# Epidemiological studies of *Leptosphaeria maculans* on canola in Australian field conditions

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## ABSTRACT
Field trials, involving nine canola cultivars, were conducted in 2000 and 2001 at two locations in South Australia and climatic data were logged with automatic weather stations. Weekly disease assessments for cotyledon and stem infection revealed variable responses between cultivars. All cultivars, except cv. Hyola 60, were highly susceptible to leaf infection. Scanning electron microscopy revealed that pycnidiospore germination and hyphal growth were severely restricted on leaves of Hyola 60. Natural infection, which was most prominent during early leaf growth, was influenced by rainfall, temperature and wind activity. Yield was correlated more closely to stem canker than to leaf or cotyledon infection. The data were used to develop preliminary models to predict stem canker potential based on climatic conditions during early leaf growth.

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

## INTRODUCTION
Blackleg disease of canola (*Brassica napus*) is caused by the fungus *Leptosphaeria maculans* (anamorph *Phoma lingam*). This fungus is the most important pathogen of canola in Australia. The area sown to canola in Australia has expanded six-fold over the past decade, resulting in increased cropping frequencies and, hence, increased risk of disease in the canola crop.

Blackleg control relies on the use of resistant canola cultivars along with stubble management, longer rotations and fungicide treatment (Barbetti and Khangura 2000). *L. maculans* is a genetically diverse fungus that has the potential to overcome resistance (Pongam *et al.* 1999, Sosnowski *et al.* 2001). Therefore, it is important to seek new sources of resistance by identifying additional mechanisms by which canola plants can resist or contain infection by the pathogen. Some commercial cultivars have resistance to the stem canker phase of blackleg, but may suffer yield or quality penalties and may not be suited to certain environments due to their time to maturity. Environmental factors can determine the extent of blackleg development in the field (West *et al.* 2001), so the risk of disease will vary between regions. Therefore, it is important to select the most appropriate canola cultivar as part of a management strategy for a particular area.

This paper reports results from field trials conducted in two locations in South Australia over two consecutive years, in which the effects of environment and canola cultivar on the development of blackleg were monitored. A mechanism of blackleg resistance in the leaves of canola was revealed using scanning electron microscopy.

## MATERIALS AND METHODS
**Epidemiology** Field trials were conducted at Kingsford Research Station, near Gawler, South Australia and Charlick Research Station, near Strathalbyn, South Australia during 2000 and 2001. In 2000, the trials, sown in May, comprised eight cultivars and three replications. The cultivars were; Dunkeld, Scoop and Mystic (resistant to blackleg), Pinnacle (moderately resistant), Monty and Karoo (moderately susceptible), Hyola 42 and Q2 (susceptible). In 2001 the highly resistant cultivar Hyola 60 was also included in the trials. The trials were inoculated late in May with barley grain, which was autoclaved and colonised with *L. maculans.* Ten seedlings were marked at random in each plot. Cotyledon, leaf and stem infection was rated separately on a weekly basis throughout the growing season. Climatic data were collected using automated weather stations.

Trays, containing 18 plants (cv. Hyola 42) at the 3-leaf stage, were placed 20m from each trial every week, throughout the season, and then returned to the glasshouse a week later. The amount of ascospore inoculum was determined by counting the number of leaf lesions on each plant after incubation for 10 days.

**Microscopy** Spore germination and hyphal growth on the leaf surface of cv. Hyola 60 and cv. Q2 were compared using a Field Emission Scanning Electron Microscope (FESEM), at the Centre for Electron Microscopy and Microstructure Analysis (CEMMSA), University of Adelaide, South Australia. Plants, at the 3-leaf stage, were inoculated by placing 10 µl droplets of pycnidiospore suspension ($10^6$ spores/ml sterile distilled water) of the virulent (A-group) isolate 66/97 of *L. maculans* on the adaxial surface of leaves. Samples were prepared for the FESEM using a standard protocol developed by CEMMSA.

## RESULTS

**Epidemiology** Fig.1 shows the incidence of blackleg infection throughout the season at the Kingsford trial site in 2001. Ranking was similar at all four trials but the percentage of plants infected varied between sites and years (not shown). Cotyledon infection ranged from 60 to 90% among cultivars. Leaf infection was first observed at the beginning of July and incidence reached 100% within 6 weeks for all cultivars. At maturity, the incidence of stem infection ranged from 75 to 100% for all cultivars except Hyola 60, which reached less than 5%.

Infection of the spore trap plants at Kingsford in 2000 was greatest during June and July, with a maximum of 37 lesions/plant in the last week of June (Fig. 2). In 2001, infection of spore trap plants was greatest during July and August, with a maximum of 33 lesions/plant in the first week of August. There was minimal infection after mid-September in both years. Weeks in which total rainfall, mean temperature and mean wind speed were high correlated with weeks in which the number of lesions on the trap plants was greatest, until mid-September.

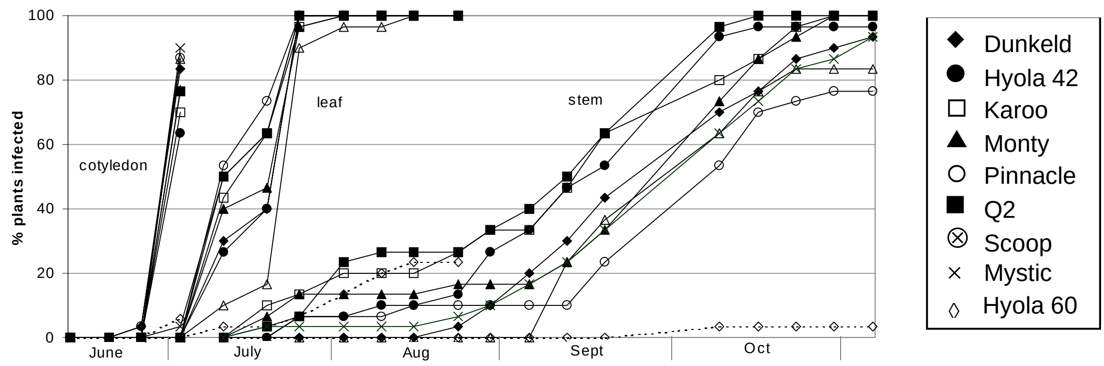


Figure 1. Mean incidence of *L. maculans* infection of cotyledons, leaves and stems at weekly intervals, at the Kingsford trial site, 2001 (sown May 31).

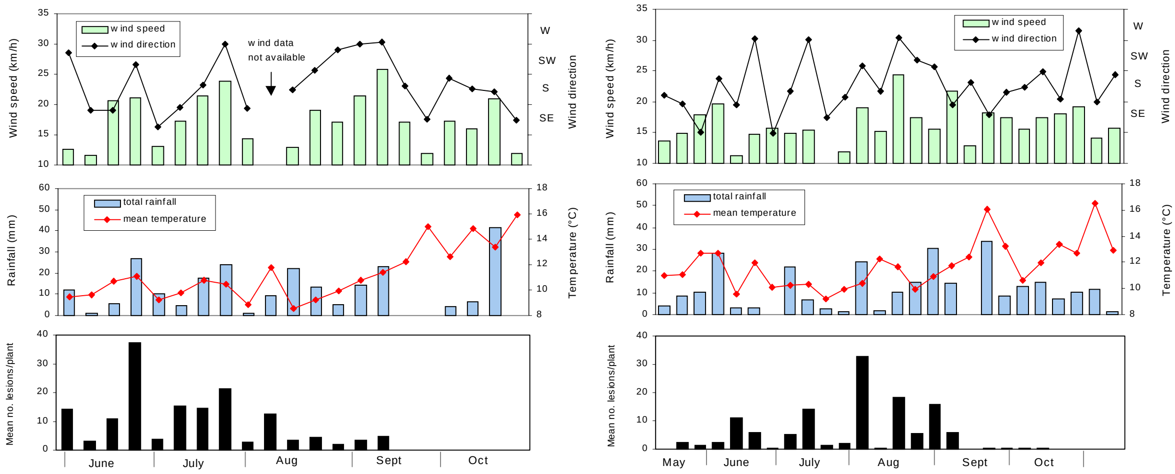


Figure 2. Total rainfall (mm), mean temperature (°C), wind speed (km/h), wind direction and number of leaf lesions on Hyola 42 trap plants at Kingsford each week throughout 2000 (left) and 2001 (right).

**Microscopy** Fourteen days after inoculation with pycnidiospores of *L. maculans*, hyphae had grown profusely over the surface of the susceptible cv. Q2, forming a mycelial mat (Fig 3). However, on the highly resistant cv. Hyola 60, pycnidiospore germination was inhibited and hyphal growth appeared severely restricted.

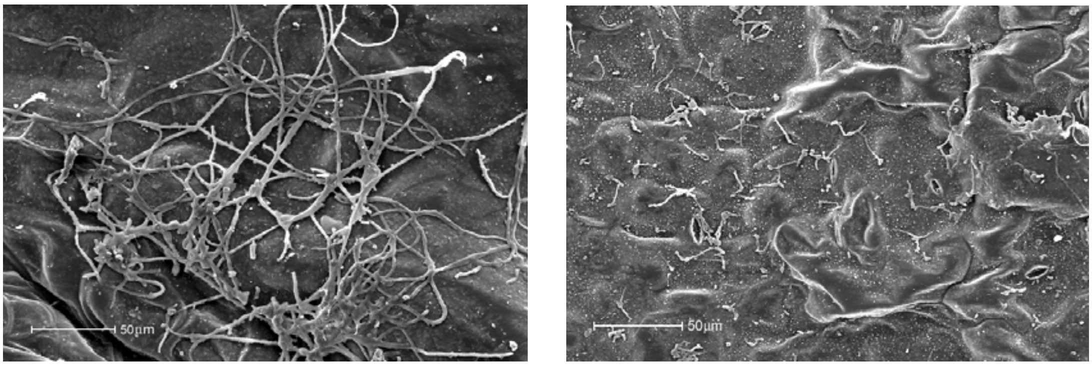


Figure 3. Scanning electron micrographs of the leaf surface of canola leaves (left cv. Q2, right cv. Hyola 60), 14 days after inoculation with a pycnidiospore suspension of *L. maculans*.

## DISCUSSION

Some cultivars had resistance to cotyledon and stem infection, but all cultivars, except Hyola 60, were highly susceptible to leaf infection, suggesting that leaf resistance does not have a major role in the overall resistance of many current cultivars. One reason for this may be that current resistant cultivars are only selected on the basis of stem canker resistance (Salisbury *et al.* 1995). Hyola 60 has resistance originating from a wild accession of *B. rapa* var. *sylvestris* (Crouch *et al*. 1994). It is possible that Hyola 60 produces an anti-fungal biochemical compound in the leaves.

Data collected in field trials were analysed using multiple linear regression. It revealed that stem canker reduced yield in cultivars with low levels of resistance. Furthermore, cultivar, rainfall and temperature influenced the incidence of stem canker. However, these relationships have been identified using a limited set of data, which produced a large amount of variability. They will need to be validated through the analysis of data obtained from a much large number of field trials.

Infection of spore trap plants was greatest between June and August. Rainfall, temperature and wind were directly related to infection of spore trap plants. In these trials, ascospore infection was greatest during early leaf growth, when plants were most vulnerable to infection. Sowing canola to avoid exposing young seedlings to the greatest infection periods may be a possible management strategy to reduce yield loss to blackleg.

## ACKNOWLEDGMENTS

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