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Components of Partial Disease Resistance in Wheat Detected in a Detached Leaf Assay Inoculated with *Microdochium majus* using First, Second and Third Expanding Seedling Leaves

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Abstract

The use of first, second and third expanding seedling leaves of wheat (L1, L2 and L3 respectively), inoculated with conidial suspensions of *Microdochium majus* (syn. *Microdochium nivale* var. *majus*) in a detached leaf assay, for detecting components of partial disease resistance (PDR) was investigated across a range of wheat cultivars. Incubation periods (period from inoculation to first appearance of symptoms; a dull grey-green water-soaked lesion) and latent periods (period from inoculation to the first appearance of sporodochia) were longest and lesions smallest on L3. The expression of PDR components on L2 was intermediate to those on L1 and L3. The longer latent periods on L3 typically occurred after leaf senescence contrasting with latent periods on L1 and L2 where sporulation most frequently occurred on relatively green leaf tissue. Cultivar differences in the first appearance of symptoms, incubation period, which occurred before any leaf senescence was observed, correlated significantly across all leaf positions. Similarly cultivar differences in latent period were correlated for L1 and L2. However, latent periods on L3, which were the least consistent between cultivars across experiments, were not correlated with those of L1 or L2 in any experiment. The results indicate that due to the delay in sporulation until after leaf senescence, observations on latent period in L3 are less representative of what occurs in the whole plant where infection of living tissue is of greatest interest. This work indicates that the selection of the first or second expanding leaf of wheat is optimal for the use in the detached leaf assay using *M. majus* for studying components of PDR.

Introduction

Partial disease resistance (PDR) is characterized by a reduced rate of epidemic development in a host population attributed to various components of PDR including lower infection frequency and a longer latent period (period from inoculation to sporulation). *In vitro* detached leaf assays have been used as a tool for investigating PDR in the whole plant for a wide range of diseases including *Septoria tritici* (Arraiano et al., 2001; Chartrain et al., 2004) rust resistance in *Salix* to *Melampsora larici-epitea* (Pei et al., 2004) and against Fusarium head blight (FHB; Diamond and Cooke, 1999; Browne and Cooke, 2004b; Browne et al., 2005). In FHB research, the detached leaf assay has the advantage of enabling more insight into complex polygenic nature of resistance than can be distinguished using whole plant evaluation alone (Browne et al., 2005). However, an important challenge is to optimize the *in vitro* technique for detecting cultivar (cv.) differences and understanding how resistance observed in individual plant organs and growth stages relates to disease resistance in the whole plant.

The level of expression of PDR is known to vary with plant development. For example, partial resistance is greater in late expanding leaves against rust in wheat (Singh and Huerta-Espino, 2003) and resistance to powdery mildew detected on leaf segments in oat (Roderick and Clifford, 1995). Conversely, Arraiano et al. (2001), using a detached leaf assay in wheat, found that secondary leaves were more susceptible against *S. tritici* than first expanding leaves. The selection of leaves for use in a detached leaf assay may, therefore, be of importance with regard to discrimination

of cultivars for disease resistance. Components of PDR have been studied in wheat using *Microdochium majus* (Wollenw.) Glynn & S.G. Edwards (syn. *Microdochium nivale* var. *majus*) through inoculation on the first expanding (Browne and Cooke, 2004b; Browne et al., 2005) and second expanding leaves (Diamond and Cooke, 1999) using a detached leaf assay. However, it was not known how leaf position affected the relative determination of PDR components in this assay.

The objective of this research was to assess components of PDR in the first, second and third expanding leaves across a range of wheat cultivars to consider utility of the different leaf classes for prescreening of PDR components using a detached leaf assay.

Materials and Methods

Wheat genotypes used in these experiments, predominantly European wheat cultivars or breeding lines, are listed in Table 1. Germplasm previously evaluated in the detached leaf assay included the wheat cvs Claire, Alexandria (Browne and Cooke, 2004b), the US genotype VA01W476 and the South American cv. Frontana (Browne et al., 2005). The parents of a Norwegian mapping population using the Swiss cv. Arina and a Norwegian breeding line NK96360 were included in Experiment 4.

In Experiments 1, 2 and 3, the detached leaf assay was conducted as described by Browne and Cooke (2004b) using leaf segments 4 cm in length from the mid-section of the first (L1), second (L2) and third (L3) expanding seedling leaves of 4-week-old plants. In Experiment 4, the six cultivars (Bjørke, Claire, Arina, Alexandria, Magnifik and NK93604 were grown for 3 weeks in a greenhouse in Norway during April–May, 2005. Supplementary light was used for 16 h during day with temperatures of 18°C–22°C and night temperature of 12°C (8 h). Five centimetre segments from

the mid-section of first, second and third leaves were cut and mounted on water agar for inoculation as described by Browne and Cooke (2004b). Conidial suspensions of *M. majus*, single-spore isolate OP2A, were used in all experiments produced as described by Browne and Cooke (2004a) with Cellophane (Sigma-Aldrich, St. Louis, MI, USA). The components of PDR measured were incubation period (days from inoculation to first symptom development, a dull grey–green water-soaked lesion), latent period (days from inoculation to sporulation) and in Experiment 4, lesion size measured as lesion length determined over a light source (length of water-soaked area) 7 days after inoculation.

Statistical analysis

ANOVA were conducted using Genstat V software. The factorial experiments were randomized block design with leaf position L1, L2 and L3, and cultivar as factors with five replicates in Experiments 1, 2 and 4, and six replicates in Experiment 3. Each experiment was considered a repeat for observations of the effect of leaf position on the expression of PDR components with common cultivars between experiments (Table 1). Correlation analyses were conducted using Microsoft Excel across Experiments 1, 2 and 3 but not for Experiment 4 in which only six cultivars were included.

Results

Incubation period

In all experiments (1–4), there were highly significant differences in incubation period due to leaf position ($P < 0.001$) and between wheat genotypes (Figs 1a, 2a,

Table 1
Wheat cultivars used in the detached leaf assay in Experiments 1–4

Experiment 1	Experiment 2	Experiment 3	Experiment 4
Lona	Lona	–	–
Greina	Greina	–	–
Fiorina	Fiorina	Fiorina	–
Nadro	Nadro	Nadro	–
Toronit	Toronit	Toronit	–
A6	A6	A6	–
A7	A7	–	–
A8	A8	–	–
Sonalika	Sonalika	–	–
Backup	Backup	Backup	–
ND2710	ND2710	–	–
Wheaton	Wheaton	–	–
	Frontana	Frontana	–
	Arina	Arina	Arina
	Orsino	–	–
	Forno	–	–
		VA01W476	–
			Claire
			Bjorke
			Magnifik
			NK93604
			Alexandria

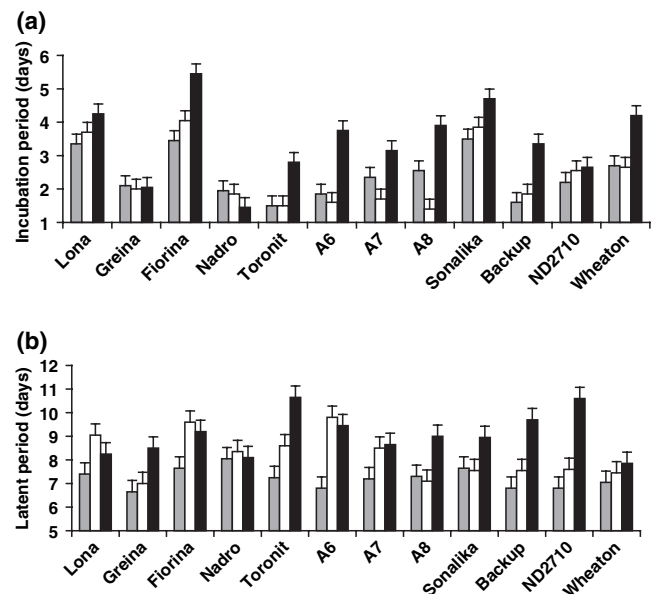


Fig. 1 Experiment 1. (a) Incubation period and (b) latent period of the first (L1; □), second (L2; ▒) and third expanding leaves (L3; ■) inoculated with *Microdochium majus* isolate OP2A. Bars represent standard error of the mean of the leaf position by cultivar interaction

3a and 4a respectively). There was no significant interaction of cultivars with leaf position for incubation period in Experiments 2, 3 or 4 although there was a significant interaction in Experiment 1 ($P < 0.001$). Incubation period was consistently higher in L3 than in L1 across all cultivars and experiments (Experiment 1: Fig. 1a; Experiment 2: Fig. 2a; Experiment 3: Fig. 3a; Experiment 4: Fig. 4a) with the exception of Nadro in Experiment 1 contributing to a significant cultivar \times leaf position interaction). This observation was not repeated for cv. Nadro in Experiment 2 or 3. The incubation periods observed in L2 were more similar to that of L1 and generally shorter than L3. In all experiments, incubation periods of L1, L2 and L3 were significantly correlated (Table 2).

The overall objective of these experiments were to evaluate leaf position in relation to PDR components detected using the detached leaf assay including a wider range of germplasm with subsets of cultivars common between experiments. Nevertheless cvs Toronit and Nadro had amongst the shortest incubation periods and cv. Orsino amongst the longest. In Experiment 4, cv. Toronit had a short incubation period similar to the winter wheat line VA01W476. Overall differences in incubation period were readily observed across all leaf positions.

Latent period

Across all experiments, the latent period of L3 was significantly longer ($P < 0.001$) than that of L1 while that of L2 was intermediate to that of both L1 and L3

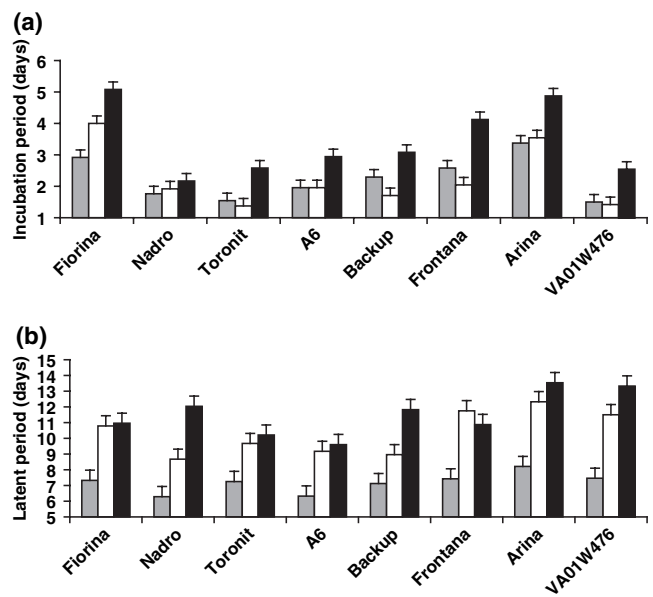


Fig. 3 Experiment 3. (a) Incubation period and (b) latent period of the first (L1; ■) second (L2; □) and third expanding leaves (L3; ■) inoculated with *Microdochium majus* isolate OP2A. Bars represent standard error of the mean of the leaf position by cultivar interaction

(Figs 1b, 2b, 3b and 4b). Latent periods of L3 increased more in cultivars with shorter latent period in L1 and L2 so that latent periods were more similar across all cultivars in L3. In Experiments 2 and 3, L1 latent period (LP L1) was significantly correlated with

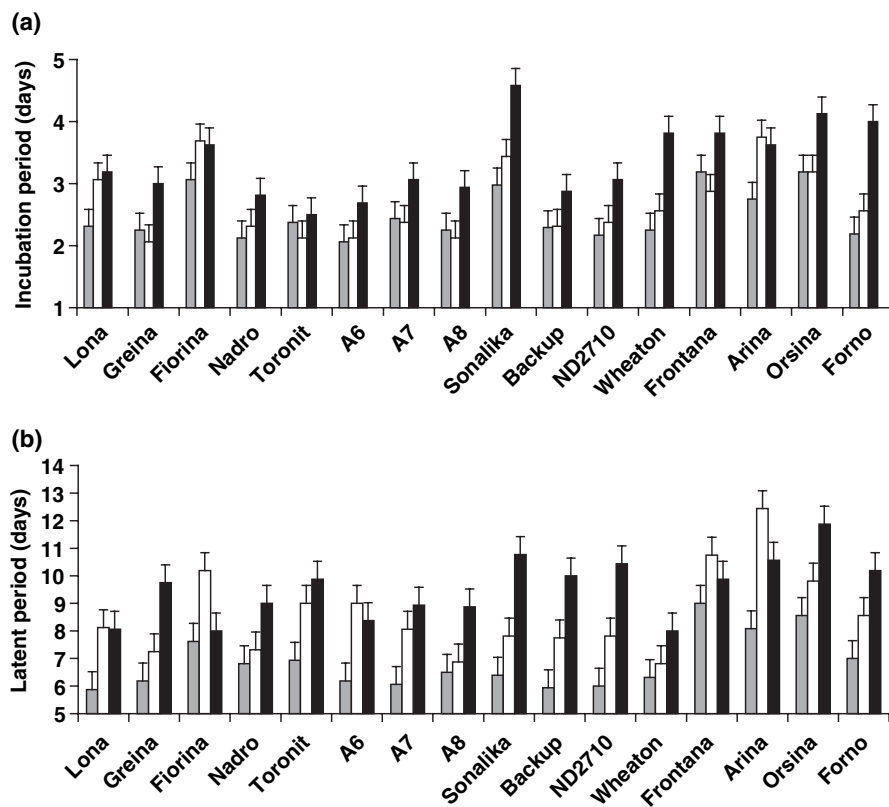


Fig. 2 Experiment 2. (a) Incubation period and (b) latent period of the first (L1; ■) second (L2; □) and third expanding leaves (L3; ■) inoculated with *Microdochium majus* isolate OP2A. Bars represent standard error of the mean of the leaf position by cultivar interaction

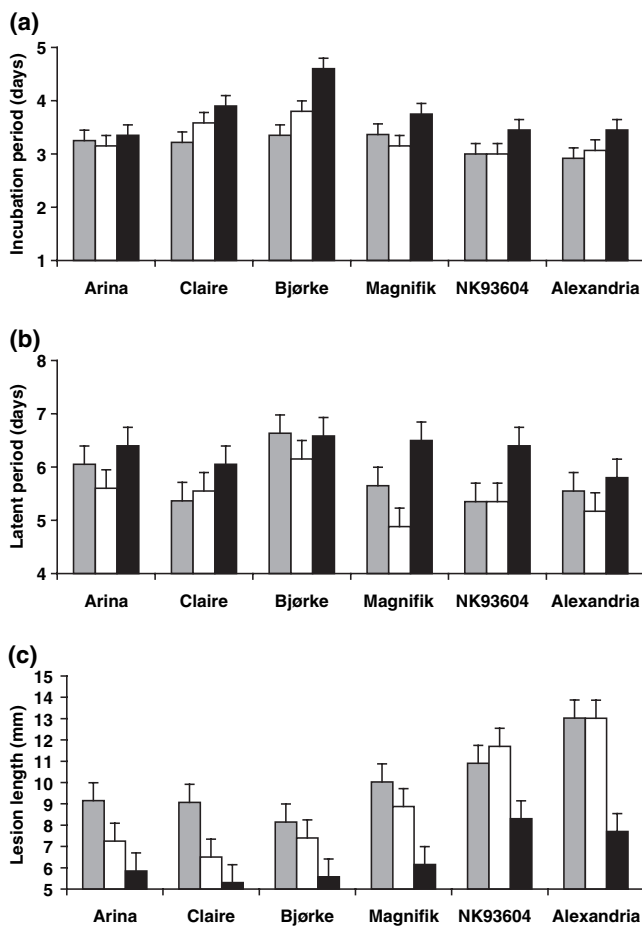


Fig. 4 Experiment 4. (a) Incubation period, (b) latent period and (c) lesion length measured on day 7 postinoculation of the first (L1; ■) second (L2; □) and third expanding leaves (L3; ■) inoculated with *Microdochium majus* isolate OP2A. Bars represent standard error of the mean of the leaf position by cultivar interaction

Table 2 Correlation coefficients between incubation periods (IP) and latent periods (LP) of wheat cultivars using the first, second and third expanding seedling leaves (L1, L2 and L3 respectively)

	IP L1	IP L2	IP L3	LP L1	LP L2
Experiment 1					
IP L2	0.88***				
IP L3	0.74**	0.67*			
LP L1	NS	NS	NS		
LP L2	NS	NS	NS	NS	
LP L3	NS	NS	NS	NS	NS
Experiment 2					
IP L2	0.76***				
IP L3	0.67**	0.71**			
LP L1	0.77***	0.53*	0.46*		
LP L2	0.63**	0.65**	NS	0.78***	
LP L3	NS	NS	NS	NS	NS
Experiment 3					
IP L2	0.87**				
IP L3	0.94**	0.89**			
LP L1	NS	NS			
LP L2	NS	NS	NS	0.85**	
LP L3	NS	NS	NS	NS	NS

*P < 0.05, **P < 0.01, ***P < 0.001; NS, not significant.

LP L2, indicating a close relationship between the resistance expressed in both L1 and L2. The lack of correlation between latent period of cultivars assessed in L3 compared with L1 or L2 was reflected in significant cultivar by leaf age interactions (Experiment 1: P < 0.001; Experiment 2: P < 0.01; Experiment 3: NS; Experiment 4: NS). It was notable that the delay in sporulation on L3 was accompanied by more extensive chlorosis and death of leaf tissue before sporulation occurred in contrast to sporulation on green leaf tissue on L1 and L2 (symptoms not presented; see Browne and Cooke, 2005b for figures of incubation period and latent period). This senescence and chlorosis appears to inhibit sporulation for a period of time until sporulation occurs profusely over dead leaf tissue. Across all experiments, differences in latent period were most clearly observed in L1 and L2. The longer latent periods in L3 did not appear to offer any advantage in discrimination between cultivars. Observations on latent periods in L3 were the least consistent for cultivars across experiments. In Experiment 4 (Fig. 4c), where lesion length was measured, L3 was found to be more resistant with more restricted lesion development on day 7 post inoculation than L1 and L2. As for latent period, discrimination between cultivars was greatest for L1 and L2.

Discussion

In all cultivars and across experiments, the third expanding leaves (L3) were more resistant to *M. majus* with longer incubation and latent periods and more restricted lesion development than L1 and L2. The current findings reflect an increase in resistance of later developing leaves against snow mould caused by *M. nivale* (Ergon and Tronsmo (in press)). In the present study, the repeatability of our observations of differences between L1, L2 and L3 have been demonstrated over several experiments and across a wide number of cultivars. Cultivar differences were more thoroughly investigated in previous publications where experiments are repeated for all cultivars at least twice. Nevertheless, observations of cultivars were in broad agreement with previous studies (Browne and Cooke, 2004b; Browne et al., 2005).

The PDR components are polygenic resistances and as yet we do not have a full understanding of the underlying mechanisms. However, for PDR components detected using the detached leaf assay described in the present experiments the underlying mechanisms are likely to be similar within each cultivar across the leaf positions. This is supported by significant correlations of the incubation period across L1, L2 and L3 and those for latent period between L1 and L2. The latent period observed in L3 differed from that on L1 due to a longer period from inoculation until sporulation and thus senescence of the detached leaves before sporulation occurred. The leaf senescence may have had an inhibiting effect on sporulation for a period before sporulation occurred profusely over leaf stomata. This differed markedly from visual observations

of sporulation on L1, which occurs predominantly on relatively green leaf tissue and may explain why there were no significant correlations for latent periods of L3 with those of L1 or L2. The utility of the *M. majus* detached leaf assay is to detect components of PDR to FHB in living tissue particularly at anthesis; therefore, the senescence in L3 before sporulation is likely to relate less well to disease resistance against FHB in whole plant. It is notable that even in Experiment 4 with more rapid symptom development there was no benefit of longer latent periods of L3 to allow discrimination between cultivars. However, where infection is more rapid, as in Experiment 4, L2 may be more useful than L1. Resistance detected in the detached leaf assay is common for both *M. majus* and *M. nivale* (Diamond and Cooke, 1999; Browne and Cooke, 2004b) both elevated to species status (Glynn et al., 2005) although *M. majus* used in this study provides more distinct symptoms.

Our results, in which senescence of leaf tissue before symptom development altered the accuracy with which PDR resistance was determined, have implications for the use of detached leaf assays to determine resistance to other diseases. Using whole plants, which prevents the senescence associated with detached leaves, Singh and Huerta-Espino (2003) found that cultivar differences were most clearly observed in later forming leaves which showed greater resistance. For *S. tritici* with a relatively long period between inoculation and symptom development in a detached leaf assay, Arraiano et al. (2001) inoculated seedling leaves prior to detaching the leaves and mounting them on water agar.

The detached leaf assay for prescreening resistance to FHB is being utilized together with methods to determine independent resistance factors that the leaf assay does not detect, including resistance against *Fusarium* spp. in a seed germination assay and morphological factors specific to the wheat spike (Browne and Cooke, 2005a), to understand the sum of PDR components against FHB within the whole plant. Our present work confirms the suitability of using of L1 (Browne and Cooke, 2004b; Browne et al., 2005) and L2 (Diamond and Cooke, 1999) to prescreen for components of PDR in the *M. majus* detached leaf assay.

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