# PATHOGENIC VARIATION IN *DRECHSLERA TERES* IN NEW ZEALAND

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## ABSTRACT

*Drechslera teres* f. sp. *teres* causes net blotch of barley. While it is usually controlled adequately by fungicides, episodes of fungicide insensitivity have led to periodic outbreaks of severe net blotch in New Zealand. Disease resistance is an alternative control method, but resistance may not be durable due to the development and spread of new pathotypes of *D. teres*. In New Zealand the use of disease resistance has been hampered by a lack of information on pathogenic variation in *D. teres*. Samples of net blotch were collected from barley crops and field trials and single conidium isolates of *D. teres* f. sp. *teres* were produced. These were inoculated onto internationally recognised differential barley cultivars. Disease reactions were assessed and pathogenic variation in the New Zealand *D. teres* population determined. Some differential cultivars were resistant to all isolates tested, but others were susceptible to one or more of the isolates. While pathogenic variation was identified in the New Zealand *D. teres* f. sp. *teres* population, the extent of variation was less than indicated in some overseas studies.

**Keywords:** *Drechslera teres* f. sp. *teres*, *Pyrenophora teres*, barley, net blotch, pathotypes.

## INTRODUCTION

Net blotch of barley, caused by the fungus *Drechelera teres* [Sacc.] Shoem. f. sp. *teres* Smedeg. is widespread in New Zealand, where high yield losses have been recorded (Sheridan & Grbavac 1985). Infected seed is the main source of inoculum of *D. teres* in New Zealand in the absence of efficient fungicide seed treatments (Hampton 1980).

While net blotch is usually controlled adequately with fungicides, episodes of fungicide insensitivity have led to periodic severe disease outbreaks in New Zealand. Net blotch was the major disease of barley in Wairarapa crops in the mid-1970s, but its incidence had dropped dramatically by 1980 with the introduction of systemic seed treatments (Cromey et al. 1980). However, resistance in *D. teres* to the fungicide triadimenol led to further severe outbreaks of net blotch in the early 1980s (Sheridan & Grbavac 1985).

Disease resistance is an alternative method of disease control but success in breeding for resistance depends on an understanding of the diversity in the pathogen population (Tekauz 1990). Pathogenic variation among isolates of *D. teres* is common, and pathotypes have been reported from many countries (Steffenson & Webster 1992). In New Zealand, use of disease resistance has been hampered by a lack of information on pathogenic variation in local populations of *D. teres*. The objective of this study was to determine the virulence spectrum of the New Zealand population of *D. teres* f. sp. *teres*.

## MATERIAL AND METHODS

Barley crops and field trials in Rangatikei, Canterbury and Southland were sampled over three growing seasons between 1999 and 2001. Leaves with characteristic net blotch symptoms were collected, dried and stored at room temperature between sheets of absorbent paper until used to prepare isolates.

Infected leaf pieces were surface sterilised in 1% sodium hypochlorite for 1 min, rinsed in sterile water, and placed on moist sterilised filter paper in sterile Petri dishes. After 2 to 3 days, conidia were mixed with a small quantity of sterile water and streaked on plates of 2% water agar that had been amended with 100 µg/ml chloramphenicol and 20 µg/ml streptomycin sulphate to prevent bacterial growth. Single germinated conidia, identified using a compound microscope, were transferred onto V8 juice agar (V8A: 177 ml V8 juice, 16 g agar/litre distilled water). Seedlings at the two to four leaf stage of the highly susceptible cultivar Regatta were inoculated with a conidial suspension from a 1 week V8A culture (see method below). Infected leaves were collected approximately 2 weeks after inoculation, dried and used to produce further inoculum. A total of 26 single conidium isolates from sampled crops were used in the study.

Thirty-one barley cultivars or lines (see Table 1) were used as differential cultivars. These included most of those used in Australia (Khan 1982), North America (Tekauz 1990; Steffenson & Webster 1992) and Europe (Jonsson et al. 1997). Four seeds of each of four differential cultivars per pot were sown in square pots (800 $cm^3$ capacity) containing potting mix. Plants were grown at ca 20°C in a glasshouse cubicle and inoculated at the three-leaf stage.

Inoculum of single conidium isolates was prepared by incubating previously prepared infected leaf pieces on moist filter paper in Petri dishes at room temperature with a 12 h photoperiod for 3 to 4 days. Sporulating leaf pieces were shaken in distilled water containing 0.005% Tween 20 (wetting agent) and conidium concentration adjusted to 1 x $10^4$ conidia/ml. Sets of differential cultivars were inoculated with conidium suspensions using an atomiser until evenly wet, and were then placed in an incubation chamber in the dark at 20°C to maintain leaf wetness for 20 h, before returning to the glasshouse at 20–25°C.

Seven days after inoculation, lesions were assessed on a 1 to 10 scale (Tekauz 1985). To characterise pathotypes, lesions rated 1 to 5 were considered to represent a resistant reaction and those rated 6 to 10 a susceptible one. Inoculations were repeated to confirm lesion types where categorisation into resistant or susceptible was not clear, such as when lesion types spanned resistant and susceptible categories. In such cases, the mode reaction was used for categorisation. Pathotypes were designated according to each unique mix of resistant and susceptible reactions of differential cultivars.

## RESULTS

All isolates were virulent on two of the differential cultivars, Herta and Rika (Table 1). Nineteen of the differential cultivars were resistant to all isolates. Virulence to Kombar and CI 11458 was present in just over half of the isolates, while virulence was less common against Algerian, Atlas, Cape, Harbin, Manchurian, Ming, Prato and CI 2330.

All differentials used in the present study have been used in at least one other study (Table 1). There were no instances where virulence to a differential cultivar occurred in New Zealand, but not in at least one overseas study. There were some instances where virulence was common in another study, but not found in the present study, and a few instances (such as virulence to Rika) where virulence was much more common in the present study than in others.

Eleven pathotypes were characterised amongst the 29 isolates using the 31 barley differential cultivars (Table 2). All pathotypes were virulent on some of the differential cultivars. The most common pathotype, Pathotype 1 (represented by six out of 31 isolates), was virulent on only two differential cultivars, and four pathotypes (Pathotypes 2–5) were virulent on only three differential cultivars. Most isolates were virulent on no more than three differential cultivars, and most pathotypes were virulent on no more than four differential cultivars. All pathotypes were virulent on Herta and Rika, while seven were virulent on Kombar and seven on CI 11458. The most virulent pathotype (Pathotype 10) was virulent on seven of the differential cultivars. The only differentiating cultivars (those resistant to some pathotypes but susceptible to others) that had an identical response to the 11 pathotypes were Harbin and CI2330.

**TABLE 1: Percentage of New Zealand isolates of *Drechslera teres* f. sp. *teres* virulent to 31 barley differential cultivars in this and other published studies.**

| | | Current | Other studies[2] | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cultivar or line | CI[1] | study | A | B | C | D | E | F |
| Algerian | 1179 | 12 | 47 | 2 | 0 | - | 0 | - |
| Atlas | 4118 | 4 | 100 | 21 | 8 | - | 51 | - |
| Beecher | 6566 | 0 | 100 | 21 | 8 | - | 38 | 30 |
| Canadian Lake Shore | 2750 | 0 | 0 | - | - | - | 0 | 22 |
| Cape | 1026 | 8 | - | - | - | - | 24 | - |
| Coast | 2235 | 0 | 12 | - | - | - | 0 | - |
| Harbin | 4929 | 8 | - | - | - | - | 1 | 30 |
| Hazera | 12673 | 0 | 100 | - | 0 | - | 41 | - |
| Heartland | 145351 | 0 | - | - | - | 58 | - | - |
| Herta | 8097 | 100 | - | - | 0 | 99 | - | - |
| Kombar | 15694 | 54 | - | - | 8 | - | 56 | - |
| Manchu | 4795 | 0 | 0 | - | - | - | - | - |
| Mancurian | 739 | 4 | - | - | - | - | 1 | - |
| Ming | 4797 | 8 | 0 | - | 0 | - | 0 | - |
| Norbert | 4214 | 0 | - | - | - | 37 | - | - |
| Prato | 15815 | 4 | - | - | 0 | - | 82 | - |
| Rabat 071 | 9776 | 0 | 12 | - | - | - | - | 4 |
| Rika | 8069 | 100 | - | - | 0 | - | 1 | - |
| Rojo | 5401 | 0 | - | - | - | - | 0 | - |
| Steptoe | 15224 | 0 | - | - | 0 | 57 | - | - |
| Tifang | 4407.1 | 0 | 0 | - | - | - | 0 | - |
| TR 473 | 4976 | 0 | 0 | - | - | 13 | - | - |
| CI 1243 | 1243 | 0 | 0 | - | - | - | - | - |
| CI2330 | 2330 | 8 | 0 | - | - | - | 1 | 37 |
| CI4922 | 4922 | 0 | - | - | - | - | 1 | 26 |
| CI5791 | 5791 | 0 | 0 | 0 | - | 2 | 0 | - |
| CI7584 | 7584 | 0 | 12 | 0 | - | - | 0 | - |
| CI9214 | 9214 | 0 | 0 | - | 0 | 81 | - | - |
| CI9819 | 9819 | 0 | 0 | - | - | - | 0 | - |
| CI9820 | 9820 | 0 | 0 | - | - | 9 | - | - |
| CI11458 | 11458 | 54 | - | - | - | - | 1 | - |

[1]CI = cereal investigation number.
[2]Reference for each study. A: Khan & Boyd (1969); B: Khan (1982); C: Gupta & Loughman (2001); D: Tekauz (1990); E: Steffenson (1992); F: Jonsson et al. (1997).

## DISCUSSION

This is the first report of pathogenic variation in *D. teres* in New Zealand. The most common pathotype, which was recorded throughout the surveyed area, was virulent to only the two differential barley cultivars that were susceptible to all isolates. The low number of differential cultivars susceptible to most isolates suggests that there has been limited selection amongst barley crops for multiple virulence in New Zealand. None of the differential cultivars have been grown commercially in New Zealand, and most New Zealand barley cultivars are susceptible to net blotch. Of the cultivars currently grown commercially in New Zealand, only Fleet is resistant (M.G. Cromey, unpubl. data). The most common pathotype was recorded throughout the surveyed area.

**TABLE 2: Characterisation of pathotypes of *Drechslera teres* f. sp. *teres* based on the reaction of 31 barley differential cultivars to 29 New Zealand isolates.**

| Genotype/ CI no. | Pathotype 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | No. virulent pathotypes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Algerian | R | R | R | **S** | R | R | R | R | R | **S** | **S** | 3 |
| Atlas | R | R | R | R | R | R | R | R | R | R | **S** | 1 |
| Beecher | R | R | R | R | R | R | R | R | R | R | R | 0 |
| Canadian LS | R | R | R | R | R | R | R | R | R | R | R | 0 |
| Cape | R | R | R | R | **S** | R | R | R | R | **S** | R | 2 |
| Coast | R | R | R | R | R | R | R | R | R | R | R | 0 |
| Harbin | R | R | R | R | R | R | R | R | **S** | R | R | 1 |
| Hazera | R | R | R | R | R | R | R | R | R | R | R | 0 |
| Heartland | R | R | R | R | R | R | R | R | R | R | R | 0 |
| Herta | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | 11 |
| Kombar | R | **S** | R | R | R | **S** | **S** | **S** | **S** | **S** | **S** | 7 |
| Manchu | R | R | R | R | R | R | R | R | R | R | R | 0 |
| Mancurian | R | R | R | R | R | R | R | R | R | **S** | R | 1 |
| Ming | R | R | R | R | R | R | **S** | R | R | R | R | 1 |
| Norbert | R | R | R | R | R | R | R | R | R | R | R | 0 |
| Prato | R | R | R | R | R | R | R | **S** | R | R | R | 1 |
| Rabat 071 | R | R | R | R | R | R | R | R | R | R | R | 0 |
| Rika | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | **S** | 11 |
| Rojo | R | R | R | R | R | R | R | R | R | R | R | 0 |
| Steptoe | R | R | R | R | R | R | R | R | R | R | R | 0 |
| Tifang | R | R | R | R | R | R | R | R | R | R | R | 0 |
| TR 473 | R | R | R | R | R | R | R | R | R | R | R | 0 |
| CI 1243 | R | R | R | R | R | R | R | R | R | R | R | 0 |
| CI2330 | R | R | R | R | R | R | R | R | **S** | R | R | 1 |
| CI4922 | R | R | R | R | R | R | R | R | R | R | R | 0 |
| CI5791 | R | R | R | R | R | R | R | R | R | R | R | 0 |
| CI7584 | R | R | R | R | R | R | R | R | R | R | R | 0 |
| CI9214 | R | R | R | R | R | R | R | R | R | R | R | 0 |
| CI9819 | R | R | R | R | R | R | R | R | R | R | R | 0 |
| CI9820 | R | R | R | R | R | R | R | R | R | R | R | 0 |
| CI11458 | R | R | **S** | R | R | **S** | **S** | **S** | **S** | **S** | **S** | 7 |
| No. isolates | 6 | 4 | 3 | 1 | 1 | 4 | 2 | 1 | 2 | 1 | 1 | |

A larger number of cultivars were used in the present study than in many overseas studies because there is no standard international set of differential cultivars. Many of the differential cultivars used in other studies were included to provide baseline information on the range of virulence in the New Zealand *D. teres* population. As shown in Table 1, the virulence spectrum of the New Zealand population differed from that recorded in any other region, but contained some similarities to each. Steffenson & Webster (1992) identified 16 pathotypes on a smaller range of differential cultivars (22), but with a larger number (91) of isolates. However, as in the present study, the most common pathotype was not particularly virulent.

Where virulence occurred in the present study, it was often present in a single pathotype, and that pathotype was often represented by a single isolate. In California, 14% of isolates were distinct pathotypes (Steffenson & Webster 1992), while Jonsson et al. (1997), who tested 27 Swedish isolates, found 11 pathotypes were represented by a single isolate and three pathotypes were represented by four, four and eight isolates. The extent of variation is not surprising, given the occurrence of the sexual state (Steffenson & Webster 1992), which allows recombination between pathotypes.

Nineteen of the 31 differential cultivars tested in the present study were resistant to all isolates. While virulence to seven of these differential cultivars was not reported in any of the other reports discussed here, virulence to the other 12 was recorded in at least one isolate in at least one of these other studies. Virulence to Beecher (not recorded in New Zealand) was found in Australia, the USA and Europe, at high frequency in some regions. Virulence to Heartland and Steptoe was also common in some regions.

The New Zealand population of *D. teres* is quite different from that in Western Australia, where isolates were commonly found to be virulent against cultivars for which there were no virulent isolates found in New Zealand, and vice versa. Virulence against Beecher in Western Australia may be explained by this cultivar being commonly grown there (Gupta & Loughman 2001). Lack of virulence to Beecher in New Zealand is probably because no cultivars grown in New Zealand contain the Beecher resistance and there is consequently no selection pressure for matching virulence.

Virulence in populations of *D. teres* can vary across time as well as space, as has been reported in Western Australian studies in the 1960s, 1970s and 1990s. For instance, virulence was common on Hazera in the 1960s (Khan & Boyd 1969), was not detected in the 1970s (Khan 1982), but was again found in the 1990s (Gupta & Loughman 2001).

In Canada, Tekauz (1990) used nine differential cultivars, which were all included in the present study. Virulence was detected against each differential cultivar in Canada, and was very common against some of these cultivars. However, of these differential cultivars, virulence only to Herta was detected in New Zealand. The New Zealand and Canadian *D. teres* populations are therefore quite distinct. The only point of similarity was the differential cultivar Herta, which was susceptible to all New Zealand pathotypes, and to all but one Canadian pathotype (this pathotype was represented by a single isolate).

There was little evidence of pairs of cultivars demonstrating matching responses to all pathotypes. One example, however, was Harbin and CI2330, which were both susceptible to only New Zealand Pathotype 9. Jonsson et al. (1997) also showed a matching response between these two cultivars, which suggests that these cultivars may share the same resistance gene(s).

There were some similarities between the present study and that of Steffenson & Webster (1992) in California. For instance, virulence to Kombar was common in both regions. However, they found no virulence to Ming and Algerian while it was found rarely in the present study, with the reverse occurring for CI 4922 and Hazera. All virulences detected in the present study were found in at least one other region.

The level of virulence detected in New Zealand and overseas indicates that resistant cultivars, if introduced, may subject the pathogen population to considerable selection pressure. *Drechslera teres* appears to be capable of rapid adaptation, as demonstrated in western Canada where the virulence spectrum is broad and changes rapidly (Tekauz 1990). Knowledge of the variation in virulence of pathogen populations is important when breeding for disease resistance. Results of the present study show that a number of sources of resistance are effective against all of the pathotypes identified in New Zealand. However, it is likely that new pathotypes could develop and spread in New Zealand given sufficient selection pressure.

Development of an internationally standard set of differential barley cultivars for *D. teres* f. sp. *tritici* would make comparisons of pathogenic variation between regions easier. It will be important, however, to include regional differential cultivars where appropriate to reflect local commercial cultivars and virulence not detected in the standard set.

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