STUDIES ON THE EPIDEMIOLOGY OF BLACKLEG (*LEPTOSPHAERIA MACULANS*) AND MECHANISMS OF RESISTANCE IN CANOLA.

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ABSTRACT

Blackleg disease of canola (*Brassica napus*) is caused by the fungus *Leptosphaeria maculans*. It has the potential to reduce crop yield by up to 80% in a severe epidemic. In this project, three aspects of the disease were examined.

Mechanisms of resistance in Australian canola cultivars were investigated using scanning electron microscopy (SEM) and histochemical stains in combination with light microscopy. SEM suggested that the major mechanism of stem resistance was located in the junction of the petiole and stem of plants and that the cultivar Hyola 60 had the ability to resist invasion by *L. maculans* at the leaf surface. Histochemical stains showed that lignin and suberin were produced in the epidermal cell walls and guard cells surrounding stomata in both resistant and susceptible cultivars in response to infection with *L. maculans*. It appeared that resistant cultivars had higher concentrations of both compounds than the susceptible cultivars.

The epidemiology of the disease was studied, both in the field and in controlled conditions. In the field, most cultivars that showed resistance to stem and cotyledon infection were susceptible to leaf infection. Infection by ascospore inoculum was favoured by periods of increased rainfall, temperature and wind activity and infection was most prevalent early in the season, when plants were most vulnerable to leaf infection. Controlled environment experiments revealed that the optimal conditions for stem infection were a temperature regime of 23°C with at least 48 h of leaf wetness, at least 10^6 pycnidiospores/ml water and infection of leaves up until the three-leaf stage.

A DNA-based method of detection of *L. maculans*, which was developed by the Root Disease Testing Service, South Australian Research and Development Institute using *L. maculans* specific primers acquired from the CSIRO, was validated for quantifying DNA in stubble and

soil samples. A selective medium was developed to enumerate colonies of *L. maculans* from infected canola stubble and a plant bioassay was developed to estimate the disease potential of infected stubble. Milled and ground canola stubble was mixed with oaten hay (*Avena sativa* L.) in a series of fractions and spread on plates of the selective medium and over plants in a bioassay. There was a strong correlation ($R^2 > 0.94$) between these results and the estimated amount of *L. maculans* DNA. After validation, the procedure was used to quantify *L. maculans* DNA in soil samples; which revealed that levels decreased as stubble decomposed and that *L. maculans* could not be detected in fields in which canola had been grown more than 2 years earlier.

Finally, during this study *L. maculans* was observed to have colonised the roots of canola plants in the field. Canola seedlings artificially inoculated via the roots or hypocotyls developed root infection and crown canker. It was concluded that *L. maculans* was able to infect the roots of canola via the stem or directly from the soil.