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Pathogenic variation in populations of *Drechslera teres* f. *teres* and *D. teres* f. *maculata* and differences in host cultivar responses

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Abstract

The current study examined the variability in the pathogenicity of populations of *Drechslera teres* f. *teres* and *D. teres* f. *maculata* (the net and spot forms of *D. teres*) from Ireland and northern Europe. A population of progeny isolates from a mating of net and spot forms was also examined. Significant variation in virulence was found both between and among net form and spot form isolates ($p < 0.001$). In the Irish population, significant differences were found between the net and spot forms, with the spot form isolates more virulent ($p < 0.05$). Progeny isolates were significantly more virulent than net form or spot form populations ($p < 0.001$). Significant differences were found in cultivar reactions, with cv. Botnia most susceptible to both forms of the pathogen ($p < 0.001$). Cultivar Boreal 94145, although quantitatively resistant, was found to be very susceptible to both forms of the pathogen and to progeny isolates. Cultivars CI 5791, CI 2330 and CI 9819 were all less susceptible to infection by both forms, but were more susceptible to spot form isolates. Significant correlations were found between whole plants and detached leaf experiments for the net form isolates only ($p < 0.001$). This study illustrates the importance of including both net form and spot form isolates in resistance studies and the need for a clearer understanding for the genetic basis of resistance to the net and spot forms. It also highlights the limitations of using a detached leaf assay for screening of net blotch of barley.

Introduction

Pyrenophora teres (anamorph *Drechslera teres*), the causal agent of net blotch disease of barley, is one of the most important diseases of barley crops causing yield losses in all cereal growing regions of the world. Two forms of the pathogen have been described based on the symptoms they incite on the host plant, namely the net and the spot form. Net blotch disease in both its forms has been reported to cause significant yield losses in the field ranging from 10 to 40% (Jordan, 1981; Parry, 1990; Steffenson et al., 1991; Wilcoxson et al., 1992; Gupta and Loughman, 2001). In the current agricultural climate where emphasis is on reducing costs and fungicide inputs, the use of resistant cultivars has become vital.

Before initiating an effective breeding programme, information on the variation and distribution of pathogenic isolates of a pathogen and variation in host resistance is desirable. Ho et al. (1996) reported inheritance of resistance to the two forms of net blotch is inherited independently. The ability of plant pathogen populations to infect host

plants can change with time as new cultivars are produced (Parry, 1990). This change in pathogenicity was reported by Khan (1982) for *D. teres* in relation to changes in cultivars grown in Australia. Khan and Boyd (1969) reported the occurrence of three physiological races but when the population was re-examined 10 years later, a new group was identified which arose presumably in response to changes in the cultivars used.

The two forms of *P. teres* have been found to mate readily in culture, the progeny segregating into either the parental forms or two recombinant forms resulting in intermediate or flecking-type symptoms on the host plant (Smedegaard-Petersen, 1971, 1977). That two forms readily intercross suggests that they must be considered as two forms of the same species rather than as different species (Smedegaard-Petersen, 1971, 1977; Scott, 1991). Wu et al. (2003) using RFLP profiles were unable to separate the two forms and reported that the genetic distance between isolates of the two forms was similar to that of isolates among the same form. However, a recent report by Leisova et al. (2005) based on AFLP data suggests that the two forms are distinct species.

Differences in pathogenicity between the net and spot forms of the pathogen have been reported (Khan, 1982; Bockelman et al., 1983). Khan (1982) found that net and spot form isolates differed in pathogenicity on two Canadian cultivars. Because the pathogenicity patterns of the two forms of the pathogen differ, attention must be given to finding new sources of resistance to both forms. The objective of this research was to examine the variation in virulence of populations of *D. teres*, to compare reactions of *D. teres* isolates on different cultivars and to assess the use of a detached leaf assay for screening possible sources of host resistance.

Materials and methods

Isolate collection and preparation of inoculum

Isolates from different countries in Europe and progeny of a mating of net form and spot form isolates were obtained from the Boreal Research Centre, Finland and host cultivar and location recorded (Table 1a–c). Leaf material was surface-sterilised with 50% ethanol for 15 s and then in 2% sodium hypochorite (NaOCl) for 30 s and finally in sterile distilled water (SDW). The material was then plated onto lima bean agar (LBA, Oxoid, UK) under aseptic conditions (Sharma, 1984; Robinson and Jalli, 1997). The Petri dishes were incubated for 14 days at 20 ± 2 °C under a near-ultraviolet (NUV) light diurnal cycle. Isolates from Ireland were obtained both from leaf material and seed from different regions of the country. Seeds were initially surface-sterilised in NaOCl for 5 min, rinsed in SDW and plated onto potato dextrose agar (PDA, Oxoid, UK). *Drechslera teres* colonies were sub-cultured onto PDA, incubated for 7 days and single-spore isolates obtained. Spore suspensions were made by flooding the plates with SDW and gently agitating the conidia with a sterile blade. A few drops of this suspension were then spread on fresh LBA plates and incubated for a further 14 days (Robinson and Jalli, 1997). Conidial suspensions for inoculum were made by flooding the LBA plates and agitating as before. These were then transferred to glass test tubes, homogenised, sieved through sterilised cheese cloth and concentrations estimated using a haemocytometer. Each isolate spore suspension was adjusted to give a final concentration of approximately 2×10^4 spores ml^{-1}.

Glasshouse experiment

The glasshouse experiment was set up in a split-block design with cultivar as the main plot and isolate as the sub-plot. The cultivars used in the current study were: Boreal 94145, Botnia, CI 2330, CI 5791, CI 9819 and CI 5822. Boreal 94145 was selected for its quantitative resistance properties, Botnia was selected as a susceptible cultivar and does not contain any known resistance genes, CI 5791, CI 2330, CI 9819 and CI 5822 were selected as resistant cultivars and all appear in the list of differentials of Afanasenko et al. (1995); in addition, CI 5791 has been found to retain its resistance to the net form of the pathogen but was susceptible to the spot form of the pathogen (Tekauz and Mills, 1974) and CI 2330 contains the resistance gene *Pta*. Three replicates of each treatment were carried out. Two seeds were sown per 5 cm dia pot and pots were arranged randomly in the glasshouse. Plants were grown under natural light conditions at 20 °C until growth stage (GS) 13 (Zadoks et al., 1974); 6 h prior to inoculation,

Table 1. Mean variation in aggressiveness of populations of net and spot form isolates of *Drechslera teres* from northern Europe (a), Ireland (b) and progeny of matings of net form and spot form isolates (c)

Isolate code	Country of origin	Cultivar of origin	Type	Mean overall symptom severity [a]	Standard error of mean values
a					
FN1	Denmark	Celtic	Net	8.0	0.6
FN2–FN4	Denmark	Regina	Net	3.0–4.1*	0.7–1.2
FN5	Norway	Artturi	Net	4.2	1.2
FN6	Norway	CI 9819	Net	2.3	1.3
FN7	Slovakia	Inari	Net	2.7	0.9
FN8	Denmark	Inari	Net	3.9	0.9
FN9	Denmark	Artturi	Net	4.3	1.2
FN10–FN11	Finland	Unknown	Net	0.7–2.7*	0.3–0.6
FN12	Sweden	Alliot	Net	3.9	1.2
FN13	Czech Republic	Kromir	Net	3.1	1.2
FN14	Finland	CI 9819	Net	4.2	1.4
FN15	Denmark	Unknown	Net	3.1	0.9
FS1	Slovakia	Inari	Spot	2.4	0.9
FS2–FS4	Slovakia	Artturi	Spot	3.8–4.2*	0.4–0.7
FS5–FS6	Slovakia	CI 9819	Spot	3.4–3.8*	0.3–0.4
FS7	Czech Republic	Kromoz	Spot	4.0	0.4
FS8–FS9	Czech Republic	Unknown	Spot	2.0–5.0*	0.3–0.5
FS10–FS11	Czech Republic	Iuran	Spot	3.4–4.2*	0.3–0.4
FS12–FS14	Czech Republic	CI 9819	Spot	1.8–3.9*	0.3–0.6
FS15	Czech Republic	Arve	Spot	3.2	0.3
b					
N1–N15		Century	Net	0.5–5.4*	0.2–0.7
N16–N21		Cooper	Net	0.1–5.2*	0.1–0.8
N22		Fractal	Net	5.1	0.8
N23–N24		Canasta	Net	3.9–5.0*	0.4–0.6
N25		Lamba	Net	3.0	0.5
N26–N27		Blenheim	Net	0.6–3.9*	0.2–0.3
N28		Newgrange	Net	5.1	0.6
N29		Regina	Net	4.1	0.5
N30–N39		Unknown	Net	1.3–4.9*	0.4–0.7
S1		Blenhiem	Spot	7.1	0.3
S2		Cooper	Spot	3.5	0.4
S3		Tavern	Spot	4.8	0.5
S4		Fractal	Spot	3.2	0.5
S5		Optic	Spot	4.8	0.5
c					
Isolate code	Origin of isolate				
M1–M12	Progeny net parent 1			3.1–6.5*	0.3–0.7
M13–M16	Progeny net parent 2			3.7–5.9*	0.6–0.8
M17	Net parent 2			2.7	0.6
M18	Spot parent			6.6	0.4

*Range of severity values for isolates from same cultivar of origin.
[a]Mean severity measurements of infection by different isolates assessed using numerical scale of Tekauz (1985), where 1 is resistant and 10 is very susceptible.

the glasshouse was subjected to 100% relative humidity using a humidifier. The glasshouse experiment was arranged in replicates according to isolate and 0.5 ml of spore suspension sprayed onto each plant using a gas humidity sprayer. The glasshouse remained under high humidity conditions for a further 24 h, after which time the glasshouse experiment was re-organised randomly into cultivars. The humidity was increased twice per day for 3 h, but otherwise the plants were maintained under normal glasshouse conditions. Symptoms were scored on the second leaf 7 days after inoculation. Symptoms were classified using the numerical scale of Tekauz (1985).

Detached leaf experiment

Plant material was produced in a glasshouse with an air filtration system and the second and third leaves selected. Five leaves were placed abaxially on benzimidazole agar plates, with two replicates of each cultivar per isolate treatment. Benzimidazole agar was prepared by dissolving 6 g of bacterial agar (Agar Technical no. 3, Oxoid UK) and 0.12 g of benzimidazole in 1 l of water and sterilising for 15 min at 121 °C. Benzimidazole was used as a senescence retarder; 10 μl drops of spore suspension (2×10^4 spores ml^{-1}) were pipetted onto each leaf and incubated for seven days under a white light diurnal cycle at 20 °C (Deadman and Cooke, 1985). Symptoms were scored 7 days after inoculation. Length and width of lesions were measured using a clear perspex ruler.

Statistical analysis

Data from both the glasshouse and detached leaf experiments for each population were subjected to analysis of variance incorporating Fisher's protected least significant difference tests (PLSD). Correlation analyses were used to establish the relationship between the glasshouse and detached leaf data. Analysis was carried out using Statview 5.0 (SAS systems, USA).

Results

Variation in virulence of Drechslera teres *populations*

Variation in virulence of isolates occurred in both populations between the net and spot forms ($p < 0.05$), but also among populations of both forms (Table 1a–c) ($p < 0.01$). Analysis of the data suggests that the isolates behaved in a similar fashion on all cultivars examined, that is they were either virulent or non-virulent on all cultivars. For example isolates FN10 and N1 produced few symptoms whereas isolates FN1 and N28 produced high overall levels of disease (Table 1a, b).

In the northern European population, no significant differences were found between overall virulence of net form and spot form isolates, with mean severity ratings of 3.6 and 3.5 respectively. Significant differences were found in overall virulence of net form and spot form isolates from the Irish population ($p < 0.05$). Overall, the spot form isolates were responsible for higher levels of disease (mean 4.8), than net form isolates (mean 3.5) (Table 1c). The collection of progeny isolates was found to vary significantly in virulence, ranging for example from 3.1 to 6.5 respectively. The overall virulence of the progeny isolates was found to be significantly greater than the other two populations with a mean severity rating of 5.3 ($p < 0.05$).

Variation in reaction of different host cultivars to inoculation with Drechslera teres *isolates*

Cultivar reactions varied significantly from each other and from inoculation with either the net or spot forms of the pathogen (Table 2) ($p < 0.001$). The susceptible cv. Botnia, was found to be susceptible to both forms of the pathogen from both populations, with all isolates able to incite symptoms. No significant differences were found in the reactions caused by the net form or spot form populations, with mean severity ratings of 3.6 and 4 for the net and spot form isolates from the northern European population and 5.2 for both forms from the Irish population respectively. Similar reactions were observed on cv. Boreal 94145 (Table 2), with no significant differences found between reactions to inoculation with net and spot form isolates.

Cultivar CI 5791 was significantly more resistant to both forms of the pathogen than cvs. Botnia and Boreal 94145 ($p < 0.001$) and also exhibited varying reactions to inoculation with net and spot forms ($p < 0.05$). Although all net form isolates caused symptoms, the resulting severity levels were relatively low (1.5 and 1.8 for northern European and Irish populations respectively). The spot form isolates however caused significantly more infection on this cultivar ($p < 0.001$) (Table 2).

Cultivar CI 2330 was also significantly less susceptible to infection by both forms of the pathogen than cvs. Botnia and Boreal 94145 ($p < 0.05$) and was also significantly more susceptible to infection from spot form isolates than from net form isolates (Table 2) ($p < 0.05$). Cultivar CI 9819 (only used in northern European study) was significantly more resistant to both forms of the pathogen than either cvs. Botnia or Boreal 94145 ($p < 0.05$);

Table 2. Overall effect of populations of net form and spot form isolates on barley cultivars after 7 days

Isolate type[a]	Mean disease severity ± SEM						
	Number of isolates (*N*)	cv. Botnia	cv. Boreal 94145	cv. CI 5791	cv. CI 2330	cv. CI 5822	cv. CI 9819
Northern European net form	15	3.7 ± 0.3	3.4 ± 0.3	1.7 ± 0.1	1.8 ± 0.2	N/a	2.2 ± 0.2
Northern European spot form	15	4.0 ± 0.3	4.0 ± 1.3	2.5 ± 0.2	3.8 ± 0.3	N/a	3.1 ± 0.2
Irish net form	38	5.2 ± 0.4	N/a	1.9 ± 0.2	2.9 ± 0.2	2.7 ± 0.3	N/a
Irish spot form	5	5.3 ± 0.5	N/a	3.9 ± 0.4	4.5 ± 0.5	4.2 ± 0.5	N/a
Progeny of mating of net and spot forms	19	7.3 ± 0.5	6.1 ± 0.3	3.5 ± 0.3	5.0 ± 0.2	N/a	4.6 ± 0.2

[a]Mean severity measurements of populations of isolates assessed using numerical scale of Tekauz (1985), where 1 is resistant and 10 is very susceptible. N/a: insufficient seed of cultivar available.

however, no significant differences were found in the reactions produced from either form of the pathogen. Cultivar CI 5822 (used in the Irish study) followed a similar pattern to CI 5791 and was significantly more susceptible to the spot form ($p < 0.001$).

Significant differences were found in cultivar reactions when inoculated with progeny isolates ($p < 0.001$) (Table 2). Cultivars Botnia and Boreal 94145 were significantly more susceptible to progeny isolates than CI 5791, CI 2330 and CI 9819 ($p < 0.001$, $p < 0.05$ respectively). None of the cultivars were immune to inoculation, but CI 5791 was again the most resistant cultivar ($p < 0.001$).

Correlation of glasshouse experiments with detached leaf experiments

Results from the glasshouse were compared with assessments of lesion size made on detached leaves for the northern European and Irish populations and progeny isolates after 7 days. In the detached leaf experiment, significant differences were found in virulence of net form and spot form isolates based on reactions of the five different cultivars (Table 3) ($p < 0.001$). Spot form isolates from the northern European population were more virulent that net form isolates on all cultivars in the detached leaf experiment, with cv. 9819 most susceptible to disease (Table 3). Cultivars Botnia and Boreal 94145 were again most susceptible to net form isolates when compared to the other cultivars examined. The spot form isolates from the Irish population were most virulent on cv. 9819 and net form isolates most virulent on cvs. Botnia, CI 5791 and CI 2330 (Table 3). Cultivar Boreal 94145 was found to be most susceptible to progeny isolates when examined using the detached leaf assay, with cv. CI 2330 most resistant to progeny isolates (Table 3).

Comparison of net form isolates from northern European and Irish populations on five cultivars inoculated in the glasshouse and the corresponding detached leaf measurements showed high correlation between the two methods ($r = 0.97$; $p < 0.05$, $r = 0.90$; $p < 0.05$). There was no significant correlation, however, between the two methods when inoculated with the spot form isolates from either population ($r = 0.28$) or when inoculated with progeny isolates ($r = 0.34$).

Table 3. Mean lesion area measurements of populations of isolates on different cultivars in mm^2 on detached leaves 7 days post-inoculation

Isolate type[a]	Cultivar					
	Number of isolates (*N*)	cv. Botnia	cv. Boreal 94145	cv. CI 5791	cv. CI 2330	cv. CI 9819
Northern European net form	15	32.8 ± 2.2	37.3 ± 2.7	9.6 ± 1.0	17.3 ± 1.8	11.4 ± 0.9
Northern European spot form	15	38.4 ± 2.2	41.0 ± 2.7	40.1 ± 3.2	21.1 ± 2.5	55.5 ± 4.7
Irish net form	38	138.8 ± 5.3	N/a	74.5 ± 3.8	85.6 ± 5.8	88.4 ± 5.0
Irish spot form	5	98.6 ± 17.3	N/a	38.4 ± 11.7	69.6 ± 9.6	128.7 ± 15.2
Progeny of mating of net and spot forms	19	107.9 ± 4.0	156.7 ± 5.0	82.7 ± 4.1	50.0 ± 2.4	87.0 ± 4.3

[a]Mean lesion area measurements of isolates on different cultivars in mm^2. N/a: insufficient seed of cultivar available.

Discussion

Variation in pathogenicity of populations of the net form of *D. teres* has been extensively examined (Khan and Boyd, 1968; Tekauz, 1990; Afanasenko et al., 1995; Cromey and Parkes, 2003). The occurrence of the spot form of the pathogen is relatively new and so more information is required on its distribution, virulence spectrum, possible sources of resistance and how it differs from the net form in these characteristics. In the current study, isolates were described in terms of virulent as defined by Van der Plank (1968). Significant interactions, although small, occurred in both experiments carried out. Similar interactions between the net form and host cultivars have been reported by Douiyssi et al. (1998), Arabi et al. (1992), Jalli and Robinson (2000) and Gupta and Loughman (2001) and these reports indicate the specificity of the host-pathogen relationship (Douiyssi et al., 1998). Such significant interactions suggest that the pathogen may exist as specialised pathological races; this implies that such races exist in the northern European populations. However, further studies on more populations containing larger numbers of both forms would be required to confirm the existence of such races.

In the current study net and spot form isolates from various locations were compared on five barley genotypes. The use of cultivar resistance in different regions exerts differential selective pressures on the pathogen populations. The large amount of variation in virulence of both populations examined in this work suggests more races may exist in both regions than is shown in this study. Tekauz (1990) suggested that variability of a population of *D. teres* depends on the number of barley cultivars examined and the differences in their genetic background, the presence of both pathogen forms in a population and the number of isolates examined.

Variation in virulence was detected between both net form and spot form isolates and within groups of both forms. The patterns of virulence in both populations examined in the current study were similar to those reported by Douiyssi et al. (1998) and Jalli and Robinson (2000). Overall, the spot form of the pathogen was more virulent on all cultivars examined, except on the susceptible cv. Botnia, which does not contain any known major resistance genes. Progeny isolates resulting from

matings of both forms were significantly more virulent than either parental form. Tekauz and Mills (1974) found that two lines, CI 5791 and BT 201, retained their resistance to the net form but were susceptible to the spot form of the pathogen. Khan and Tekauz (1982) and Bockelman et al. (1983) found that a high degree of resistance to the spot form was rare in cultivars examined. Wu et al. (2003) reported conflicting results and found that the spot form isolates were less pathogenic than net form isolates and suggested different cultivars would be more suitable for detecting patterns of pathogenicity in the spot form than those used to examine populations of the net form. Brandl and Hoffman (1991) also found differences in pathogenicity between the net and spot forms, also among net form isolates but not among spot form isolates. This trend was also evident in the current study, as both net form populations were more variable in virulence on all cultivars than the spot form isolates.

In the current study, all cultivars developed symptoms following inoculation and so were not resistant to either the net form or spot form isolates. Resistance to net blotch is thought in most barley cultivars to be regulated by one to three major genes (Arabi et al., 2003). However, there were differences in the degree to which the disease developed on the different host genotypes. Robinson and Jalli (1997) reported that partial resistance and the average effect of alleles can be inherited and that these may be important factors in conditioning resistance to net blotch. Screening for resistance to net blotch have been conducted for several decades (Jonsson et al., 1997; Jalli and Robinson, 2000). Such studies are limited in their usefulness as different experiments examined different genotypes. Afanasenko et al. (1995) identified a set of differentials for characterising *Drechslera* populations for international use based on their pathogenicity. The resistant cultivars (CI 2330, CI 5791, CI 5822 and CI 9819) used in the current study also appear in the list of Afanasenko et al. (1995). These cultivars exhibited differential reactions to different isolates, but were found to be moderately resistant to all isolates tested, with CI 5791 exhibiting the highest resistance. This result corresponds with the data from the current experiment; CI 5791 was significantly more resistant to both forms of the pathogen. Arabi et al. (1992) also used cv. CI 5791 in their study and found variations in the reaction to net form and spot form isolates on leaves; they found that this previously resistant cultivar was highly susceptible to one net form and one spot form isolate. However, only a small population of isolates was examined. CI 5791 has been reported to contain two known major resistance genes, one of which is allelic to *Pta*, the first resistance gene identified (Khan and Boyd, 1969). A breakdown in resistance may be less likely with this cultivar than with the other cultivars used in these experiments.

In the current study, cv. CI 2330, previously classified as resistant to *Drechslera* isolates, was found to be moderately resistant to the net form of the pathogen from the northern European population. The spot form isolates in both experiments were more pathogenic on this cultivar; the majority of isolates caused a score >4 which is rated as moderate. Cultivar CI 2330 contains one known major resistance gene *Pta* and expression of resistance in this cultivar has been found to be affected by environmental conditions (Douglas and Gordon, 1985). Khan (1969) reported that resistance in this cultivar was expressed only with high pre-inoculation temperatures and bright light during the incubation period. In the current experiment, optimum conditions for expression of resistance may not have occurred as plants were maintained in the glasshouse under natural light conditions.

The glasshouse and detached leaf experiments were found to be correlated in symptom expression with net form isolates but not with spot form isolates or progeny of the mating of the two forms. Deadman and Cooke (1986) and Sharma (1984) reported similar correlations in their studies with net form isolates. This is the first comparison of whole plant and detached leaf methods using spot form isolates or their progeny. The lack of correlation may be due to differences in the genetic make-up of the net and spot forms, resulting in two different symptom types; different genes are thought to control symptoms expression caused by the two forms (Smedegaard-Petersen, 1971). The lack of correlation may also be due to the amount of chlorosis which characteristically accompanies the spot type lesions. This chlorosis is generally more diffuse throughout the area of the host affected and so measurements of the extent of damage caused by the spot form may be overestimated. Estimations of fungal biomass in the

host tissue either by ergosterol levels or quantitative PCR could clarify the relationship between visible symptoms and damage to the host. Bates et al. (2001) developed a real-time PCR-based assay for the quantification of *Pyrenophora* species including *P. teres* in infected seeds. This could be adapted to examine the fungal biomass in leaf tissue in resistance studies.

In conclusion, this study illustrates the importance of including both net form and spot form isolates in resistance studies, as the different forms produced significantly different virulence spectra on the cultivars used in these experiments. Resistance to both forms of the pathogen may not be genetically linked in the barley cultivars used in the current study where three of the cultivars examined showed more susceptibility to the spot form than the net form. The large amount of variation in virulence among parental and progeny isolates may be of some concern as this variation probably represents only a fraction of the total amount of variation present in both northern European and Irish populations. The increased virulence of the spot form and progeny isolates may have important implications for the spread of the spot form of the pathogen in Europe.

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