Epidemiological studies of *Leptosphaeria maculans* on canola in a controlled environment

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ABSTRACT

The effect of temperature and leaf wetness on disease development was studied in a controlled environment chamber. Seedlings (3-4 leaf stage) of canola cultivar Hyola 42 were inoculated with pycnidiospore suspensions and incubated at 8, 13, 18 or 23°C with leaf wetness periods of 0, 3, 9, 24, 48, 72 or 96 h. Lesions appeared on leaves from 6 to 25 days after inoculation as temperature decreased from 23°C to 8°C. Leaf infection increased with wetness period and this effect was most significant at 23°C. As temperature decreased, so too did the severity of leaf infection and at 8°C the wetness period had no significant effect on leaf infection. Six weeks after inoculation, canola plants incubated at 8°C and 13°C had little or no stem infection but at 18°C, incidence of stem infection ranged from 8% to 60% as the wetness period increased from 0 to 96 h and at 23°C, incidence of stem infection ranged from 25% to 80% as wetness period increased from 0 to 96h.

KEYWORDS blackleg - *Brassica napus* - *Phoma lingam*

INTRODUCTION

Blackleg caused by the fungus *Leptosphaeria maculans* is the most economically important disease of canola (*Brassica napus*) in Australia. This disease has the potential to devastate crops (Bokor et al. 1975). The area sown to canola in Australia has expanded six-fold over the last decade, due to the development of cultivars adapted to Australian conditions and the increased profitability of the crop on acid soils and in lower rainfall areas (Colton and Potter 1999). This expansion has brought rotations closer together and, hence, has increased the risk of disease on the canola crop.

The fungus normally enters plants via stomata on the leaf, colonises the mesophyll and spreads through petioles into the stem (Chen and Howlett 1996, Hammond and Lewis 1987). Once at the base of the stem it can cause a canker that restricts the supply of nutrients and water.

Temperature and wetness period are important factors which affect the germination of ascospores and pycnidiospores, as well as the colonisation of the leaves and stems of canola plants by the fungus. As West *et al.* (2001) revealed in a review of the epidemiology of *L. maculans,* temperature and wetness period also play a crucial role in the severity of leaf and stem infection. Although the factors which affect blackleg infection have been studied extensively in the past, it is important to determine if the effect of these factors has changed with the development of new cultivars and more virulent isolates of *L. maculans*. Hence, a series of controlled environment experiments was carried out to investigate this possibility.

MATERIALS AND METHODS

A series of experiments was conducted in an Environ Air controlled environment chamber to test the effect of temperature and wetness period on infection of canola by *L. maculans*. Light was provided by four metal halide lights and two cool white fluorescent tubes which were set at a 12 h photoperiod.

The trial consisted of a series of eight experiments, each of which involved 28 pots (140 mm diameter) filled with potting soil. Each pot was sown with five seeds of cv. Hyola 42, maintained in a glasshouse (18-29ºC), and the seedlings thinned to three per pot. Once plants reached GS 2.3, a suspension of 10^6 pycnidiospores/ml of the *L. maculans* isolate 66/97 (A-group) was sprayed over the plants until run-off occurred (50 ml per replication). Pots were then placed in white trays inside the controlled environment chamber and tap water was poured into trays every few days to keep soil moist for the duration of the experiments. Each experiment was arranged in a randomised complete block design with four replications and six wetness periods were produced by covering plants with plastic bags immediately after inoculation then removing them 3, 9, 24, 48, 72 and 96 h later. One pot in each replication was left uncovered as a control (0 h).

The entire trial was arranged as a split plot design with two replications. For each experiment, the temperature was set to one of the following four regimes; 8/6°C day/night, 13/10°C, 18/15°C or 23/20°C, and maintained for 6 weeks. Plants were checked every day until the first leaf lesions were observed. Plants were then assessed each week for disease symptoms, depending on the stage of the plant, by either i) counting the number of leaf lesions, ii) rating leaf and petiole infection on a scale from 0-4, or iii) counting plants with stem infection.

RESULTS

Leaf infection The greatest number of leaf lesions occurred on canola seedlings which were incubated at 18/15°C for a wetness period of 96 h. At higher temperatures (18/15°C and 23/20°C), significantly fewer ($P<0.05$) leaf lesions occurred at wetness periods of between 0 h and 48 h than wetness periods of 72 h and 96 h. At 8/6°C and 13/10°C there was no significant difference ($P>0.05$) between wetness periods. The regression between latent period (days from inoculation to appearance of first leaf lesions) and temperature was exponential (Fig. 1).

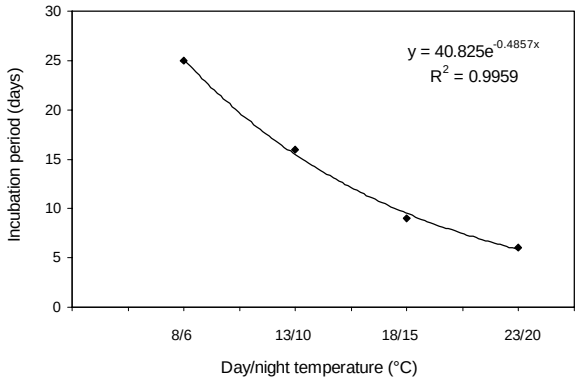

Figure 1. Regression analysis of latent period (days from inoculation to appearance of first leaf lesions) on canola cv. Hyola 42 and temperature (day/night).

Stem infection Fig. 2 shows the effect of temperature and wetness period on the incidence of stem infection on plants 42 days after inoculation. At 8/6°C, no stem infection developed on the plants at any wetness period, nor was stem infection observed on plants incubated at 13/10°C, except for the 96-h wetness period, after which 10% of the plants developed stem infection. At 18/15°C, as the wetness period increased from 0 h to 96 h, the incidence of stem infection increased from 10% to 60%, but at wetness periods 24 h and 48 h, only between 15% and 20% of plants developed stem infection. At 23/20°C, as wetness period increased from 0 h to 48 h, the incidence of stem infection increased from 20% to over 80% and then remained constant at 72 h and 96 h.

DISCUSSION

These experiments have revealed that a temperature of 18/15°C and leaf wetness period of 96 h are required for maximum leaf infection. Previous studies showed that optimal conditions for leaf infection were 15-20°C at 48 h leaf wetness (Biddulph *et al.* 1999; Toscano-Underwood *et al.* 2001). The difference in requirement for leaf wetness may have been due to the different choices of cultivar between the studies. The optimal conditions for the development of stem canker were 23/20°C with at least 48 h of leaf wetness, which corresponded with previous studies (Barbetti 1975; McGee and Petrie 1979; Gladders and Musa 1980). At 18/15°C, there was only a small incidence of stem infection at 24 h and 72 h, compared to 9 h and 48 h, which was most likely due to experimental error, such as the plastic bags not being completely sealed resulting in less than 100% relative humidity. One of the reasons for the greater prevalence and severity of blackleg stem canker in Australia than in Europe and Canada may be that average temperatures are greater in the former. These results provide information on some of the conditions which are conducive to blackleg infection, and should now be carried out on a range of cultivars with different resistance to blackleg.

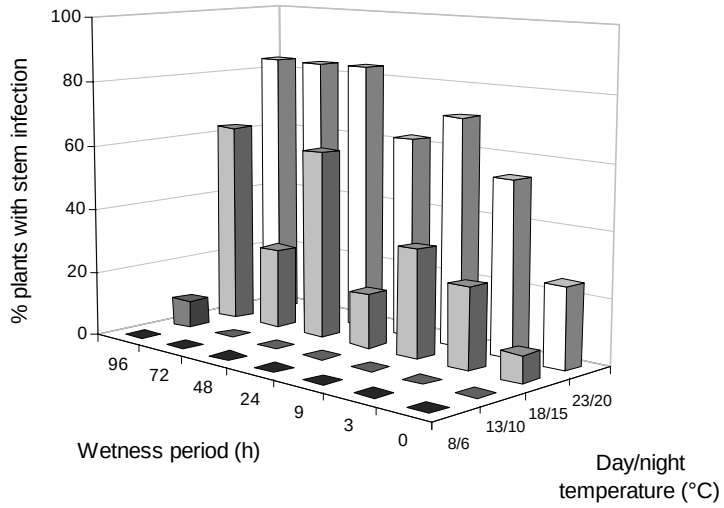

Figure 2. Effect of temperature (day/night) and wetness period on the percentage of plants (cv. Hyola 42) with stem infection, 42 days after inoculation.

ACKNOWLEDGMENTS

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