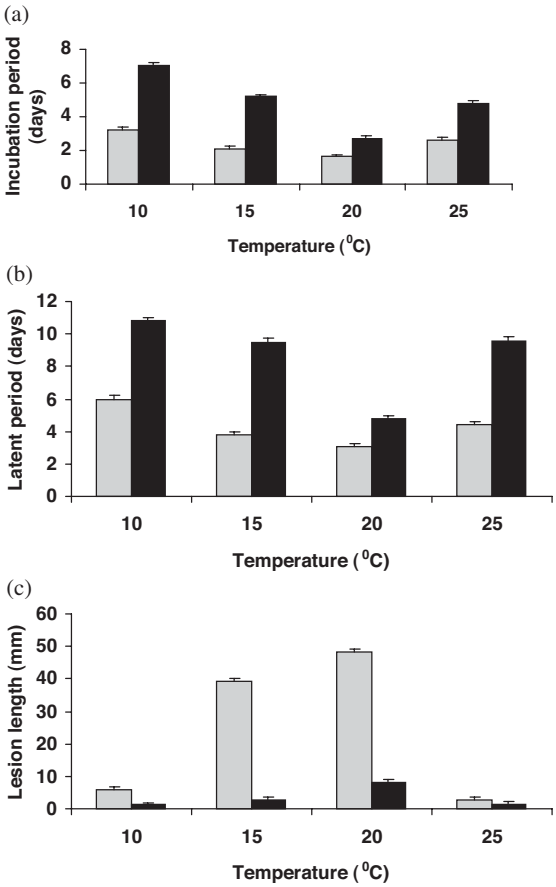

Figure 1. Effect of incubation temperature on incubation period (a), latent period (b) and lesion length (c) of the isolates Dard1/M (▒) and 44/S/N (■) on detached wheat leaves. Bars represent standard errors of the mean and apply to all data.

the shortest incubation (Figure 1a) and latent periods (Figure 1b) and greatest lesion length (Figure 1c). The isolate *M. nivale* var. *majus* Dard1/M was more pathogenic than the var. *nivale* isolate 44/S/N at all temperatures, causing shorter incubation and latent periods and greater lesion lengths ($P < 0.001$). Across all wheat genotypes, incubation and latent periods were longest at 10 °C, however while lesion length was smaller at 10 °C than at 15 and 20 °C it was greater than at 25 °C. At 25 °C, infection was identified by dark necrotic lesions restricted to the area of the initial droplet making it difficult to identify sporulation. This contrasted with 10 °C, where infection was observed as dull grey-green water-soaked areas of the leaf with the lesions extending beyond the initial droplet of inoculum, allowing sporulation to be clearly observed. There

were significant isolate × temperature interactions for incubation period, latent period and lesion length ($P < 0.001$) with differences in incubation period and latent period smallest at 20 °C; no significant temperature or isolate interaction occurred with the wheat genotypes.

All isolates of *M. nivale* var. *majus* and var. *nivale* were pathogenic to all wheat genotypes, although there was significant variation ($P < 0.001$) in incubation period (Figure 2a), latent period (Figure 2b) and lesion length (Figure 2c). There was no significant wheat genotype × isolate interaction. The var. *majus* isolates were the most pathogenic, causing shorter incubation and latent periods and greatest lesion lengths. OP1A/M was the most pathogenic and 44/3/M the least pathogenic of the var. *majus* isolates; var. *nivale* isolate SO28/2/N was the least pathogenic of all the isolates. In addition to lower pathogenicity, the var. *nivale* isolates appeared to differ from those of var. *majus* in that initial infection was not clearly observed under macroscopic examination; mycelial growth could be seen before obvious damage to leaf tissue and sporulation was much less pronounced often around the base of the leaf trichomes. In order to allow the PDR components to be clearly observed, particularly incubation and latent period, an incubation temperature of 10 °C and isolates of intermediate pathogenicity of *M. nivale* var. *majus*, omitting the most pathogenic isolate Dard1/M, were selected for evaluation of the commercial cultivars and CIMMYT genotypes.

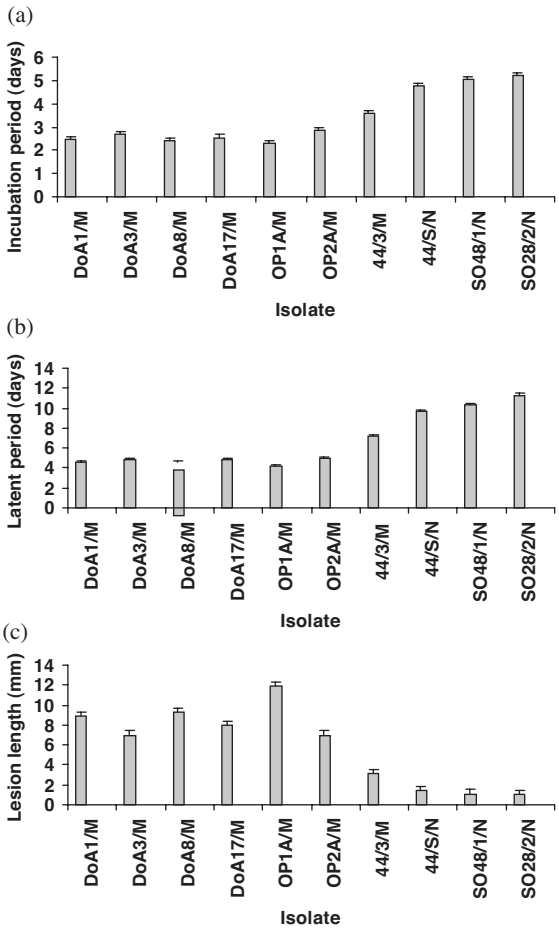

Figure 2. Incubation period (a), latent period (b) and lesion length (c) caused by seven isolates of *M. nivale* var. *majus* (M) and three isolates of *M. nivale* var. *nivale* (N) on detached wheat leaves. Bars represent standard errors of mean.

Detached leaf assay: experiment 1 – commercial cultivars

There were highly significant differences ($P < 0.001$) between commercial cultivars for each of the components of PDR: incubation (Figure 3a) and latent periods (Figure 3b) and lesion length (Figure 3c). However, correlations between the components of PDR, although significant, were low, particularly between incubation period and latent period ($r = 0.606$; $P < 0.001$) and lesion length ($r = -0.466$; $P < 0.01$). The correlation between latent period and lesion length was higher ($r = -0.825$; $P < 0.001$). The most resistant cultivars, with the longest incubation and latent periods and shortest lesion length in the detached leaf assay were, in order of ranking, Frontana, Tanker and Biscay for incubation period, Claire, Solstice and Frontana for latent period and Frontana, Claire and Solstice for lesion length. Spearman rank correlations between UK recommended list FHB ratings were significant for both incubation period ($r_s = 0.53$; $P < 0.05$) (Figure 4a) and latent period ($r_s = 0.70$; $P < 0.01$) (Figure 4b) but not for lesion length ($r_s = -0.26$) (Figure 4c).

In order to compare resistances measured in the detached leaf assay for genotypes with UK 2003 recommended list FHB resistance ratings, wheat genotypes were identified that showed the highest resistance across all three components of PDR. These were identified as genotypes that had longer incubation and latent periods or shorter lesion lengths than the mean of the three most resistant genotypes within each of the components of PDR, minus the least significant difference in the case of incubation period (LSD = 0.49) and latent period (LSD = 0.39),

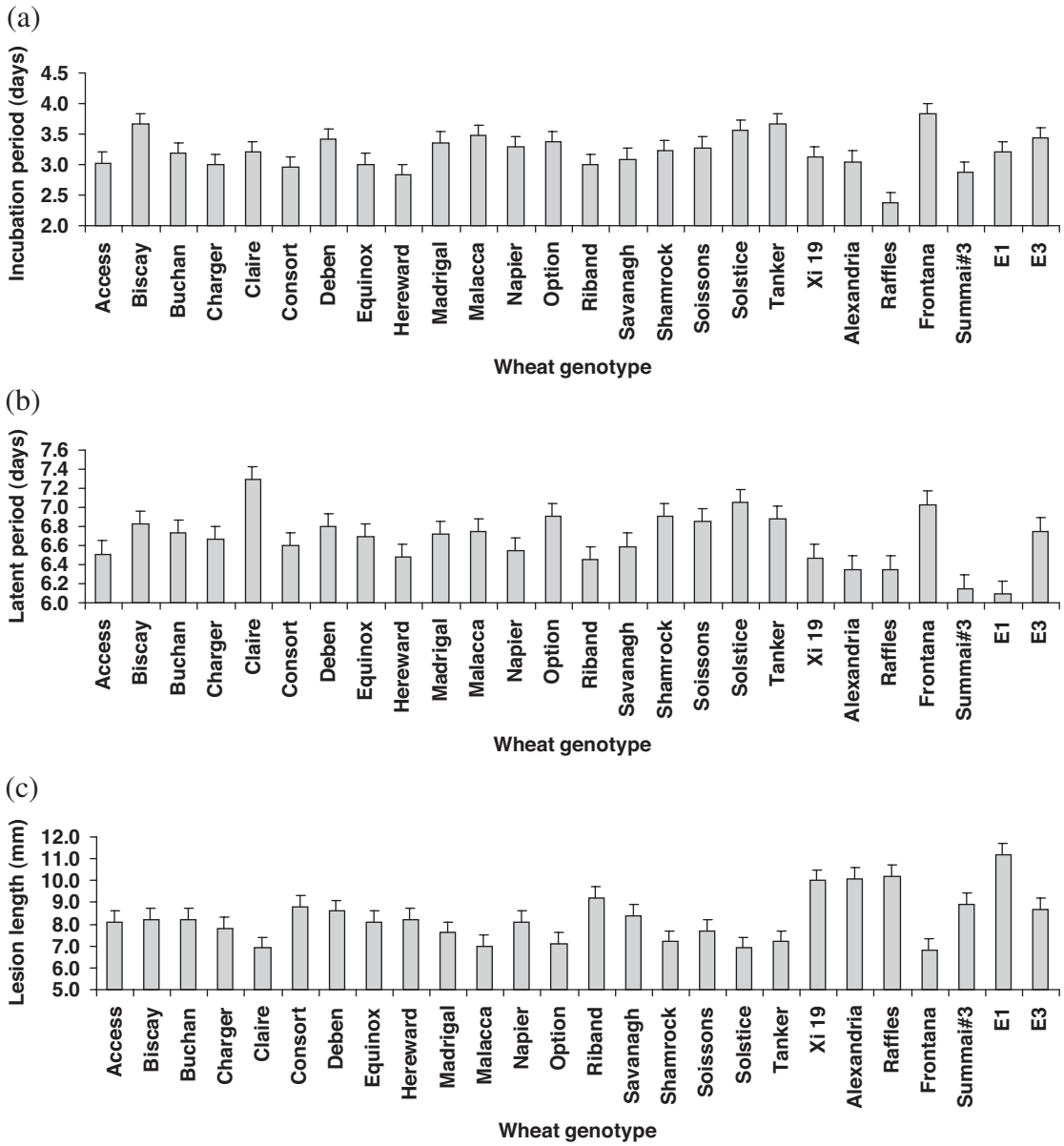

Figure 3. Incubation period (a), latent period (b) and lesion length (c) of cultivars on the UK recommended list 2002, spring wheat cvs Alexandria and Raffles, breeding germplasm Frontana and Summai #3 and CIMMYT entries E1 and E3, inoculated with isolates of *M. nivale* var. *majus* on detached wheat leaves. Bars represent standard errors of the mean.

plus the least significant difference for lesion length (LSD = 1.46). In the grouping for moderate levels of resistance in order of ranking, were Frontana, Tanker, Biscay, Solstice, Malacca, E3, Deben, Option, Madrigal, Napier, Soissons, Shamrock, Claire and E1 for incubation period (Figure 3a); Claire, Solstice, Frontana, Option, Shamrock, Tanker, Soissons, Biscay, Deben, E3 and Malacca for latent period (Figure 3b) and Frontana, Claire, Solstice, Malacca, Option, Shamrock, Tanker, Madrigal, Soissons, Charger, Equinox, Napier, Access, Biscay, Buchan and Hereward for lesion length (Figure 3c).

In the most resistant grouping for each component of PDR in the detached leaf assay, were Biscay with

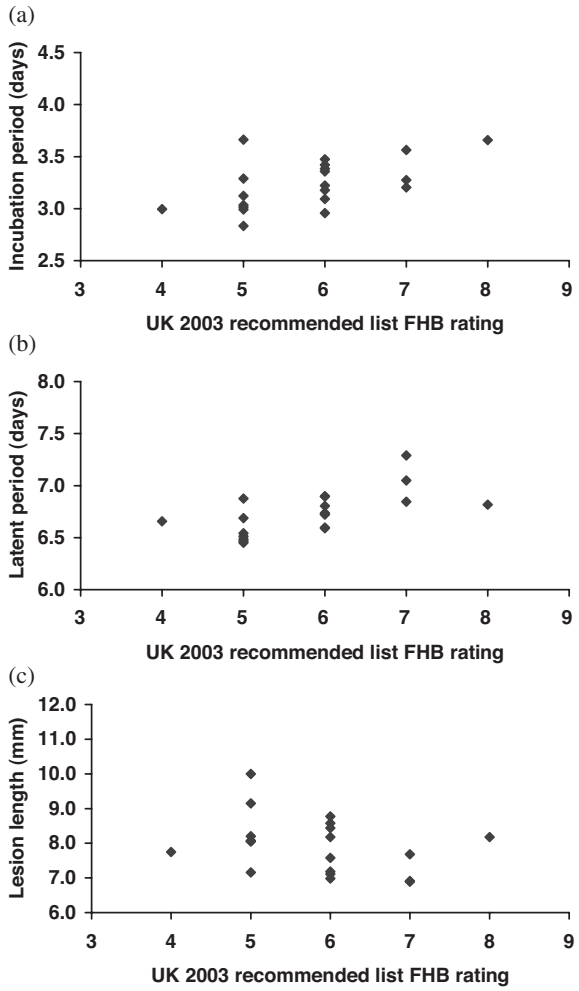

Figure 4. Incubation period (a), latent period (b) and lesion length (c) plotted against UK 2003 FHB ratings of the commercial cultivars.

a UK 2003 recommended list FHB rating of 8, Claire, Solstice and Soissons with ratings of 7 and Option, Malacca and Shamrock with ratings of 6. However, this group also included Tanker with a more susceptible UK list rating of 5. In the intermediate group in the detached leaf assay, with two components only of PDR in the resistant groupings, were Deben and Madrigal with a UK list rating of 6 and Napier with a rating of 5. The most susceptible cultivars in the detached leaf assay, with one or none of the components of PDR in the resistant groupings were Charger with a UK list rating of 4, Xi 19, Access, Hereward, Riband and Equinox with ratings of 5 and Consort, Savanagh and Buchan with ratings of 6. The spring wheat cvs Alexandria and Raffles also showed high susceptibility in the detached leaf assay. The genotype Frontana, known to have high field resistance to FHB, showed high resistance in the detached leaf assay; however Summai #3, also with high FHB field resistance, was amongst the most susceptible genotypes for all three components of PDR in the leaf assay.

Detached leaf assay: experiment 2 – CIMMYT genotypes

Within the group of genotypes comprised mainly of CIMMYT entries, there were highly significant differences between genotypes for incubation periods (Figure 5a), latent periods (Figure 5b) and lesion length (Figure 5c) ($P < 0.001$). As with the commercial cultivars, incubation period was again poorly correlated with latent period ($r = 0.498$; $P < 0.01$) and lesion length ($r = -0.685$; $P < 0.001$) but the correlation was again higher between latent period and lesion length ($r = -0.848$; $P < 0.001$). The most resistant genotypes were, in order of ranking, Frontana, E6 and E11 for incubation period, Biscay, E17 and E6 for latent period and Biscay, E6 and Soissons for lesion length. As with the commercial cultivars, genotypes were identified that had similar or longer incubation and latent periods or shorter lesion lengths than the mean of the most resistant genotypes within each of the components of PDR, minus the least significant difference in the case of incubation (LSD = 0.45) and latent periods (LSD = 0.43) plus the least significant difference for lesion length (LSD = 1.51). In the grouping for some level of resistance in order of ranking, were Frontana, E6, E11, E13, Soissons, E9, E17, E5, E8, E16, E1, Biscay, E14 and E10 for incubation period (Figure 5a); Biscay E17, E6, Frontana, E11, Soissons, Raffles, E9, E3, E16 and E15 for latent period (Figure 5b) and Biscay, E6, Soissons, E17, Frontana, E16, E14, E13, E9 and E4 for lesion length (Figure 5c).

Of the CIMMYT entries, E6, E9, E17 and E16 were in the most resistant grouping for each component of PDR in the leaf assay as were Frontana, Soissons and Biscay. E11 and E13 were in the intermediate grouping with two components only of PDR in the resistant groupings. Entries E2, E12, E18, E7 and E19 were the most susceptible, with none of the components of PDR in the resistant groupings as again were Summai #3 and Alexandria. Genotypes common to experiments 1 and 2, Frontana, Biscay and Soissons were among the

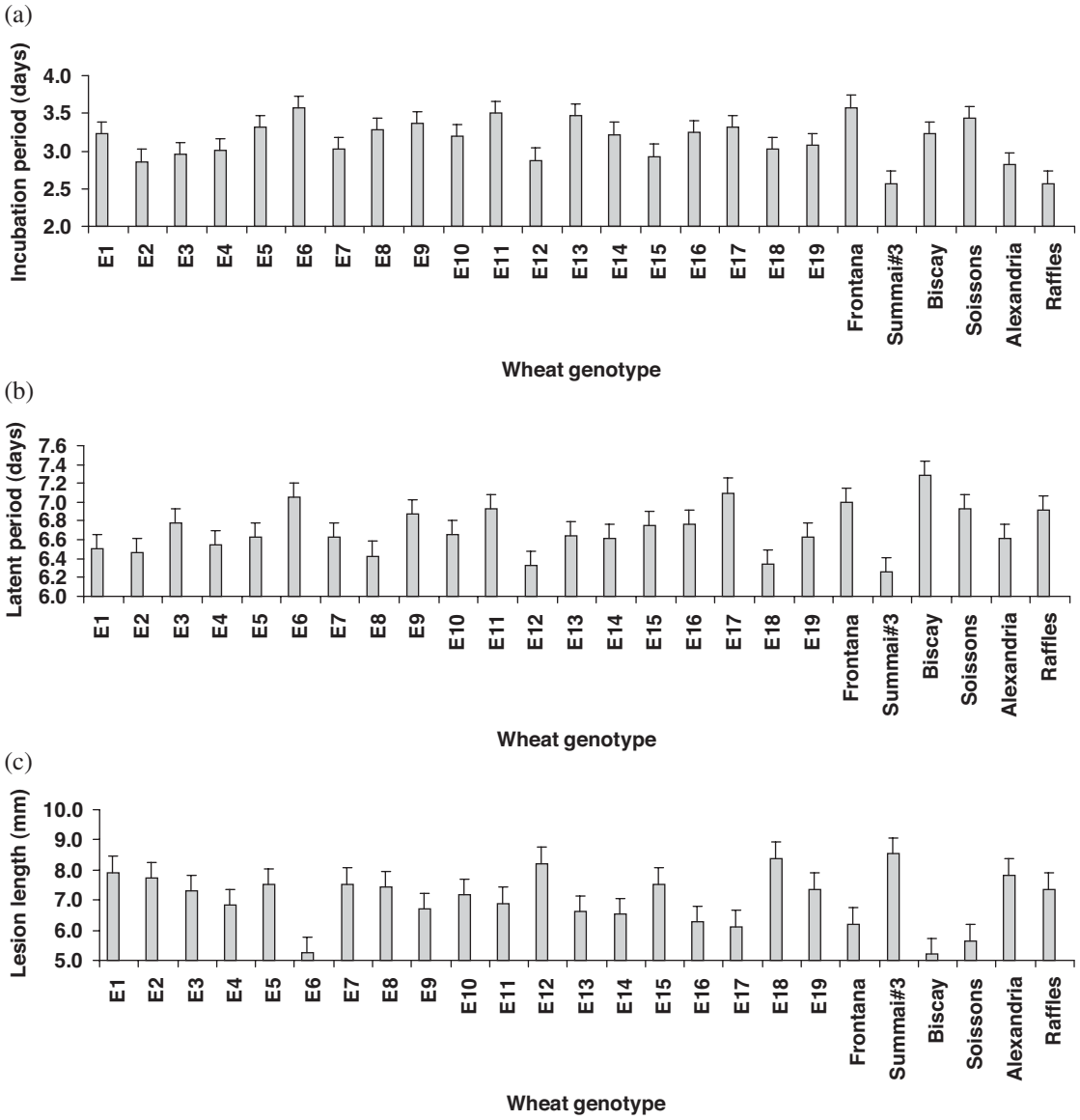

Figure 5. Incubation period (a), latent period (b) and lesion length (c) of CIMMYT genotypes E1–E19, breeding germplasm Frontana and Summai #3, and commercial cvs Biscay, Soissons, Alexandria and Raffles inoculated with isolates of *M. nivale* var. *majus* on detached wheat leaves. Bars represent standard errors of the mean.

most resistant and Alexandria, Raffles, Summai #3 and E1 the most susceptible. Cultivar Raffles was amongst the most susceptible for all three components of PDR but had a longer latent period relative to Alexandria in experiment 2 than in experiment 1. It was notable that the components of PDR of the most resistant commercial cvs Claire, Solstice and Biscay measured in the detached leaf assay were not significantly different to those of the most resistant unadapted germplasm evaluated, including Frontana.

There were significant correlations, although weak, between PDR components in the detached leaf assay and FHB severity of the 19 CIMMYT entries (Table 1). However, in contrast to what was observed in the commercial cultivars, higher resistances in the PDR components were associated with higher FHB susceptibility

(incubation period $r = 0.52$; $P < 0.01$, latent period $r = 0.53$; $P < 0.01$, lesion length $r = -0.49$; $P < 0.01$). This was particularly evident in two of the most FHB resistant genotypes E2 and E12 which were amongst the poorest genotypes for all three PDR components in the detached leaf assay.

Discussion

Investigations were carried out to investigate an appropriate *in vitro* protocol for manipulating *M. nivale* and the environment to maximise wheat host differences in PDR components. Although all isolates were most pathogenic at 20 °C, the development of the pathogen was too rapid to differentiate between wheat genotypes, particularly those with intermediate resistance. For the same reason, the most pathogenic isolates of *M. nivale* var. *majus* were also avoided. Isolates of *M. nivale* var. *majus* were more pathogenic than those of var. *nivale*, agreeing with previous reports (Diamond and Cooke 1997; 1999) and allowed components of PDR to be more clearly observed. For the pre-screening purposes outlined in this paper, the authors consider an incubation temperature of 10 °C and inoculation with isolates of intermediate pathogenicity of *M. nivale* var. *majus*, to be most suitable conditions for detecting variation in PDR components, particularly incubation and latent period, in the detached leaf assay. There was no evidence that varying incubation temperatures and isolates affected the relative resistances of the wheat genotypes in the detached leaf assay.

Relationships between individual components of PDR are not clear; these require further investigation as to their relative importance with regard to FHB resistance in whole plants. Clearly pathogen development, sporulation and lesion size on detached leaf segments are not simply a function of initial infection or incubation period, as both latent period and lesion length were relatively poorly correlated with this component. Resistance factors in each component of PDR may therefore be under separate genetic control. Until the relationships between individual components of PDR and FHB resistance are investigated further, it is probably more appropriate to consider all three PDR components simultaneously.

There were significant correlations between the PDR components, incubation period and latent period, and FHB resistance ratings with higher resistances in the detached leaf assay associated with higher resistance to FHB. Importantly, all commercial cultivars with high whole plant resistance ratings to FHB showed high levels of resistance in the detached leaf assay, suggesting that resistances measured in the leaf assay are under the same genetic control as a major component of the FHB resistances expressed in commercial cultivars. There was, however, a notable exception in cv. Tanker which had a higher susceptibility rating than results from the detached leaf assay suggest, indicating that a susceptibility factor, not detected by the detached leaf assay, may be present. In addition, cultivars with an intermediate FHB resistance rating of 6 were present in groups with the highest, intermediate and lowest resistances in the detached leaf assay. This may be due to variation in FHB resistance of cultivars with the inoculation method used for detecting Type I resistance (John Clarkson, NIAB, Cambridge, UK, personal communication) and the difficulty in separating the cultivars into distinct groups on the basis of PDR components in the detached leaf assay and for whole plant FHB ratings. However, while resistances measured in the detached leaf assay may be important components of whole plant resistance to FHB, and unexplained variation may reflect in part the difficulty in determining small differences in FHB, other resistance mechanisms and susceptibility factors may also be present in commercial cultivars. Plant characteristics which would confer disease escape include plant height, absence of awns (Mesterhazy, 1995) and flowering in the boot stage (Cook, 1981); the detached leaf assay does not detect such characteristics. It is not known how resistance to FHB determined by point inoculation and often termed Type II relates to resistance presented in the UK recommended list or to that detected in the detached leaf assay. Nevertheless, with the exception of the higher susceptibility FHB rating of cv. Tanker, there was no major reranking of cultivars, and therefore the effects of other resistance mechanisms, if present, were subordinate to those measured in the detached leaf assay. It would however, be desirable to further elucidate additional resistance mechanisms and susceptibility factors if present.

In addition to the commercial cultivars in experiment 1 the world renowned resistant genotypes Frontana and Summai #3 were included for comparison. The poor resistances detected in the detached leaf assay for Summai #3, which was as susceptible as the most susceptible commercial cultivars, contrasted with the high resistance found for Frontana, both of which are reported to have different resistance backgrounds (Singh and van Ginkel, 1997). The current work supports the view that the resistance backgrounds

of both genotypes are different, Summai #3 not possessing resistances detected by the detached leaf assay. However, while Frontana had high resistances in the detached leaf assay these were not superior to the most resistant commercial cultivars. Biscay, the commercial cultivar with the highest resistance rating of UK commercial cultivars, is known to be considerably inferior to the resistance found in highly resistant germplasm (Akos Mesterhazy, Cereal Research non profit Co., Szeged, Hungary, personal communication); therefore it is unlikely that resistances detected in the detached leaf assay alone are responsible for the high FHB resistance of Frontana, although they are likely to play some part in overall resistance. Again, this view is consistent with a number of separate resistances being responsible for FHB resistance in different genotypes and which could be present in a number of combinations.

In contrast to the commercial cultivars, higher resistances in the detached leaf assay were associated with higher FHB susceptibility in the CIMMYT genotypes, although correlations were weak. This pattern was particularly evident in genotypes E2 and E12, identified as having potential for use in a breeding programme due to their high whole plant FHB resistance (Dardis and Walsh, 2003), and as with Summai #3, showed very low levels of resistance in the detached leaf assay. It appears that in germplasm used for breeding including Frontana, Summai #3, E2 and E12, there are highly effective FHB resistance mechanisms expressed in adult plants that do not appear to be present in the commercial cultivars evaluated here. It is not clear what these resistance mechanisms may be or why they do not appear to be present in Irish and UK commercial cultivars. However, Chen et al. (1997) found that high-yielding progenies of crosses from Summai #3 did not reach the level of resistance of the resistant parents, a view supported by Mesterhazy et al. (1999). It is possible, therefore, that the resistance mechanism or a significant component of the resistance mechanism in unadapted germplasm such as Summai #3 may not be compatible with a high-yielding genotype. This is of particular concern, as substantial parts of current breeding efforts against FHB are concentrated on incorporating resistance derived from Summai #3 (Ruckenbauer et al., 2001).

Within the CIMMYT genotypes, E11 is notable as it had higher levels of resistance than cv. Alexandria in the detached leaf assay, yet was found by Dardis (2000) to be more susceptible in whole plant evaluation. The reason for this discrepancy is unclear; it may be due to an added susceptibility factor, different responses to *F. culmorum* and *M. nivale*, or more importantly, all resistances expressed in detached leaves not being expressed in the head in all genotypes. The interaction of resistances detected in the detached leaf assay with more effective resistances in highly resistant germplasm therefore requires further investigation.

Previous studies have failed to show a relationship between juvenile plant resistance and whole plant resistance (Miedaner, 1997). In the present paper, the genotypes were comprised of distinct groups, the commercial cultivars and the unadapted apparently resistant germplasm. In a breeding nursery, resistances measured in the detached leaf assay are likely to be combined with mature plant resistances (not detected by the detached leaf assay); this might explain the lack of correlation in previous studies with *F. culmorum* between reaction of leaves of intact seedlings and whole plant FHB resistance (Miedaner, 1997). The detached leaf assay offers the advantages that conditions are more controlled, assays can be replicated and repeated more readily than whole plant work, and the individual measurement of three or more components of PDR is possible rather than disease severity or incidence only. Whereas, in the present work, *F. culmorum* was evaluated for use in the detached leaf assay and showed growth on the leaf surface, it did not readily cause discrete necrotic lesions even when growth of mycelium was quite extensive over the leaf surface. Measuring components of PDR in the detached leaf assay using *F. culmorum* would therefore be problematic; other members of the FHB complex may also be unsuitable for the same reason.

The results of the current research raise doubts that selection of FHB resistant breeding material to minimise mycotoxin levels in grain should concentrate solely on visual symptoms of FHB in whole plants, as some resistance mechanisms may not be compatible with the agronomic requirements of commercial cultivars. Evidence that resistances measured in the detached leaf assay are those expressed in the head is circumstantial; it would be desirable to further elucidate the resistance mechanisms expressed in detached leaves and mature plants to clarify any relationship between them. Nevertheless, the detached leaf assay shows good potential for use in breeding programmes for pre-screening FHB resistance, particularly where adult plant specific resistance, not detected by the detached leaf assay, does not appear to be compatible with agronomic requirements. It is clear that the

detached leaf assay does not lessen the necessity for whole plant evaluation of FHB resistance under field conditions; this is required to ensure resistance is expressed in the head and to avoid possible susceptibility factors. While many of the cultivars reported here were very susceptible or had only intermediate resistance, it is notable that the most resistant commercial cultivars had a level of resistance in the detached leaf assay comparable to the best unadapted germplasm evaluated. It is not known if superior levels of resistance detectable by the detached leaf assay are available. At present, germplasm with the most promising levels of resistance may not have been selected, as this may have been masked by the FHB resistance expressed in adult plants such as Summai #3 not detected by the detached leaf assay. In addition to its potential as a rapid pre-screening tool in breeding, the detached leaf assay has the advantage that it accurately measures specific resistances; this may allow greater potential for elucidating the genetic nature of resistance to FHB.

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