A *Leptosphaeria biglobosa* 'canadensis' isolate induced resistance in *Brassica juncea* L. and *Brassica napus* L. against *Leptosphaeria maculans* (Desm.) Ces. et de Not.

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ABSTRACT

Blackleg disease severity of *Brassica napus* (canola) was reduced when canola plants were artificially inoculated with a non-aggressive *Leptosphaeria biglobosa* 'canadensis' isolate prior to inoculation with an aggressive *L. maculans* isolate. The reduction in blackleg severity by inoculating plants with a non-aggressive Leptosphaeria isolate consistently occurred for both *B. napus* and *B. juncea* cultivars. The only cultivars that were not protected were the susceptible control cvs. Karoo and Q2. Inoculation of plants with the *L. biglobosa* 'canadensis' isolate 48 hours after inoculation with the aggressive *L. maculans* isolate was less effective, with internal infection severity not significantly reduced compared to inoculation with just the *L. maculans* isolate alone. The use of *L. biglobosa* 'canadensis' as a biological control offers a potential management practice that could complement host resistance.

Key Words: Systemic acquired resistance - SAR.

INTRODUCTION

Blackleg, the most important disease of *Brassica napus* L. (canola) worldwide, is caused by a species complex of *Leptosphaeria maculans* (Desm.) Ces. et de Not. and *L. biglobosa*. *L. biglobosa* is not known to be associated with the damaging stem cankers caused by *L. maculans* (Van de Wouw et al. 2008), however disease symptoms such as leaf lesions and less damaging upper stem lesions do occur. Under Australian conditions yield loss due to *L. biglobosa* infection has not been reported.

Juncea canola (canola quality *B. juncea*) was first commercially cultivated in Australia in 2007 (Burton et al. 2008). Although *B. juncea* is currently thought to have a superior level of resistance to *L. maculans* than *B. napus*, it is likely that as juncea canola is more widely grown, blackleg may also become a problem for this crop.

Infection with an avirulent strain of *L. maculans* has been reported to induce a systemic acquired resistance (SAR) response in *Brassica* species to more virulent strains of the fungus (Li et al. 2006). (Ballinger et al. 1991) showed that when cultivars were inoculated with a mixture of isolates, one tended to dominate over the others. They showed that a non-*B. juncea* attacking isolate dominated over two *B. juncea* attacking isolates causing the *B. juncea* cultivars to be resistant. (Mahuku et al. 1996) showed that canola plants that were inoculated with *L. biglobosa* displayed an induced resistance to *L. maculans*. They reported that leaves inoculated with a weakly virulent isolate first, then followed by highly virulent isolate, produced significantly smaller leaf lesions compared to leaves inoculated with only the highly virulent isolates. It was also shown that after inoculation with the highly virulent isolate, induced resistance could be observed as long as the weakly virulent isolate infected the plant within the next forty eight hours.

Li et al. (2006) found that in a mixture isolates, the non-virulent types only had to be 10% the volume of the mixture for a hypersensitive reaction to be formed on the *B. napus* cultivar Surpass 400, even though the virulent isolate alone could cause a fully susceptible reaction.

With the recent release of juncea canola in Australia, and the expected increase in blackleg disease severity within this new crop over time, new management options need to be investigated. As *L. biglobosa* 'canadensis' is already present in northern New South Wales growing areas of juncea canola, and causes minimal damage compared to *L. maculans*, this pathogen is an ideal candidate for investigation into systemic acquired resistance. Hence, the aim of this study was to determine if seedling inoculation with *L. biglobosa* 'canadensis' could reduce or prevent adult plant stem cankers which cause yield loss.

MATERIALS AND METHODS

Two *Leptosphaeria* isolates were selected for this study. They were isolated from *B. juncea* stubble and were previously screened over three *B. juncea* and three *B. napus* lines during 2006. These isolates were one *L. biglobosa* 'canadensis' (less aggressive) and one *L. maculans* (more aggressive). The *L. maculans* isolate previously caused a mean internal infection of 58% on juncea canola line JC05007. The *L. biglobosa* 'canadensis' isolate chosen caused no internal infection on the same *B. juncea* line, however it caused a mean internal infection of 48% on the *B. napus* cultivar ATR Beacon and 28% on the *B. napus* susceptible cultivar Q2. The isolates were grown on 10% V8 agar and pycnidiospore solution (1×10^7 spores/ml) was used as the inoculant. Four weeks prior to inoculation, pots were sown with juncea canola and canola seeds and placed in the shadehouse facility at Horsham. In 2007, three replicate pots were sown for each treatment and each pot contained five plants. Each of the pots contained four plants of JC05007 (*B. juncea*), with a Q2 plant (susceptible *B. napus* control) in the middle position. In 2008 six different *Brassica* lines were used with three replicates per treatment. The juncea canola lines used were JC05007, Dune and Oasis CL and the *B. napus* lines were AV Sapphire, Q2 and Karoo. JC05007 was used as all isolates had been previously screened on this juncea canola breeding line. Dune and Oasis CL are the first two juncea canola cultivars commercially available in Australia.

Five different inoculation treatments were applied to the plants during 2007 and 2008;

I. Inoculated only with the *L. maculans* isolate.
II. Inoculated only with the *L. biglobosa* 'canadensis' isolate.
III. Inoculated with the *L. maculans* isolate and then 48 hours later with the *L. biglobosa* 'canadensis' isolate.
IV. Inoculated with a mixture of both the *L. maculans* and *L. biglobosa* 'canadensis' isolates.
V. Inoculated with the *L. biglobosa* 'canadensis' isolate and then 48 hours later with the *L. maculans* isolate.

The inoculant was sprayed onto the four week old plants. The pots were then placed into a humidity chamber for 48 hours. The second inoculation was then carried out on the plants for treatments IV and V. All plants remained in the humidity chamber for a further 48 hours and were then transferred to the shadehouse facility for the remainder of the growing season.

Approximately ten days before plants were due to be harvested, at windrowing stage, the plants were scored for internal infection. They were cut at the crown using a pair of secateurs and the cross sectional area was then scored for the percentage of internal infection; 0, 5, 10, 15…..90, 95, 100%.

The data were analysed by ANOVA (MINITAB® Release 14.13). Treatments were compared using least significant difference tests. Log (base 10) transformations were used prior to analysis in order to normalize the data.

RESULTS

When the *L. maculans* isolate alone was used to inoculate plants, it consistently caused the greatest amount of internal infection or was in the group that caused the greatest amount of internal infection compared to the other treatments (Table 6.1). When the *L. biglobosa* 'canadensis' isolate was used alone to inoculate plants it was in the group that caused the least internal infection for all lines except for the susceptible cultivar Q2.

Table 1. Effect of five inoculation treatments on blackleg internal infection severity (%)

Year	Line	Species	Treatment				
			I. *L. maculans*	II. *L. biglobosa*	III. *L. maculans* then *L. biglobosa*	IV. *L. maculans* and *L. biglobosa*	V. *L. biglobosa* then *L. maculans*
2007	JC05007	*B. juncea*	60.8b	0.4a	59.6b	5.0a	9.2a
2008	JC05007	*B. juncea*	46.0b	0.0a	29.0b	6.0a	2.7a
2007	Q2	*B. napus*	93.3b	0.0a	100.0b	15.0ab	0.0a
2008	Q2	*B. napus*	91.3c	45.4b	86.0c	39.0b	16.4a
2008	Dune	*B. juncea*	10.7b	0.0a	9.7b	1.0a	1.3a
2008	Oasis	*B. juncea*	28.6c	0.0a	7.3b	0.0a	3.7b
2008	Karoo	*B. napus*	61.1b	31.8a	54.3ab	63.5b	55.0ab
2008	AV Sapphire	*B. napus*	41.3b	20.3a	23.0ab	9.3a	16.3a

Letters denote significance horizontally only at p≤0.05

[1] Greatest reduction is the greatest reduction (%) of internal infection in treatments III to V compared to treatment I.

Inoculation with a *L. biglobosa* 'canadensis' isolate prior to or simultaneously with a *L. maculans* isolate resulted in significantly less internal infection severity in most *Brassica* lines (Table 6.1). When the *L. biglobosa* 'canadensis' isolate was used to inoculate the plants 48 hours after the *L. maculans* isolate, similar levels of disease were observed compared to the *L. maculans* only treatment, the only exception was *B. juncea* cultivar. Oasis which recorded less disease than the *L. maculans* only isolate. When the *L. maculans* isolate was used to inoculate the plants 48 hours after the *L. biglobosa* 'canadensis' isolate, the severity of internal infection was reduced relative to the *L. maculans* alone. This occurred in all lines over both years except for cultivar Karoo. This treatment reduced internal infection severity to similar levels as when the plants were only inoculated with the *L. biglobosa* 'canadensis' in all cultivars except Oasis. The internal infection severity in Q2 in 2008 was significantly lower using this treatment than the *L. biglobosa* 'canadensis' isolate alone. When both the *L. maculans* and *L. biglobosa* 'canadensis' isolates were used to inoculate plants simultaneously there was significantly reduced infection compared to the *L. maculans* isolate alone in virtually all cultivars (except Q2 in 2007 and Karoo in 2008, both susceptible *B. napus* lines).

DISCUSSION

This study has shown that the inoculation of juncea canola and *B. napus* lines with a *L. biglobosa* 'canadensis' isolate, capable of causing only low disease severity, prior to, or simultaneously with, an inoculation of an aggressive *L. maculans* isolate can reduce blackleg severity. In some cases the treatment can reduce infection to the same level as when the plants were only inoculated with the *L. biglobosa* 'canadensis' isolate. The same *L. biglobosa* 'canadensis' isolate was capable of inducing resistance across a range of lines and in both *B. juncea* and *B. napus*.

No induced resistance occurred in Karoo which is a susceptible check cultivar in blackleg disease nurseries. It appears that the use of a *L. biglobosa* 'canadensis' isolate is not sufficient to prevent high disease severity in susceptible lines. However, in the lines containing effective resistance, both inoculation with a *L. biglobosa* 'canadensis' isolate prior to and simultaneously with a *L. maculans* isolate resulted in a reduced severity of internal infection compared to the *L. maculans* isolate alone. Cultivar Q2 contains the resistance gene *Rlm3* which is not effective in Australia against *L. maculans*, however, it does appear to have some effectiveness against *L. biglobosa* 'canadensis'. This is most likely why some level of induced resistance was achieved in this cultivar. Although resistance can be induced or activated earlier using a *L. biglobosa* 'canadensis' isolate, if the resistance is ineffective it will not reduce disease severity. Although the same isolate and seed source were used in both 2007 and 2008, the mean internal infection severity in Q2 was much higher in 2008 than in 2007 when inoculated with only the *L. biglobosa* 'canadensis' isolate. As both host and pathogen were tested in both years, environmental conditions are the most likely cause of this difference.

The level of internal infection caused by blackleg plays a major role in the crop yield. (Marcroft et al. 2004) showed that internal infection levels needed to be greater than fifty per cent in order to significantly reduce yield in a single plant. This study demonstrated that although treatments did not often completely stop internal infection from occurring, levels of internal infection were significantly reduced. By reducing internal infection severity below the threshold of fifty per cent, the disease is less likely to significantly reduce yield.

Due to the ability of *L. maculans* to overcome host resistance quickly under Australian conditions (Sprague et al. 2006), biological controls may play an important role in the future control of blackleg disease. As *L. biglobosa* 'canadensis' is endemic to Australia it is a potential candidate for a biological control of blackleg disease. However, different subclades of *L. biglobosa* are present in different areas of Australia (Van de Wouw et al. 2008; Vincenot et al. 2008). It may be important to prevent the introduction of some of these subclades to new areas if they are found to be more damaging than the *L. maculans* isolates already present, although it is unlikely that *L. biglobosa* would cause greater disease severity. The potential effect of *L. biglobosa* on the Australian juncea canola industry needs to be investigated further, as it appears that at least the *L. biglobosa* subclade 'canadensis' can cause some levels of stem canker on juncea canola (Van de Wouw et al. 2008). If significant disease levels are possible in juncea canola due to these isolates, alternative biological control agents would need to be investigated.

It is unknown whether this system could be adapted to a field scale application as the pathogenic variability of *L. maculans* within a field is very large. Further investigations into whether a single isolate of *L. biglobosa* 'canadensis' could induce resistance against such a variable *L. maculans* population would be required. Induced resistance could be taken further in the future by identifying the mechanisms that control this induced resistance and a chemical could potentially be produced that activates the same mechanisms without the use of a *L. biglobosa* 'canadensis' isolate. This would prolong shelf life of the inducing chemical and make this disease management option much more practical for broad acre applications.

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