Induced systemic resistance in Australian canola

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ABSTRACT

A range of rhizobacteria are able to stimulate a plant's natural defence system producing what is known as induced systemic resistance (ISR). ISR is a promising mechanism that could be exploited to reduce losses in canola due to blackleg caused by the highly variable and aggressive fungal pathogen, *Leptosphaeria maculans*. Bacterial factors that trigger ISR remain to be clearly determined, which makes identification of suitable isolates a difficult and time-consuming process. The aim of this study was to isolate and identify bacterial isolates able to induce resistance in canola to *L. maculans*. Whilst also attempting to identify if any links exist between an isolates' ISR ability and particular characteristics such as the use of colloidal chitin. Intact soil cores were collected from paddocks with a history of extended canola production. Rhizosphere and endophytic bacteria were isolated from the roots of canola and wheat grown in these cores at 15°C for 3 weeks. Using both non-selective and selective media *Pseudomonas* and *Bacillus* bacterial species were isolated from the root systems. Generally bacteria showed a clear preference for wheat roots compared to canola, with a significant difference in the number of bacteria isolated from the two root systems. Of the two bacterial types isolated, *Pseudomonas* species made up over 50% of the entire bacterial population. Approximately 600 isolates have been collected and assessed for utilisation of colloidal chitin, salicylic acid and 1-aminocyclopropane-1-carboxylic acid. *In vitro* antagonism of bacterial isolates to *Leptosphaeria maculans* and canola seedlings has also been determined. These same isolates have also been evaluated for their ability to trigger ISR towards blackleg in canola.

Key words: Induced systemic resistance – *Leptosphaeria maculans* – *Pseudomonas* - Rhizobacteria

INTRODUCTION

Blackleg of canola is caused by the fungus, *Leptosphaeria maculans*, which causes severe losses in canola crops worldwide. The Australian environment is conducive to the fungus outcrossing which leads to a high degree of variation and may attribute to the aggressive nature of Australian isolates of the blackleg fungus (Howlett et al., 1999). As a result of the increased blackleg pressure, some growers are experiencing economic loss, especially during the seedling stage. Germination of canola can coincide with the release of blackleg spores hence canola is particularly vulnerable at the seedling stage. Control within canola crops relies heavily on integrated use of fungicides, resistant varieties and cultural practices. As the fungal population changes fungicides become less useful and resistance is lost within specifically bred canola varieties (Salisbury et al., 1995). As current methods of integrated control slowly breakdown new methods are being sought out and studied with the intention of integrating them into current practices.

All plants posses active defence mechanisms against pathogen attack, but these mechanisms fail when a virulent pathogen avoids and evades the plant's resistance reactions and defences. A plant's defences can be enhanced against a broad spectrum of pathogens by stimulation and can be of two forms, systemic acquired (SAR) and induced systemic (ISR) resistance (Osbourn, 2001). Induced systemic resistance (ISR) activates a plants latent defence system and primes the individual for pathogen attack and the mechanism is generally considered to be triggered by a plant – rhizobacterium interaction (van Loon et al., 1998).

This study was undertaken to isolate rhizobacteria capable of inducing systemic resistance in canola to the blackleg fungus to improve seedling survival and establishment under field conditions.

MATERIALS AND METHODS

Bacterial isolation: Intact soil cores were removed from three separate farms. All three sites used a rotation of pasture followed by varying lengths of a tight canola and wheat rotation. Site one was in its first year of canola, site two was in the third year of canola growth and site three had grown canola for five years. Both canola and wheat were grown in the intact soil cores at ambient temperature for 3 weeks. Root systems were removed and gently washed to remove excess soil. Rhizobacteria were isolated using two methods. The first method was used to isolate rhizosphere bacteria by washing the roots in bacto peptone water. A second method was used to isolate endophytic bacteria by surface sterilising root material and crushing in bacto peptone water. Serial dilutions of the two isolation methods were plated onto general nutrient media, a *Pseudomonas* selective media and a boiled subsample on nutrient media to isolate *Bacillus* species.

Bacterial characterisation: Approximately 600 bacterial colonies were selected from the above isolation process. These were grown on media plates to test their ability to use three particular compounds, colloidal chitin, salicylic acid and 1-aminocyclopropance-1carboxylic acid (ACC).

In vitro antagonism of canola: Canola seed was germinated in petri dishes lined with filter paper and soaked for 24 hours in a bacterial suspension. After 14 days percentage germination, root and shoot length and dry weight was recorded for each seedling.

In vitro antagonism of *Leptosphaeria maculans*: Bacterial rings were grown around mycelial plugs of Leptosphaeria maculans on ¼ Potato Dextrose Agar. After ten days the distance from the front of fungal growth to the bacterial ring was measured.

Induced systemic resistance: A hydroponic system containing Hoagland's solution was used to determine each bacterial isolate's ability to induce systemic resistance in canola. Pre-germinated canola seedlings were grown in the system for five days before the bacterial isolate was introduced into the solution. After two days a blackleg suspension was sprayed onto the seedlings. Disease was assessed 17 days after inoculation. A disease rating scale of 0 to 10 was used where 0 was no disease symptoms and 10 was complete seedling death.

RESULTS

Results have not been included in this document for the *in vitro* antagonism of both canola and *L. maculans* as the data set is not complete and does not offer any further insight in this study.

Bacteria isolated from internal and external root tissue showed a clear preference for wheat root systems compared to canola (Figure 1 and 2). With the exception of rhizosphere *Bacillus* bacteria which preferred canola root tissue (Figure 1). This was the only rhizosphere group to be significantly different. However all three groups of endophytic bacteria showed a significant difference when compared between canola and wheat (Figure 2).

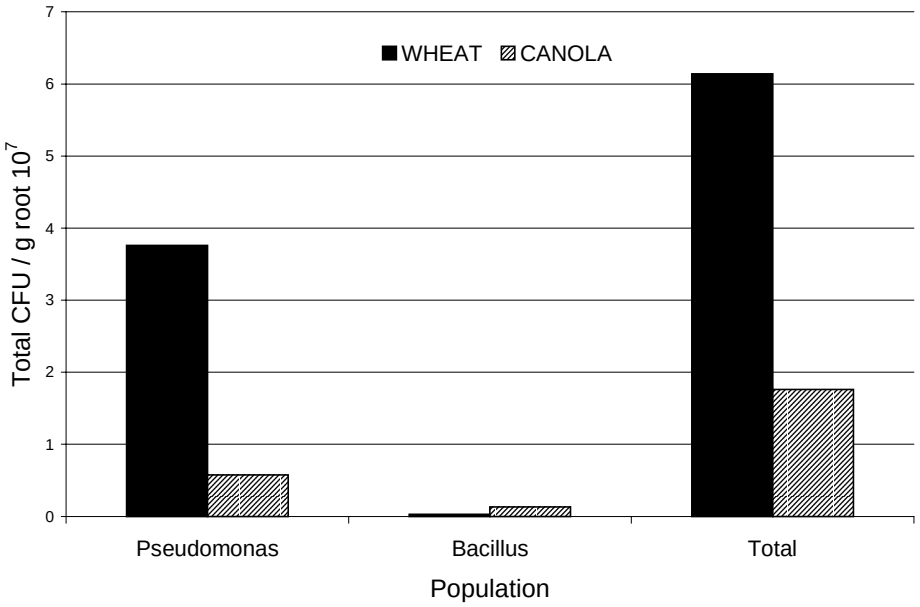

Figure 1: Number of *Pseudomonas* sp, *Bacillus* sp and total bacteria isolated from canola and wheat rhizospheres. (Only Bacillus between canola and wheat showed a significant difference at P<0.05)

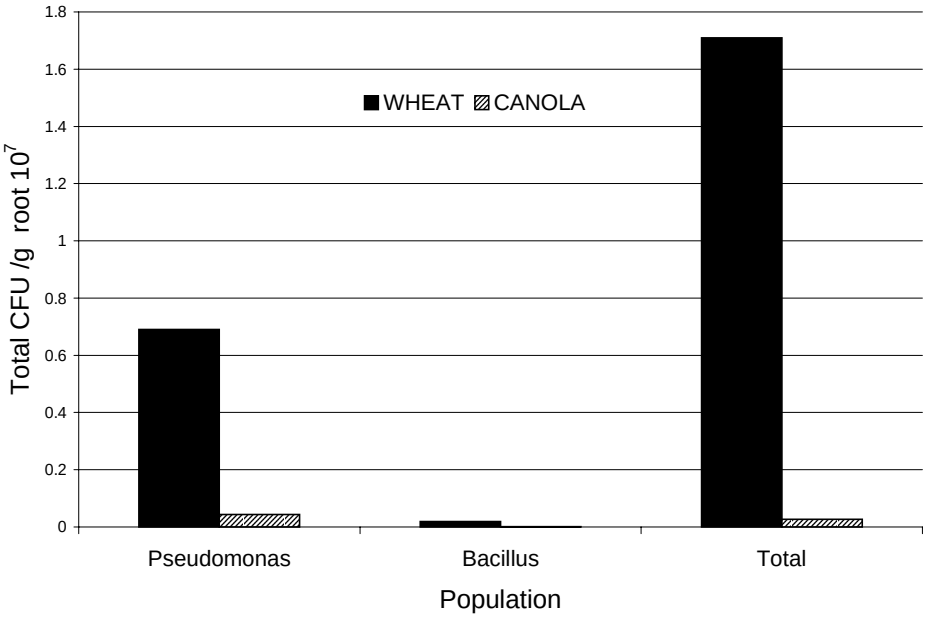

Figure 2: Number of *Pseudomonas* sp, *Bacillus* sp and total bacteria isolated from internal tissue of canola and wheat as endophytes. (All bacteria between canola and wheat showed a significant difference at P<0.05)

All of the bacterial groups had a percentage of individuals that tested positive for utilisation of the three compounds that were assayed (Table 1). However one group of bacterial, rhizosphere *Bacilli* from wheat showed no positive result for any of the compounds. When the same bacterial isolates were tested for their ability to induce systemic resistance in canola a similar spread of results was observed across the groups of bacteria (Table 2). With the majority of the bacteria falling into the medium category of ISR ability.

Table 1: Percentage of bacterial isolates able to utilise three compounds: 1-aminocyclopropane-1-carboxylic acid (ACC), salicylic acid (SA) and colloidal chitin (CC).

	Bacillus				Pseudomonas				General			
	Endophytic		Rhizosphere		Endophytic		Rhizosphere		Endophytic		Rhizosphere	
Assays	C[1]	W[2]	C	W	C	W	C	W	C	W	C	W
ACC	12	16	14	0	15	12	24	8	8	16	15	8
SA	8	4	0	0	6	6	10	17	11	2	0	4
CC	24	52	14	0	19	20	24	21	15	20	15	12

[1] Bacteria isolated from canola root systems
[2] Bacteria isolated from wheat root systems

Table 2: Percentage of bacterial isolates that produced low, medium and high ISR activity in canola

	Bacillus				Pseudomonas				General			
	Endophytic		Rhizosphere		Endophytic		Rhizosphere		Endophytic		Rhizosphere	
	C[1]	W[2]	C	W	C	W	C	W	C	W	C	W
Low	0	0	0	0	0	6	0	4	0	2	0	0
Medium	92	64	100	100	72	84	71	75	77	78	78	96
High	8	36	0	0	28	10	29	21	23	20	22	4

[1] Bacteria isolated from canola root systems
[2] Bacteria isolated from wheat root systems

DISCUSSION

To colonise the internal root tissue of any plant a bacterium needs to be specialised and adapted to the environment (Hallmann, 2001). With this in mind it is not especially surprising that the number of bacteria isolated from the internal tissue of both canola and wheat is considerably lower (Figure 2), approximately 4×10^7 cfu/g tissue, compared to the number of rhizosphere bacteria (Figure 1). A greater number of Bacilli rhizosphere bacteria were isolated form canola roots than wheat. This may be attributed to *Bacilli* being capable of surviving the unfavourable conditions of the canola rhizosphere by producing spores. However *Bacilli* cannot rely on the use of spores in the internal tissue to survive so they remain at a lower number in the canola tissue compared to the wheat.

There was little difference in the way that the bacterial groups reacted to the three compounds tested. The only significant trend was the absence of any positive bacteria within the rhizosphere *Bacilli* from wheat. It seems that the bacteria from this group do not require the competitive attributes of being able to use CC, SA and ACC. Environmental conditions may be such that these attributes are not necessary for the bacteria to be competitive and survive.

In general bacteria capable of inducing high levels of systemic resistance in canola have been isolated from canola originally.

From this work so far it appears that to identify a bacterial isolate that would act as a good inducer of systemic resistance in canola it would be advisable to target organisms that are isolated directly from internal and external root tissue of canola. It doesn't seem to be important if the organisms are capable of using the three particular compounds that have been tested in this study. However these characteristics may further aid the organism in a field environment to be competitive and to colonise effectively and should not be dismissed as futile characteristics.

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