THE WINSTON CHURCHILL MEMORIAL TRUST

CHURCHILL FELLOWSHIP 2002b

DELETERIOUS RHIZOBACTERIA AS WEED CONTROL AGENTS.

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Contents

I would like to dedicate this Churchill Fellowship Report to Jenan Nichols who lost her battle with cancer during the time of my visit to Columbia, Missouri. Jenan was the technician who ran the laboratory where I worked and knew about everything within the lab and a fair amount outside it. She was a wonderful person and my only regret is that I did not get to know her further. Thank-you Jenan and may you rest in peace.

1. Precis and Acknowledgements

This report details the findings from a Churchill Fellowship visit to Columbia, Missouri, USA investigating the role of Deleterious Rhizobacteria (DRB) as weed control agents. During this fellowship;

♦ a series of experiments was undergone to test known deleterious bacterial isolates against Australian crop cultivars and West Australian crop weeds. These isolates are deleterious to the growth of several US weeds but do not affect the growth of their winter wheat cultivars. It was decided to test our weeds and crop varieties to ascertain if the US bacterial isolates could be used in Australia.

♦ Methods associated with isolating these bacteria, testing their effects on crop and weed seeds under sterile and non-sterile conditions as well as inoculating soil were learned in order to isolate bacteria specific to Australian conditions.

My visit to the USA and the experiences I have gained would not have been possible without;

♦ The financial assistance from the Winston Churchill Memorial Trust.

♦ The support of the Department of Agriculture (WA) both financially and allowing me to develop my professional expertise in the area of weed control.

♦ Dr Robert Kremer (USDA, Columbia, Missouri) who kindly allowed me to come and visit to share his expertise of deleterious rhizobacteria. Dr Kremer proved a very worthy and helpful mentor.

♦ The assistance and friendliness of all those in the laboratory made my stay in Columbia a very happy one. Special thanks to Heidi Lewis and Michael Atkinson who helped me with some of my experiments.

2. Programme

Columbia, Missouri, USA **17 May – 4 July**

♦ Department of Natural Resources – laboratory

♦ University of Missouri Weed Science Field Day – The annual Field Day at Bradford Farm showing recent research results.

♦ Tour of US Geological Survey (Columbia Environmental Research Centre) with Dr Mike Mac (Director) – Effects of herbicides and other chemicals on river health and aquatic life.

♦ Seminar by Dr Rob Myers - Alternative Grain Crops for Missouri

♦ Seminar by Dr Sally Peltzer – My life as a Weed Woman in Western Australia

3. Executive Summary

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Fellowship objective

To investigate the use of Deleterious Rhizobacteria (DRB) as a form of control of Australian cropping weeds.

Fellowship highlight

Columbia, Missouri, USA

Worked in the laboratory of Dr Robert Kremer testing known DRB isolates on a range of Australian crop cultivars and Western Australian weed species. Also gained valuable experience of the many techniques associated with isolating and testing these bacteria for future work in Australia.

Findings

♦ Some of the deleterious rhizobacterial isolates from USA were successful in reducing the growth of some of Australia's weed species. They also however, reduced the growth of Australia's canola varieties. It is not recommended that these US isolates be used in Australia.

♦ Relevant methods associated with isolating these bacteria, testing their effects on crop and weed seeds were learned in order to isolate similar bacteria in Australia. It is recommended that Australian DRB's are isolated that target Australian weeds and are specific to Australian conditions.

Seminars

Invited seminar presentations reviewing the results of this fellowship and targeting the potential for the use of DRB within Australia will be made at;

♦ The Department of Agriculture, Albany – Albany Hour (a seminar series), September, 2003.

♦ The Department of Agriculture, South Perth for the Weed Science Society of WA, November, 2003.

♦ A Public Seminar hosted by the University of Western Australia, Albany, late 2003.

Publications

It is expected that one refereed article detailing the results from testing American DRB's on Australian crop cultivars and weed species will be published. Several articles are also intended, including those specifically written for e-weed (a weekly email publication targeting the farmers of Western Australia), the Weed Science Society of WA newsletter, Weed It and Reap (the CRC for Weed Management Systems newsletter) as well as at least one rural newspaper.

Research

There are plans for a research programme to address the recommendations from this report and investigate further into possible Australian DRB's targeting Australian weeds under Australian conditions. A research proposal will be submitted to the relevant funding bodies for a 3-5 year project to be conducted at the Department of Agriculture, Albany, WA.

4. Introduction

Background

Western Australia has the world's worst herbicide resistance in weeds due predominantly to the multi-resistance of Annual Ryegrass (*Lolium rigidum*). Annual Ryegrass has shown resistance to six major herbicide groups including glyphosate. Wild radish (*Raphanus raphanistrum*) is now resistant to four herbicide groups. The historical reliance on herbicides has brought about the need for integrated weed management using a variety of non-herbicide weed management techniques. New techniques need to be investigated and integrated into this overall programme. One technique is to utilize bacteria as a biocontrol agent to reduce weed growth.

Non-parasitic bacteria that are associated with plant roots (in the rhizosphere or area just around the roots) are described as rhizobacteria. Those that inhibit or reduce plant growth are deleterious rhizobacteria (DRB). These DRB usually inhibit growth by producing phytotoxins however, they may also reduce plant growth by competing with the plant for nutrients or by indirectly affecting the root colonization of other beneficial microorganisms such as rhizobia or mycorrhizas.

Many of these DRB are very specific in their level of plant growth reduction, at both a species and a cultivar level. This means that one bacterial isolate may reduce the growth of one plant (e.g. a weed) while not affecting the growth of another (e.g. a crop plant). The main mechanism behind this specificity is likely to be due to the proliferation and competitiveness of the bacteria with other microorganisms in the rhizosphere. This depends on the type and volume of exudates (or chemicals) that the plant releases from its root which attract the bacteria. Different plants exude different chemical substrates, which in turn encourage different rhizobacteria. There is also some evidence to suggest that the composition of root exudates may affect the production of the toxins by the bacteria. Finally, the plants may differ in their response to the phytotoxic substances produced by these bacteria.

Rhizobacteria that reduce the germination or growth of weeds but do not affect the growth of the crop plant are potential weed control agents. They can reduce the weed's competitive ability and give the desired crop the competitive advantage resulting in yield increases. One DRB strain (isolated from winter wheat roots) reduced the growth of downy brome and increased yields of winter wheat by up to 35% (Kennedy *et al*., 1991).

Once a desired DRB is isolated, however, it needs to be tested on other crop species and cultivars. Western Australian farmers use a wide range of crops in their crop rotation programme and it is not desirable for a DRB to reduce the growth of the crop species sown the following year. The DRB also needs to be able to be distributed over the paddock in a cheap and efficient manner, targeting the weed as it is germinating or emerging from soil and gaining the most competitive advantage for the crop. The DRB needs to be able to survive in the soil, be compatible with agronomic practices such as the application of herbicides and be in sufficient numbers to be able to reduce weed growth.

DRB's in USA

Dr Robert Kremer, Columbia, Misssouri has a range of DRB's that were previously isolated and shown to be deleterious to various weeds. It was decided to test these bacteria against a range of Western Australian crop weeds. Due to the necessity for these DRB's to not affect the growth of the crop species used in rotations on Western Australian farms, it also was decided to test the DRB's on the growth of various crop cultivars.

DRB's in Australia

Western Australian broadacre cropping conditions vary considerably from those in the USA with a different climate, different soils and different weed problems. Consequently, it is unlikely that the DRB's designed for Missouri conditions will be suitable for Australia.

Missouri, USA has a temperate climate with average winter temperatures of –1.3ºC and average summer temperatures of 23.7ºC. The average rainfall is 975 mm of which 70% falls between April and September, or over summer. The soils consist mainly of well-drained heavy loam soils or heavy loams over heavy clay subsoils. Missouri crops twice during the year and the major weeds are the Waterhemps (*Amaranthus* spp.), Foxtails (*Setaria* spp.), Ragweeds (*Ambrosia* spp.), Nutsedges (*Cyperus* spp.) and Morning glories (*Ipomoea* spp.) Western Australia has a Mediterranean climate with hot, dry summers and wet, cooler winters. The soils in WA are generally poor and often sandy. WA grows its' crops only during winter. The major weeds are Annual Ryegrass, Wild Radish, Silvergrass, Wild Oats and Brome Grass.

A research project in WA on DRB's as possible weed control agents should focus on WA's major weed species as well as the main crop cultivars. One of the major concerns for the survival of DRB's in Missouri are the freezing conditions which occur over winter which affect the survival of the bacteria. In WA, it is likely that the hot, desiccated soils over summer may be the major survival factor. Isolating DRB's that can withstand these WA conditions should also be a primary focus.

Whilst in Missouri, I learnt the necessary techniques associated with isolating DRB's from plant roots and testing them for weed growth suppression. I wish to use these techniques in a comprehensive research programme to isolate and test Australian DRB's (funding pending).

5. Major Findings

A detailed description of the Materials, Methods and Results can be found in the Appendix (page 11).

Materials and Methods

The effect of four deleterious rhizobacteria (DRB) isolated from US weed species was tested on 10 Australian crop cultivars and six weed species. Three methods were used; the effect of each isolate on plant growth on sterile agar plates, in sterile growth pouches and in non-sterile soil.

Results

a) Agar plates

Two of the rhizobacterial isolates, LC-19 and G2-11, reduced the root growth of all of the crop cultivars and weed species. Isolate D1 also reduced root growth of most of the crop cultivars and weed species but to a lesser extent compared to the other two isolates. There was no effect of the isolate D2-11.

b) Growth Pouches

The rhizobacterial isolates, had a lesser effect on the growth of the crop cultivars and the weed species when grown in growth pouches compared to water agar. The two most aggressive isolates, LC-19 and G2-11, reduced the growth of canola and Silvergrass (*V. myuros*). The root growth of *B. hordaceus* was also reduced by isolate LC-19. Silvergrass growth was also reduced by isolates D1 and D2-11. These isolates did not affect any of the other weed species.

c) Non-sterile soil

The rhizobacterial isolates did not affect the growth of any of the crop cultivars tested except for Surpass 501 canola. The effect of the same isolates varied between weed species. Isolates LC-19 and G2-11 and D1 reduced the growth of Silvergrass. Isolate LC-19 reduced the growth of *B. diandrus* but did not affect the other two *Bromus* spp. Isolate G2-11 did not affect any of the *Bromus* species.

6. Discussion

The rhizobacterial isolates from USA were deleterious to the growth of some of the Australian plant species tested. The most probable reason for this is the production of phytotoxins. Under sterile conditions and on agar, all of the US isolates tested reduced plant growth to some degree. As the plants were inoculated as ungerminated seed, it is unlikely that competition for nutrients or a reduction in beneficial microorganisms was the cause of growth reduction. The level of plant growth reduction varied with the DRB inoculant, Isolates LC-19 and G2-11 were the most aggressive isolates throughout the experiments. Assays done on these isolates in the US have confirmed that isolate G2-11 produces considerable amounts of hydrogen cyanide (Kremer, and Souissa, 2001) and overproduces 3-indoleacetic acid, which can be toxic in high concentrations. Isolate LC-19 does not produce these compounds, however, it and G2-11 produce a fair amount of iron siderophores, so there's a possibility for competition for iron in the rhizosphere as the seedling grows.

Canola and *Vulpia myuros* were the most affected across the whole of the project, exhibiting growth reduction on agar, in growth pouches and in soil. The extent of growth reduction attained with the inoculation of DRB's, however, varied with inoculation method (sterile agar, sterile growth pouches and non-sterile soil), plant species and cultivar. In non-sterile soil, there was a reduction in growth in canola cv. Surpass 501, *Vulpia myuros* and *Bromus diandrus* after inoculation with some of the isolates. There was no reduction in growth of any of the other species in soil although these same species showed a marked reduction in growth in sterile conditions on agar. Under non-sterile conditions, the isolates must compete with other microorganisms in the rhizosphere. This is the main mechanism for the specificity of the DRB's to reduce growth and the reason why we can manipulate the system and select bacteria that will reduce the growth of a weed but not a crop plant. Different plants exude different chemicals into the rhizosphere affecting the type and magnitude of associated microorganisms (Nehl *et al.,* 1996 and Kennedy *et al*, 2001). In the case of canola and Silvergrass, the DRB's successfully competed with the other rhizosphere microorganisms resulting in sufficient numbers with enough phytotoxin production to affect growth. The rhizosphere microorganisms associated with the other species out-competed the DRB's and no growth reductions occurred. Similarly, in previous US studies, the growth of 47 plant species varied considerably after inoculation with a DRB isolate (Kennedy *et al*., 2001).

Although there was no effect of any of the isolates on the barley or wheat cultivars when grown in soil, there were response differences between the species and to some degree between the cultivars when tested on agar. Isolates LC-19 and G2-11 substantially reduced the germination of barley cultivars on agar. Once germinated, however, their subsequent growth was less affected. The severity in the reduction in germination differed between cultivars. The germination of the wheat cultivars was either unaffected or only slightly reduced but their subsequent plant growth was severely reduced. When the same isolates were inoculated onto pre-germinated seedlings in growth pouches the growth reduction in both barley and wheat was not as severe. Canadian studies (Campbell *et al.,* 1986), have suggested that timing of inoculation is important. In field studies, canola growth was affected by pseudomonad isolates but then grew out of it after 2-3 weeks.

This study showed that although the US isolates were successful in reducing growth of some of the Australian weeds, they also reduced the growth of some of the crop varieties. This has implications for the application of DRB's selected for use in Australia. The DRB isolates used in this study were selected from US weeds grown in US soils and conditions. There is a need to select isolates that target Australian cropping weeds and are able to withstand the different soils and conditions that exist in our major cropping zones. The selected isolates need to be able to persist for a few weeks after application to reduce the early growth of the target weed but not persist in high enough numbers to affect any of the other crops in future rotations. The DRB could be sprayed like a herbicide in high numbers early in the season, which then reduce to minimal numbers over the year.

Alternatively, persistent but very selective DRB isolates could be applied. These isolates should be initially selected so as not to reduce the growth of any of our crop varieties. After persisting in the soil from season to season, these isolates would then need to multiply quickly at the season break.

7. Conclusions and Recommendations

- Some of the deleterious rhizobacterial isolates from USA were successful in reducing the growth of some of Australia's weed species. They also however, reduced the growth of Australia's canola varieties. It is therefore necessary to isolate Australian DRB's targeting Australian weeds rather than introduce US isolates.

- The extent of growth reduction attained with the inoculation of US DRB's varied with inoculation method (whether the plant was inoculated in sterile conditions or in soil), plant species and cultivar. The difference between the response of each plant species is the reason why we can select DRB's that will reduce weed growth and not affect the growth of crops.

- Relevant methods associated with isolating these bacteria, testing their effects on crop and weed seeds were learned in order to isolate bacteria specific to Australian conditions. It is recommended that Australian DRB's are isolated and tested under Australian conditions. Due to the chance that these DRB's may affect crop rotations in the future, the selection of non-persistent isolates would be the preferred approach. These can be applied at high numbers targeting the early growth of weeds. The bacterial numbers would then reduce to levels not deleterious to the choice of rotation in the following year. Another approach would be to isolate a persistent DRB that would only need to be applied once. These isolates would need rigorous testing against Australia's crop cultivars. The likelihood of finding a DRB that will over-summer in Australia's harsh conditions and then rapidly grow to deleterious numbers at the break of the season makes this approach less favorable.

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APPENDIX

Introduction

Previously isolated, known deleterious rhizobacteria (DRB) were tested on a range of Australian crop cultivars and weed species. Three methods were used. The first was an initial screening of the phytotoxic effects of each isolate on seed germination and early root growth on agar plates. This was done under sterile conditions. The second method involved assessing each bacteria on the root growth of two-day old seedlings in sterile growth pouches. Finally, the effect of the DRB isolates on plant growth was tested in non-sterile soil.

Four DRB isolates were used in this study; LC-19 and G2-11 (both *Pseudomonas flourescens* isolated from Giant foxtail), D2-11 (*Aeromonas hydrophila* isolated from Field bindweed) and D1 (*P. putida*). The Australian crop cultivars included three spring wheat cultivars (*Triticum aestivum* cv. Janz, Camm and Wyalkatchem), three barley cultivars (*Hordeum vulgare* cv. Gairdner, Stirling and Yagan), two triazine-tolerant canola cultivars (*Brassica napus* cv. Karoo and Surpass 501), one narrow-leafed lupin cultivar (*L. angustifolius* cv. Merrit) and one yellow lupin cultivar (*Lupinus luteus* cv. Wodjil). There were six Australian weed species: three Brome Grasses (*Bromus diandrus, B. rubens, B, hordaceus*), Annual ryegrass (*Lolium rigidum*), Wild Radish (*Raphanus raphanistrum*) and Silvergrass (*Vulpia myuros*).

Materials and Methods

a) Agar Plates

The phytotoxic effects of four DRB isolates (L2-19, DRB-D1, D2-11, G2-11) were tested on three spring wheat cultivars (cv. Janz, Camm and Wyalkatchem), three barley cultivars (cv. Gairdner, Stirling and Yagan), two canola cultivars (cv. Karoo and Surpass), one narrow-leafed lupin cultivar (cv. Merrit) and one yellow lupin cultivar (cv. Wodjil).

The seeds were surface sterilized (90 seconds in 70% ethanol, rinsed in sterile water, 90 seconds in 0.6% sodium hypochlorite then rinsed twice in sterile water), blotted dry on sterile paper towel, distributed evenly onto 1.0% water agar (16/plate for the grasses and canola seeds and 8/plate for the lupin seeds) and germinated overnight at 20°C. Cultures of each isolate were suspended in 0.1M $MgSO_4$ and a 30 μL suspension was delivered to each seed except for the lupin seeds, which received 60 μL of each isolate. The bacterial suspensions contained approximately $1x10^{-9}$ CFU/ml. A control was included where the seeds received the same volumes of sterile water. The plates were incubated at 20°C and root length and germination was determined after 48 hours for all species except the lupins, which were incubated for 96 hours. The seed germination and root length for each plate was meaned and treated as one replicate. There were three replicate plates for the wheat cultivars and four replicate plates for the other crop species.

The phytotoxic effects of the same bacterial isolates were tested on six Australian weed species (*Bromus diandrus, B. rubens, B, hordaceus, Lolium rigidum, Raphanus raphanistrum* and *Vulpia myuros*). The methods were the same as described above except that all species were incubated in the dark at 18°C for 3 days and then left on the bench at ambient temperature (approximately 20°C) for an additional 2 days.

b) Growth Pouches

The four DRB's isolates (L2-19, DRB-D1, D2-11, G2-11) used above were tested on five Australian crop cultivars (spring wheat cv. Camm, barley cv. Gairdner and Yagan, canola cv. Surpass 501 and yellow lupin cv. Wodji) and five weed species (*B. diandrus, B. rubens, B, hordaceus, Lolium rigidus* and *Vulpia myuros*). Surface-sterilised seed was pre-germinated on 1% water agar for 48 hours. The seedlings were then transferred into growth pouches saturated with 50 ml of nutrient solution plus nitrogen (Broughton and Dilworth, 1970); (two seedlings/pouch for wheat and barley, three seedlings/pouch for canola and only one seedling/pouch for lupins). The pouches were either inoculated with 1 ml of a suspension of each isolate (approximately 5 x 10^{-8} CFU/ml) or left uninoculated and grown under growth tubes (12 hour light/12 hour dark) for 5-7 days. Root length and Shoot fresh weights were measured. The growth pouch technique used was based on one developed by Somasegaran and Hoben (1985) modified by Kremer *et al*. (1990)

c) Non-sterile soil.

Three of the DRB isolates (L2-19, DRB-D1, D2-11, G2-11) were tested against the same crop cultivars and weed species as in the growth tubes but in unsterile soil. Seeds of each species were sown directly into small pots of 50% sieved field soil (Mexico silt loam – fine, montmorilonitic, Mesic, Vertic, Epiqualf) mixed with 50% potting mix. Each pot was either inoculated with 5 ml of a suspension of each isolate (approximately 5 x 10^{-8} CFU/ml) or left uninoculated and plants were grown under growth tubes (12 hour light/12 hour dark) for 7-14 days depending on growth rate. Root and shoot fresh weights were measured for all crop species, except the canola, and all weed species where whole plant fresh weights were taken.

Results

a) Agar plates

Two of the rhizobacterial isolates, LC-19 and G2-11, reduced the root growth of all of the crop cultivars and weed species tested (Tables 1 and 2). Isolate D1 also reduced root growth of most of the crop cultivars and weed species but to a lesser extent compared to the other two isolates. There was no effect of the isolate D2-11.

The effect of the DRB's varied between the plant species (Tables 1 and 2). Isolate D1 reduced root growth of the crop cultivars to a minor extent but substantially reduced root growth of the *Bromus* species, especially *B. rubens*. This isolate had only a minor effect on the *Vulpia* species. The effect of the most deleterious isolates (LC-19 and G2-11) was also variable (Tables 1 and 2). The germination of the three spring wheat cultivars after inoculation was unaffected but the growth of the roots was reduced quite substantially (Table 1 and Figure 9). The same isolates (LC-19 and G2-11) reduced root growth and reduced the germination of Stirling and Yagan barley (Figure 10). Viewing the plates after 5 days, it appeared that the wheat and canola seedlings inoculated with isolates LC-19c and G2-11had died while the barley seedlings were still growing. These isolates also resulted in poor root growth in the two canola cultivars while reducing the growth of the yellow lupin and the narrow-leafed lupin cultivars to a lesser extent. Isolate D2-11 had little effect on any of the crop cultivars or weed species except for *B. diandrus and hordaceus* where a reduction in root growth was noted.

There were also some differences between the crop cultivars. The germination of Yagan barley was unaffected by isolates G2-11 and LC-19 while Gairdner and Stirling were (Table 1). Isolate D1 appeared to suppress root growth in vars. Gairdner and Stirling but have little effect on Yagan.

Low levels of germination of Wild Radish (*R. raphanistrum*) were recorded across the experiment. Consequently it is difficult to ascertain the effects of each isolate although there appears to be an overall trend of root growth reduction by LC-19, G2-11 and D1.

Table 1: The effect of four rhizobacterial isolates on the germination number and root length (RL) of three spring wheat cultivars grown on water agar (mean of three replicates), three barley cultivars, two canola cultivars and two lupin cultivars (mean of four replicates).

	Spring Wheat variety					
	Wyalkatchem		**Janz**		**Camm**	
Treatment	**Germ no.**	**RL (mm)**	**Germ no.**	**RL (mm)**	**Germ no.**	**RL (mm)**
Control	16.0	11.0	15.7	8.5	16.0	11.2
LC-19c	16.0	1.9	14.7	1.3	14.7	1.8
G2-11	12.0	1.6	13.7	1.3	15.7	2.1
D1	15.3	7.5	14.3	4.0	15.7	7.1
D2-11	16.0	9.0	16.0	7.2	16.0	9.9
LSD (p=0.05)	**1.0**	**0.6**	**ns**	**1.6**	**0.8**	**1.6**
	Barley variety					
	Gairdner		**Stirling**		**Yagan**	
Treatment	**Germ no.**	**RL (mm)**	**Germ no.**	**RL (mm)**	**Germ no.**	**RL (mm)**
Control	16.0	19.1	15.8	10.9	13.5	11.5
LC-19c	14.3	10.5	10.8	2.5	8.0	5.5
G2-11	13.0	9.6	12.8	6.0	7.8	6.8
D1	15.0	14.5	14.3	8.4	13.0	12.0
D2-11	15.3	16.8	16.0	11.2	13.5	11.4
LSD (p=0.05)	**1.3**	**2.9**	**0.9**	**1.6**	**2.6**	**3.0**
	Canola variety					
	Surpass 501		**Karoo**			
Treatment	**Germ no.**	**RL (mm)**	**Germ no.**	**RL (mm)**		
Control	15.8	9.1	15.5	7.0		
LC-19c	11.8	2.1	15.5	1.6		
G2-11	12.5	2.4	14.5	2.4		
D1	15.3	5.3	15.5	4.2		
D2-11	14.3	8.2	14.8	6.4		
LSD (p=0.05)	**2.1**	**1.9**	**ns**	**1.5**		
	Yellow Lupins -Wodjil		**Narrow-leafed Lupins -Merrit**			
Treatment	**Germ no.**	**RL (mm)**	**Germ no.**	**RL (mm)**		
Control	7.8	13.3	7.0	11.3		
LC-19c	7.5	8.1	6.5	6.8		
D1	7.8	10.5	6.3	7.3		
G2-11	7.3	7.4	7.0	11.3		
D2-11	7.5	12.6	6.8	13.9		
LSD (p=0.05)	**ns**	**ns**	**ns**	**2.4**		

Table 2 : The effect of four rhizobacterial isolates on the germination and root length (RL) of six weed species.

Weed Species	Treatment	Germ no.	RL (mm)
Bromus diandrus	Control	4.7	21.4
(8 seeds/plate)	LC-19	3.7	5.0
	D1	1.3	1.6
	D2-11	7.0	19.6
	G2-11	2.7	4.4
	LSD (p=0.05)	**1.8**	**10.1**
B. rubens	Control	7.8	16.2
(8 seeds/plate)	LC-19	8.0	3.6
	D1	6.3	4.5
	D2-11	7.3	8.6
	G2-11	5.8	2.9
	LSD (p=0.05)	**1.4**	**1.8**
B. hordaceus	Control	15.5	13.8
(16 seeds/plate)	LC-19	2.8	0.6
	D1	12.5	3.4
	D2-11	14.0	8.1
	G2-11	0.7	0.1
	LSD (p=0.05)	**2.3**	**2.2**
Vulpia myuros	Control	14.5	5.7
(16 seeds/plate)	LC-19	13.8	1.9
	D1	15.0	3.9
	D2-11	14.5	4.8
	G2-11	12.8	2.1
	LSD (p=0.05)	**ns**	**1.1**
Lolium rigidum	Control	6.0	6.6
(16 seeds/plate)	LC-19	1.0	0.3
	D1	2.8	2.0
	D2-11	4.5	4.6
	G2-11	0.5	0.1
	LSD (p=0.05)	2.8	3.0
Raphanus	Control	3.0	3.8
raphanistrum	LC-19	0.7	0.2
(12 seeds/plate)	D1	3.0	0.7
	D2-11	3.7	2.5
	G2-11	0.7	0.2

b) Growth pouches

Rhizobacterial isolates LC-19 and G2-11 severely reduced both the root and shoot growth of Surpass 501 canola in growth pouches ($p<0.05$). The same isolates however, had a lesser effect on the growth of the crop cultivars when grown in growth pouches compared to water agar (Figures 1 and 2). There appeared to be minor reductions in growth in Gairdner barley and Camm wheat after inoculation with these isolates but these were not significant ($p<0.05$). Visually these isolates seemed to reduce the number of roots in both wheat and barley. None of these reductions were reflected in the wheat or barley shoot growth. Isolates LC-19 and G2-11 had no effect on Yagan barley. The growth of Wodjil lupins in the growth pouches was quite variable however both isolates appeared to reduce their root and shoot growth (results not shown). There was no effect of isolate D1 on any of the cultivars tested ($p<0.05$).

Figure 1: The effect of four rhizobacterial isolates on the root length of four crop cultivars grown in growth pouches (mean of three replicates) (LSD @ 0.05: canola = 49.4, other cultivars not significant).

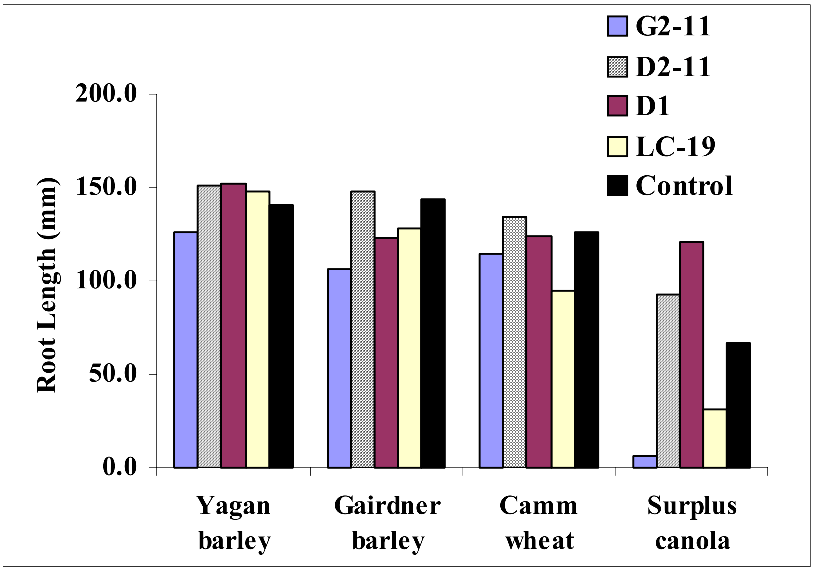
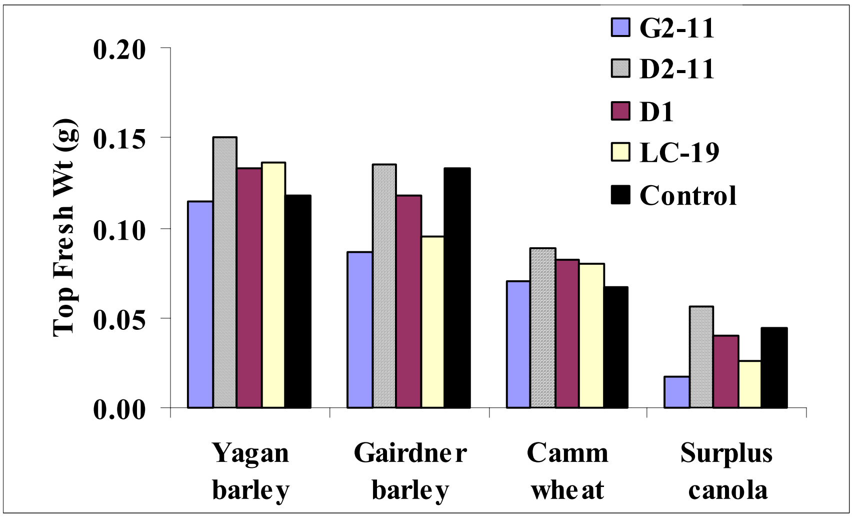
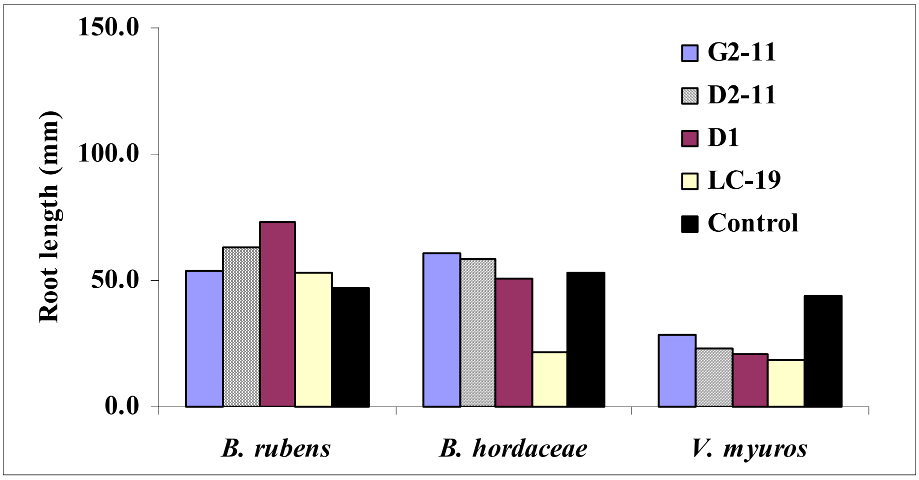


Figure 2: The effect of four rhizobacterial isolates on the shoot fresh weight (g) of four crop cultivars grown in growth pouches (mean of three replicates) (LSD @ 0.05: canola = 0.09, other cultivars not significant).



The resulting root growth of the weed species in the growth pouches varied from the agar plates (Figure 3). As on the agar plates, the root growth of *V. myuros* was substantially reduced by three isolates, LC-19, G2-11 and D1 ($p<0.05$). However, in the growth pouches, isolate D2-11 also reduced the growth of *V. myuros* ($p<0.05$). Isolates D1 and D2-11 did not affect any of the other weed species. Isolate LC-19 reduced root length in *B. hordaceus* but had no effect on *B. rubens* ($p<0.05$). Although there were many missed replicates in the *L. rigidus* pouches, isolate LC-19 also appeared to cause a substantial reduction in Annual Ryegrass root growth (results not shown). Isolate G2-11 had no effect on either of the same *Bromus* species. There were many missed replicates within both the *B. diandrus* and, however it appeared that none of the isolates were detrimental (results not shown).

Figure 3: The effect of four rhizobacterial isolates on the root length of three weed species grown in growth pouches (mean of three replicates). (LSD @ 0.05: *B. rubens* =27.3, *B. hordaceus* = 14.0, *V. myuros* =19.0).



c) Non-sterile Soil

The rhizobacterial isolates did not affect the growth of any of the crop cultivars tested except for Surpass 501 canola when grown in non-sterile soil ($p<0.05$) (Figures 4, 5 and 6). There was a reduction in growth in Surpass 501 canola after inoculation by isolate LC-19 but not by isolates D1 and G2-11 (Figure 6) which previously had reduced canola growth on agar. None of the isolates reduced the growth of any of the cereal grain cultivars (Gairdner, Yagan and Camm) (Figure 4) or Wodjil lupins (Figure 5).

The effect of the rhizobacterial isolates in soil varied between weed species (Figures 7 and 8). Isolates LC-19 and G2-11 reduced the growth of *V. myuros* in non-sterile soil ($p<0.05$). These same isolates similarly reduced the growth of Vulpia *sp.* in the growth pouches. Isolate D1 also caused growth reduction in the *Vulpia* species in soil (Figure 8), to an extent similar to the other two isolates but had no effect on any of the other species ($p<0.05$). Isolate LC-19 reduced the growth of *B. diandrus* but did not affect the other two *Bromus* spp ($p<0.05$). Isolate G2-11 did not affect any of the *Bromus* species. There was a visual reduction in L. rigidus growth caused by all three of the isolates but these were not significant ($p<0.05$).

Figure 4: The effect of three rhizobacterial isolates on the whole plant fresh weights (g) of three grain cereal cultivars grown in non-sterile soil (mean of three replicates) (LSD @ 0.05 =not significant).

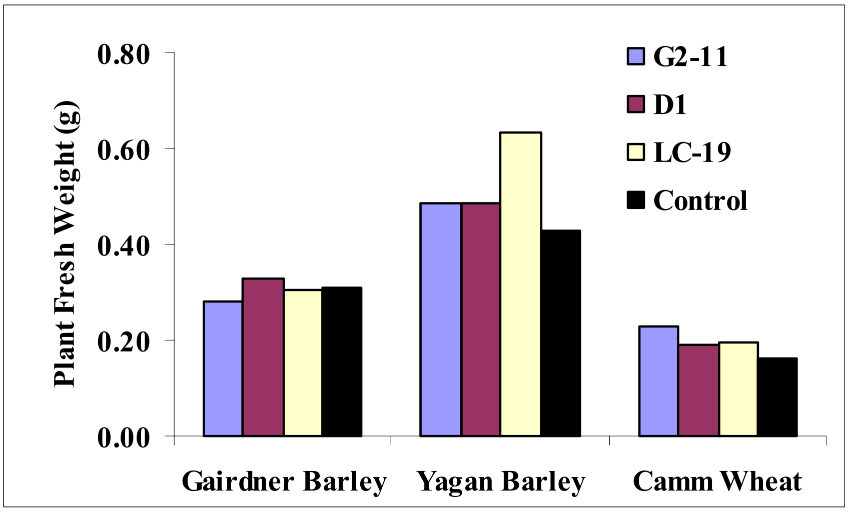


Figure 5: The effect of three rhizobacterial isolates on the root and shoot fresh weights (g) of Wodjil lupins grown in non-sterile soil (mean of three replicates) (LSD @ 0.05 =not significant).

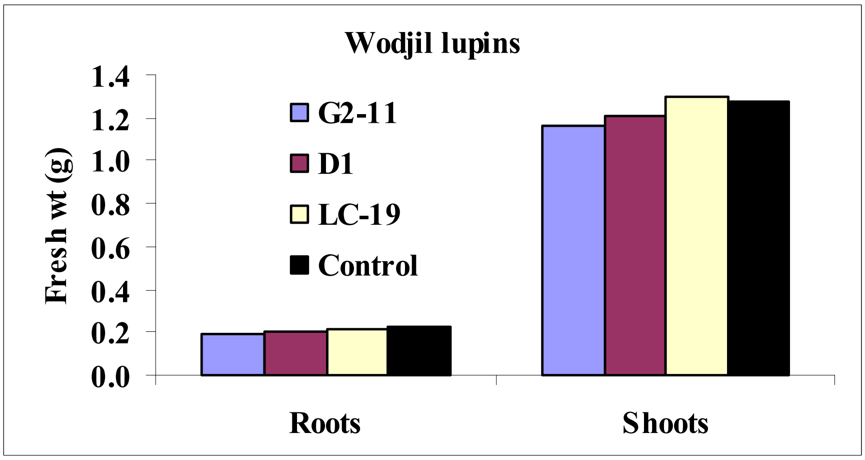


Figure 6: The effect of three rhizobacterial isolates on the whole plant fresh weights (g) of Surpass 501 canola grown in non-sterile soil (mean of three replicates) (LSD @ 0.05 = 0.052).

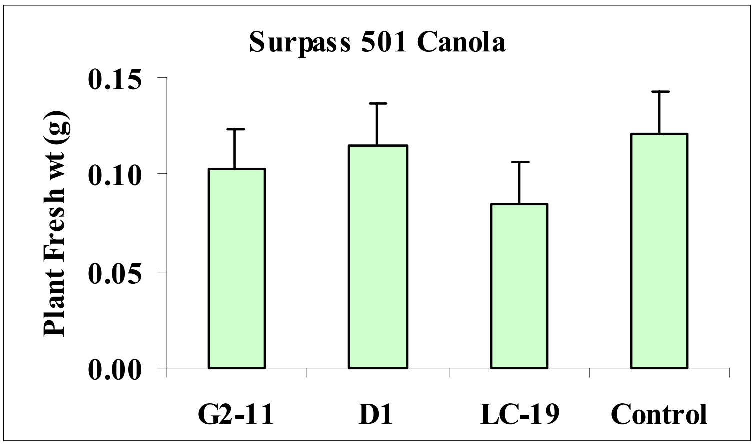


Figure 7: The effect of three rhizobacterial isolates on the whole plant fresh weights (g) of four weed species grown in non-sterile soil (mean of three replicates) (LSD @ 0.05: *L. rigidum* = 0.29, *B. hordaceus* = 0.011, *B. rubens* =0.005, *B. diandrus* =0.021).

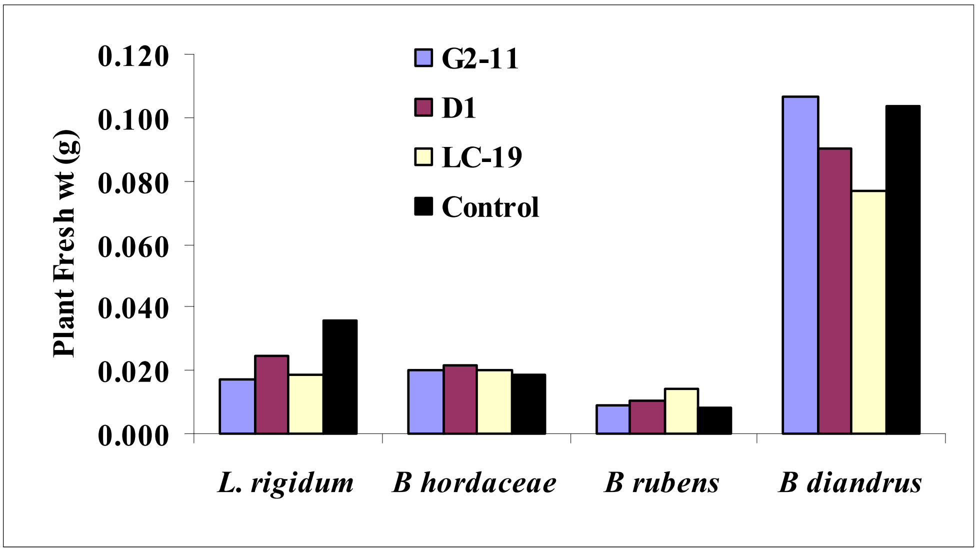


Figure 8: The effect of three rhizobacterial isolates on the whole plant fresh weight (g) of *Vulpia myuros* grown in non-sterile soil (mean of three replicates) (LSD @ 0.05 = 0.002).

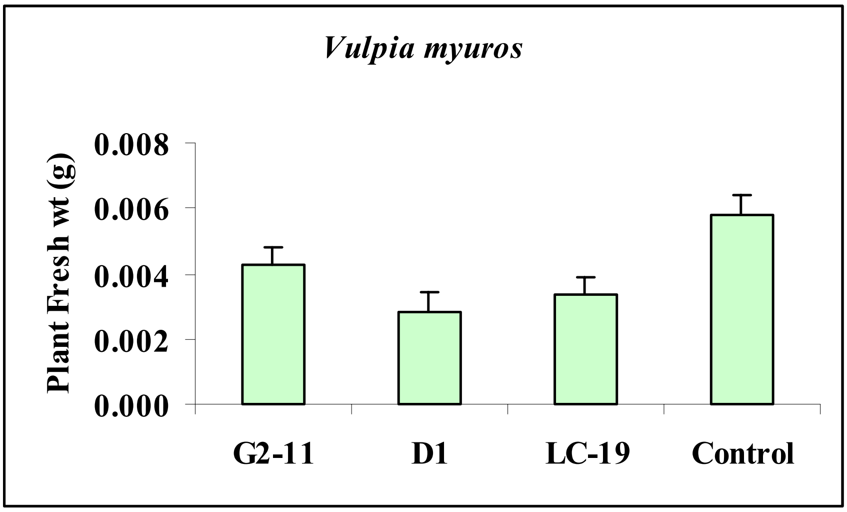


Figure 9: The germination and early growth of Camm wheat on agar after inoculation with DRB isolate G2-11.

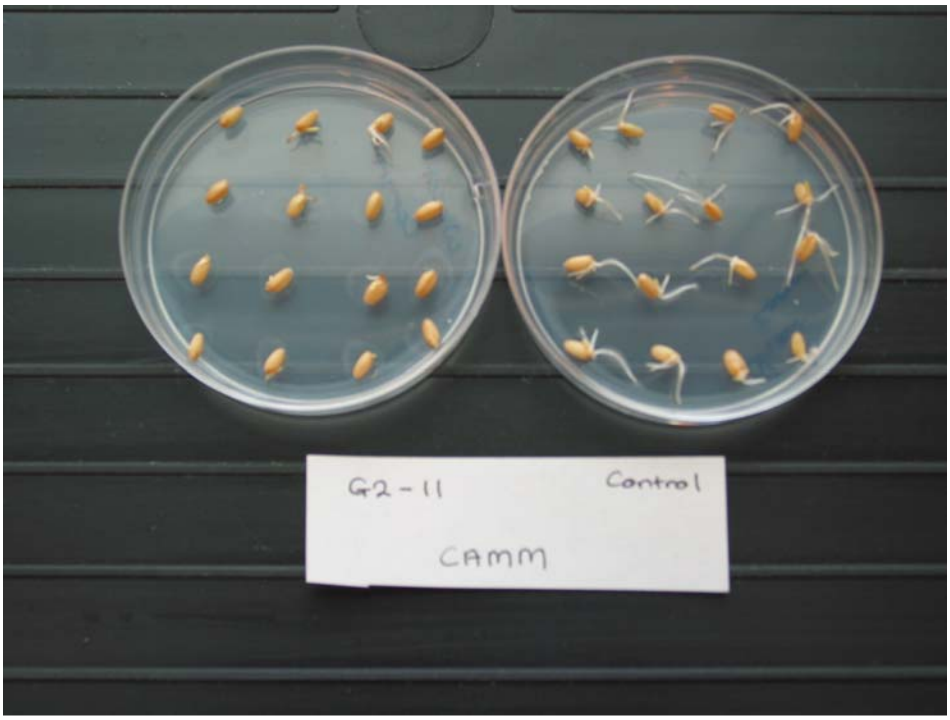


Figure 10: The germination and early growth of Yagan barley on agar after inoculation with DRB isolate G2-11.

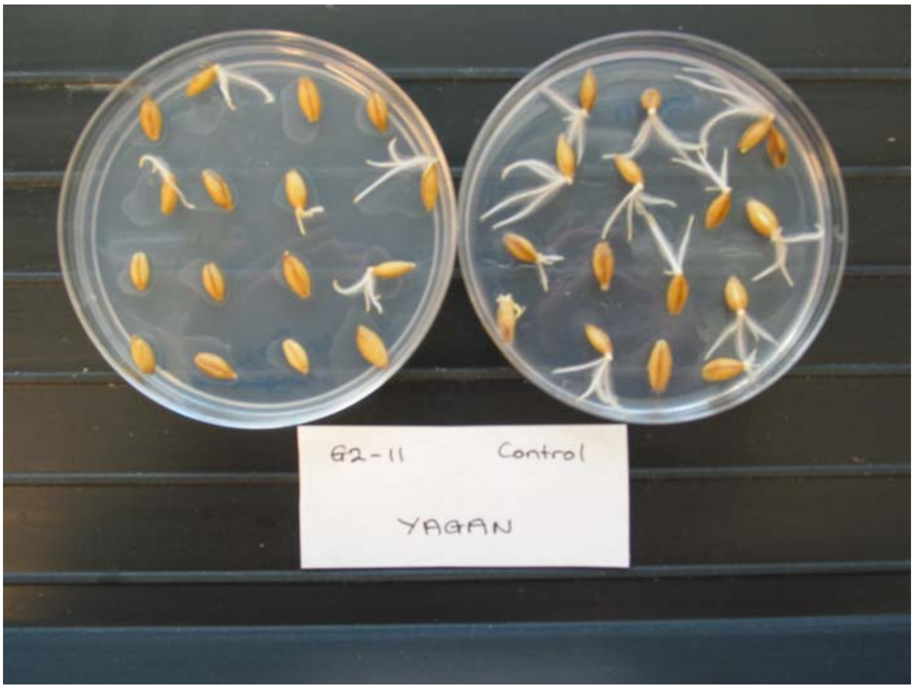


Figure 11: The germination and early growth of Yagan barley on agar after inoculation with DRB isolate D2-11. (Note that this isolate had little effect on barley germination or growth)

Figure 12: The growth pouches

Figure 13: The germination and early growth of Yagan barley after inoculation with DRB isolate G2-11.