The current situation in the *Brassica juncea*- *Leptosphaeria maculans* pathosystem and how future changes will be monitored

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Abstract

Canola quality *Brassica juncea* will be released for commercial production in Australia during 2007. Previous research has shown *B. juncea* is more resistant to the disease blackleg than commercially available *Brassica napus* cultivars. However, field surveys have found that *B. juncea* attacking blackleg isolates are already present in Australian blackleg populations. Consequently, the durability of *B. juncea* resistance is not known. This study will assess the change in blackleg population structure as a result of the introduction of *B. juncea* as a commercial crop in Australia.

Blackleg disease pressure on *B. juncea* was present in all areas of the canola growing regions. The sites could be grouped into two different levels of disease pressure with the Merriwagga, Thuddungra and Wagga Wagga sites in the group showing the lower levels of blackleg disease pressure and all other sites in the group showing higher disease pressure. Mean levels of internal infection and external canker over all sites in *B. juncea* were found to be higher than the *B. napus* varieties. The individual sites at Coonamble, Frances and Tamworth showed significantly higher levels of internal infection within the *B. juncea* varieties than the *B. napus* varieties.

Key words: Blackleg, *Leptosphaeria maculans*, *Brassica juncea*

Introduction

Canola quality *Brassica juncea* will be released commercially in Australia in 2007. *B. juncea* is an allotetraploid species resulting from the hybridisation of *B. rapa* L. as the male parent and the female parent *B. nigra* (L.) Koch and followed by chromosome doubling of the F1 generation. *Brassica napus*, the most widely cultivated *Brassica* species, is a result of hybridisation between *B. rapa* and *B. oleracea*. Hence *B. juncea* and *B. napus* share one common genome. *B. juncea* has increased drought tolerance, shatter tolerance and early vigour compared to canola (*Brassica napus*).

There are three million hectares of low rainfall cropping regions in the Southern Australian wheatbelt (areas with an annual rainfall of below 350mm) (Burton *et al.* 2003), and in these regions in 2004 less than one percent was sown to oilseeds (Norton *et al.* 2004). These areas would benefit from an oilseed suitable to be cultivated in low rainfall to provide cereal weed and disease break advantages. Due to this, in Australia *B. juncea* is expected to become a successful oilseed crop in the Mallee areas of the southern wheat belt as well as possibly in northern New South Wales and parts of Queensland (Oram *et al.* 2005).

Brassica juncea is thought to have superior resistance to the fungal pathogen *Leptosphaeria maculans* which is the causal agent of blackleg disease. Blackleg throughout the world is the most economically important disease of *Brassica* species. (Balesdent *et al.* 2006). The primary inoculum of this fungal pathogen is ascospores which are produced from sexual reproduction on crop residue over summer (Howlett 2004; Rouxel and Balesdent 2005). The ascospores are released after rain events in autumn, which corresponds to the most susceptible stage of the crop (Rouxel and Balesdent 2005; West and Fitt 2005). Symptoms of blackleg disease include cotyledon and leaf lesions, seedling death and stem cankers which can cause lodging of the plant. In the early 1970's when the Australian canola industry was based on susceptible *B. napus* varieties, blackleg caused its temporary destruction (Khangura and Barbetti 2001; Marcroft *et al.* 2003). Only when more resistant varieties were released did the Australian canola industry begin to re-establish itself. In 2003 blackleg resistance that had been introgressed into *B. napus* from *B. rapa* spp. *Sylvestris* was overcome by *L. maculans*, once again causing widespread devastation within the canola industry (Hua Li *et al.* 2003). Isolates of *L. maculans* capable of attacking *B. juncea* have been found in Australia (Ballinger *et al.* 1991), although the level of disease that they cause is thought to be less than that caused by *L. maculans* on Australian *B. napus* varieties (Burton *et al.* 1999; Oram *et al.* 1999). In France, *B. juncea* resistance that was introgressed into a *B. napus* cultivar was overcome in three years after being exposed to their own inoculum (Brun *et al.* 2000), although it is now considered likely that not all of the *B. juncea* resistance genes were introgressed into the *B. napus* cultivar. Resistance to *L. maculans* is difficult to maintain due to the immense genetic variability within the pathogen population, allowing it to quickly overcome new resistance sources.

Although *B. juncea* is currently accepted to have a greater resistance to *L. maculans* than *B. napus*, the durability of this *B. juncea* resistance is unknown. With the imminent release of canola quality *B. juncea* in Australia it is essential to understand the current pathogenic environment into which it will be released, as well as to monitor any changes within the *L. maculans* population in order to predict any major loss of resistance, and hence prevent any major crop losses and confidence in the canola industry. This will be done by characterising the pathogen population by obtaining isolates off *B. juncea* stubble and screening them over *B. juncea* and *B. napus* varieties, as well as conducting a disease survey of the Advanced *B. juncea* trial sites. Both methods will allow monitoring of the *B. juncea-L. maculans* pathosystem for changes, and will continue through 2007 and 2008. This paper will focus on the 2006 disease survey.

Methods

Six *B. juncea* and four *B. napus* varieties were surveyed for blackleg infection at field sites across Australia. Two Victorian sites (Horsham and Walpeup), one South Australian site (Frances) and eight New South Wales sites (Merriwagga, Trangie, Coonamble, Rowena, Bellata, Tamworth, Thuddungra and Wagga Wagga) were assessed as part of this survey. Three replications (where possible) had twenty plants assessed, and when only two replications were available, thirty plants were assessed within each. Each plant was scored for plant death, external infection and internal infection. External infection was scored on a 0-4 scale where; 0 = no visible external canker, 1 = external canker visible but less than 25% of stem diameter affected, 2 = external canker visible and between 26-50% of stem diameter was affected, 3 = external canker visible and between 51-75% of stem diameter was affected, 4 = external canker visible and between 76-100% of stem diameter was affected. Internal infection was scored by cutting the plant stem with secateurs at the crown and assessing the percentage of infected tissue (5%, 10%, 15….95%, 100%). Plants that were visibly dead were scored as 4 for external canker, and 100 for internal infection. The external canker and plant mortality data were transformed via a square root transformation, and the internal infection data were transformed via a log transformation.

Results

Two of the main objectives to this *B. juncea* disease survey were to assess the general level of infection across different regions of Eastern Australia's canola growing areas, and to determine if blackleg disease levels in *B. juncea* varieties differed to those in *B. napus* varieties. The following are preliminary results from the survey, from all sites except Horsham (yet to be analysed).

Differences in both external canker levels and internal infection levels were found between the ten sites.

Further analysis found that significant differences in external canker of the cultivars were found at Walpeup, Merriwagga, Trangie, Coonamble, Tamworth and Frances. The same sites, as well as Wagga Wagga also showed significant differences between cultivars for internal infection levels.

Over all the sites significant differences were found between the mean disease levels of the different cultivars for both external canker and internal infection. Further analysis discovered that the *B. napus* variety AV-Jade was the only cultivar that did not have differ significantly between sites for internal infection. All cultivars showed significant differences between sites for external canker.

Differences in disease pressure were seen among the sites for both external canker and internal infection, as shown by comparing the mean infection levels of the three cultivars that were present at all the sites (JCO5002, JCO5006 and JR055) (see Figures 1 and 2).

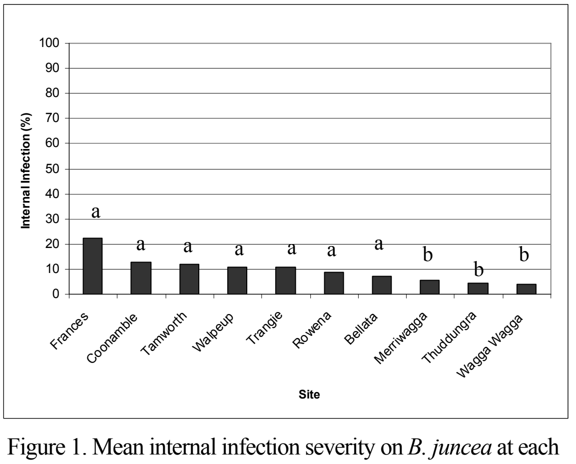

Figure 1. Mean internal infection severity on *B. juncea* at each field site. Values are the means of three common cultivars scored at each site.

(letters denote significant differences)

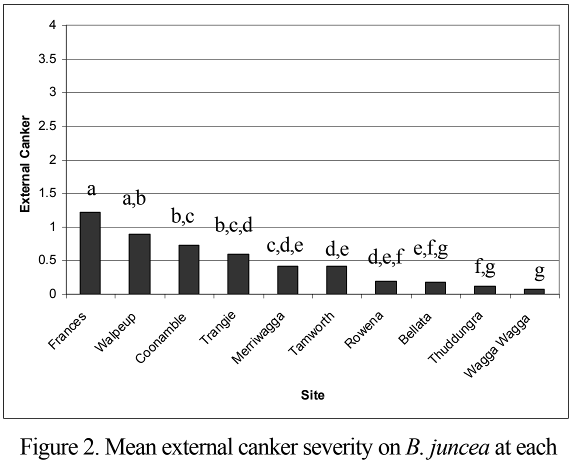

Figure 2. Mean external canker severity on *B. juncea* at each site. Values are the mean of three common cultivars scored at each site.

(letters denote significant differences)

Even though mean infection levels were relatively low, there were significant differences in mean external canker and mean internal infection found between the *B. napus* varieties and the *B. juncea* varieties, with *B. juncea* showing significantly higher levels of both. Further analysis showed that these differences were also noted for external infection at Coonamble, Frances, Tamworth, Trangie and Walpeup. For Internal Infection these differences were seen at Coonamble, Frances and Tamworth (see Figure 2).

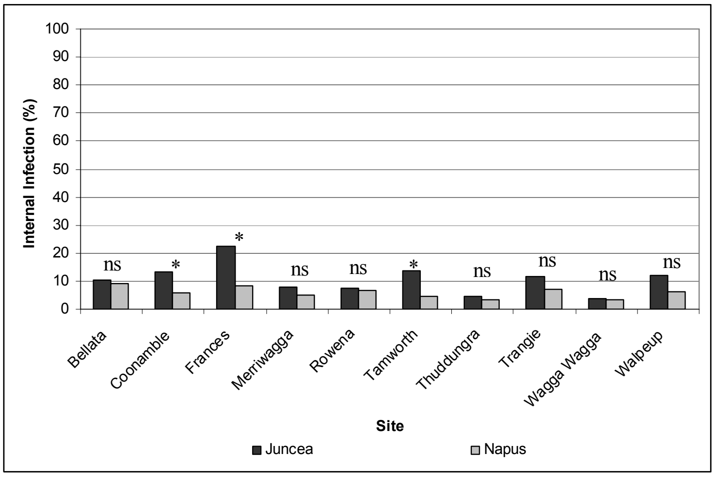

Figure 3. Comparison of internal infection caused by *Leptosphaeria maculans* in *B. juncea* and *B. napus* at each of the sites. Values are the mean of all *B. juncea* and all *B. napus* varieties at each site. Differences non-significant (ns) or significant at P<0.05 (*)

Discussion

B. juncea varieties displayed symptoms of blackleg in all sites in eastern Australia that were surveyed. This is the first time in Australia that a widescale survey has been undertaken to determine levels of blackleg in *B. juncea* within its own growing regions. The survey has shown that blackleg isolates that are virulent on *B. juncea* already exist even before the commercial release of *B. juncea* to farmers. Surveys in future years will be able to use this initial survey as a benchmark to determine if blackleg severity increases after *B. juncea* has been commercialised. However, these data were collected in 2006 which was a drought year, and therefore additional data needs to be collected in a wetter year to determine blackleg severity under normal growing conditions.

In previous work completed by the Victoria *B. juncea* breeding program it was shown that *B. juncea* is significantly more resistant to *L. maculans* compared to *B. napus*. However, this survey found as high or slightly higher levels of blackleg in *B. juncea* compared to *B. napus*. The disease level in *B. napus* was however very low due to the drought, future surveys will determine in wetter years if *B. juncea* is more resistant than *B. napus* when *B. napus* has higher levels of blackleg.

It is well documented that *L. maculans* has a high evolution potential to adapt to different sources of blackleg resistance, it is therefore crucial to monitor blackleg severity in *B. juncea,* so that if required, breeding and disease management strategies can be utilised before growers experience yield loss due to blackleg.

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References

Balesdent, M. H., Louard, K., Pinochet, X. and Rouxel, T. (2006). A large-scale survey of races of *Leptosphaeria maculans* occurring on oilseed rape in France. *European Journal of Plant Pathology* 114(1): 53-65.

Ballinger, D. J., Salisbury, P. A. and Kadkol, G. P. (1991). Race variability in *Leptosphaeria maculans* and the implications for resistance breeding in Australia. Proceedings of the Eighth Australian Research Assembly on Brassicas, Horsham, Victoria.

Barrins, J. M., Ades, P. K., Salisbury, P. and Howlett, B. J. (2004). Genetic diversity of Australian isolates of *Leptosphaeria maculans*, the fungus that causes blackleg of canola (*Brassica napus*). *Australasian Plant Pathology* 33: 529-536.

Brun, H., Levivier, S., Somda, I., Ruer, D., Renard, M. and Chèvre, A. M. (2000). A field method for evaluating the potential durability of new resistance sources: application to the *Leptosphaeria maculans-Brassica napus* pathosystem. *Phytopathology* 90(9): 961-966.

Burton, W., Salisbury, P. and Potts, D. (2003). The potential of canola quality *Brassica juncea* as an oilseed crop for Australia. 13th Australian Research Assembly on Brassicas, Tamworth, New South Wales, Australia.

Burton, W. A., Pymer, S. J., Salisbury, P. A., Kirk, J. T. O. and Oram, R. N. (1999). Performance of Australian canola quality *Brassica juncea* breeding lines. Proceedings of the 10th International Rapeseed Congress, Canberra, Australia.

Howlett, B. J. (2004). Current knowledge of the interaction between *Brassica napus* and *Leptosphaeria maculans*. *Canadian Journal of Plant Pathology* 26: 245 - 252.

Hua Li, Barbetti, M. J. and Sivasithamparam, K. (2003). Responses of *Brassica napus* cultivars to *Leptosphaeria maculans* field isolates from Western Australia. *Brassica* 5(3&4): 25-34.

Khangura, R. K. and Barbetti, M. J. (2001). Prevalence of blackleg (*Leptosphaeria maculans*) on canola (*Brassica napus*) in Western Australia. *Australian Journal of Experimental Agriculture* 41: 71-80.

Marcroft, S., Sprague, S., Salisbury, P. and Howlett, B. (2003). Survival and dissemination of *Leptosphaeria maculans* in south-eastern Australia. 13th Australian Research Assembly on Brassicas, Tamworth, New South Wales, Australia.

Norton, R., Burton, W. A. and Salisbury, P. (2004). Canola quality *Brassica juncea* for Australia. 4th International Crop Science Congress, Brisbane, Australia.

Oram, R. N., Kirk, J. T. O., Veness, P. E., Hurlstone, C. J., Edlington, J. P. and Halsall, D. M. (2005). Breeding Indian mustard [*Brassica juncea* (L.) Czern.] for cold-pressed, edible oil production - a review. *Australian Journal of Agricultural Research* 56: 581-596.

Oram, R. N., Salisbury, P. A., Kirk, J. and Burton, W. A. (1999). *Brassica juncea* breeding. In: Canola in Australia - the first thirty years. P. A. Salisbury, T. D. Potter, G. McDonald and A. G. Green, (eds.), Published for the 10th International Rapeseed Congress, Canberra, Australia.

Rouxel, T. and Balesdent, M. H. (2005). The stem canker (blackleg) fungus, *Leptosphaeria maculans*, enters the genomic era. *Molecular Plant Pathology* 6(3): 225-241.

Salisbury, P., Ballinger, D. J. and Hannah, M. (1989). Blackleg races in Australia. Eighth Australian Research Assembly on Brassicas, Horsham, Victoria.
West, J. S. and Fitt, B. D. L. (2005). Population dynamics and dispersal of *Leptosphaeria maculans* (blackleg of canola). (Adapted from a keynote address at the 15th Biennial Conference of the Australasian Plant Pathology Society, 26-29 September 2005, Geelong). *Australasian Plant Pathology* 34: 457-461.