

# The Effects of Wet Period, Spore Concentration and Genotype on Infection of Barley by *Bipolaris sorokiniana*

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## Introduction

The fungus *B. sorokiniana* (Sacc. in Sorok.) causes spot blotch of barley which is a significant foliar disease of barley in northern New South Wales and Queensland. In the Northern barley region, spot blotch is more common in central Queensland and the sub-coastal areas and can also become widespread in inland areas when environmental conditions are suited to its development or in warmer and wetter seasons as occurred in 1998. In the 1998 growing season, spot blotch caused severe yield loss in several commercial cultivars with some crops rendered unharvestable.

Resistance to spot blotch is a breeding objective of the Northern Barley Improvement Program. The regime currently used at the Hermitage Research Station (HRS) is a wet period of 24 hours with a concentration of 20,000 conidia(c) mL<sup>-1</sup>. This regime appears to give adequate infection response on some lines but more accurate separation may be obtained from a more refined inoculation regime.

This experiment was conducted to ascertain optimal conditions for separation of seedling host responses to facilitate pathotype detection within the spot blotch population of the Northern barley region. A pathotype determination of the spot blotch population has not been undertaken in Australia however pathotype variation has been documented in the USA (Valjavec-Gratian and Steffenson 1997).

Combinations of six wet periods, (6, 12, 24, 36, 48 and 72 hours) and five conidial concentrations (5, 10, 20, 40 and 80 x10,000c mL<sup>-1</sup>) were selected to separate genotype responses of the six selected lines.

## Materials and Methods

*B. sorokiniana* conidia of the WI 1902 isolate, the standard culture used for screening material for spot blotch resistance at the Hermitage Research Station, were germinated on water agar overnight. Then single conidia were picked and placed onto Czapeks agar (Johnston and Booth 1968) and incubated for 48 hours with no light.

A single colony was then sub-cultured to starch nitrate agar plates and incubated at 25°C for 14 days with no light. Conidia were washed from the agar plates and filtered. The concentration of the filtrate suspension was determined by counting conidia using a haemocytometer. This provided the stock suspension which was then diluted with the appropriate volume of distilled water to give the five target spore concentrations with a final volume of 25 mL.

Five barley lines and a wheat line (resistant control) were selected, from a preliminary spot blotch differential set, to cover a spectrum of resistances to *B. sorokiniana*.

- |                     |  |
|---------------------|--|
| 1. Larker           | resistant                                      |
| 2. Klaxon/Tallon-34 | moderately resistant                           |
| 3. Gilbert          | moderately resistant to moderately susceptible |
| 4. Klaxon/Tallon-45 | moderately susceptible                         |
| 5. Skiff            | susceptible                                    |
| 6. Hartog           | resistant wheat                                |

Three replicates of the experiment were sown totalling 270 pots by 2 lines per pot. The plants were kept in a glasshouse where temperatures ranged between 13°C and 26°C and were watered daily. The plants were ready for inoculation 12 days after sowing (growth stage 13, Zadoks 1974).

Plants were inoculated with the conidial suspensions using a Paasche® air brush connected to an air compressor with the pressure set at 185kPa and immediately placed in incubation cabinets with mist generated by ultrasonic humidifiers. Temperature within the growth chamber was set at 21°C, humidity at 70% and the day length at 12 hours at a light intensity of 0.048 Watt m<sup>-2</sup>. Plants were removed from the incubation chamber after 6, 12, 24, 36, 48 and 72 hours and returned to the glasshouse.

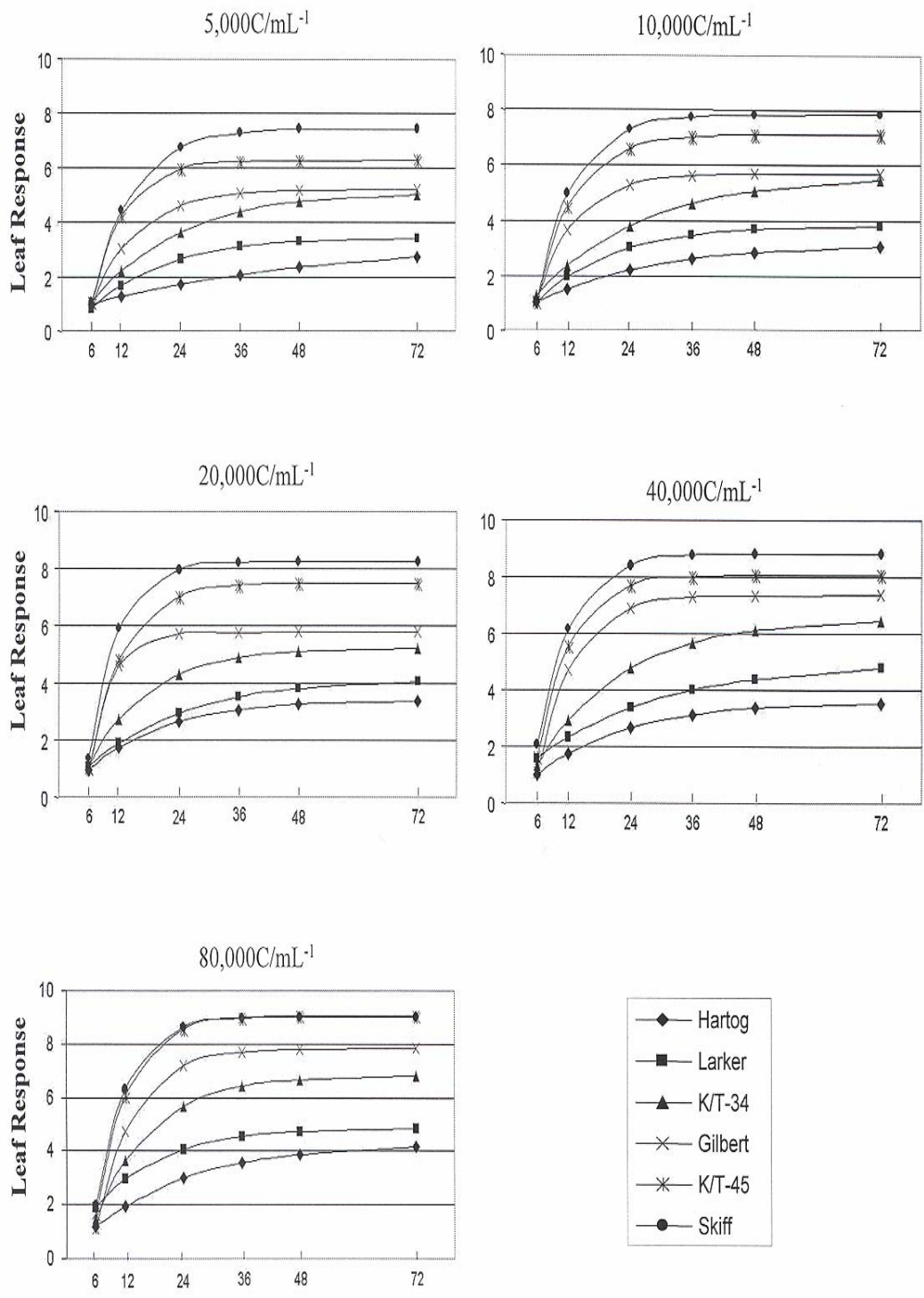
The response of the second leaf of each line to *B. sorokiniana* was recorded 12 days after inoculation at growth stage 15 (Zadoks 1974) using a numerical scale of 1 to 9 with 1 being the most resistant and 9 very susceptible (Fetch 1999).

The raw data was grouped and analysed using an Analysis of Variance in order to determine the relationships between the conidial concentrations and incubation periods for each selected line. Data were graphed as lines of best fit for each line in relation to conidial concentration and incubation period. The exponential formula of  $Y=A+B*R^X$  was used to determine the line of best fit where

- Y = Y axis value (Infection type)
- A = Asymptote of the exponential line
- B = Scaling of the X axis
- R = Curvature of the Line
- X = X axis value (Wet period in hours)

## Results

The exponential curves in Figure 1 refer to the values expressed in the analysed data detailing the different leaf infection response for each of the barley and wheat lines tested. These exponential curves make it possible to view differentiation between lines at incubation times that were not used specifically in the experiment, therefore making it possible to select a regime that offers best separation of the test lines.



**Figure 1.** Leaf infection response across conidial concentrations.

## Discussion

The regime that gave maximum separation of the test lines, in particular the lines expressing moderate infection types, was obtained at 36 hours wet period with a concentration of 10,000c mL<sup>-1</sup>. However as 36 hours of incubation would require the removal of the plants from the incubation chamber during the early hours of the morning, the closest regime using the conditions of this study needs to be chosen.

The regime currently used at the HRS is a wet period of 24 hours with a concentration of 20,000c mL<sup>-1</sup>. Another regime used in the USA, has a wet period of 16 hours with a concentration of 5,000c mL<sup>-1</sup> (Fetch and Steffenson 1994). As shown in Figure 1 neither regime would maximise differences among the genotypes tested under inoculation conditions used at HRS.

Through a process of elimination certain incubation times and conidial concentrations can be eliminated as they give infection responses that are atypical for the genotypes chosen. Figure 1 shows the optimal infection and genotype separation occurs at approximately 36 hours of wet period, and a concentration of 10,000c mL<sup>-1</sup>. As 36 hours of incubation is impractical, a more suitable wet period must be found. At 10,000c mL<sup>-1</sup> and 40 hours of wet period, Figure 1 shows the leaf responses of most lines have reached their asymptotes therefore optimal gene expression and leaf infection occurs during this inoculation regime making it a practical regime for the pathotype survey.

## References

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