Biological control of weeds in vineyards

FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **MU 00/1**

Principal Investigator: **Dr Graham O'Hara**

Research Organisation: **Murdoch University**

Date: **30th June 2005**

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Centre for Rhizobium Studies, Murdoch University

Date: 30th June 2005

1. Abstract

This project aimed to investigate naturally occurring soil bacteria for potential as biological control agents for weeds in vineyards. A total of 442 strains of bacteria were isolated from soil and roots of three major weeds: wild radish, annual ryegrass and capeweed. Seventy-four strains inhibited weeds in the laboratory and these were studied in the glasshouse for effects on weeds, grapevines and the legume cover crop subterranean clover. Nineteen strains reduced growth of annual ryegrass and/or wild radish. Strains had no deleterious effects on grapevines. Ten weed-inhibiting strains promoted growth of subterranean clover. Inhibitory isolates were identified using biochemical and molecular techniques as *Pseudomonas fluorescens* and *Alcaligenes xylosoxidans*, both relatively common soil and plant associated bacteria.

2. Executive Summary

Laboratory and glasshouse studies were conducted over three years to isolate bacteria from the root systems of weeds and characterise their ability to inhibit major weeds without having adverse effects on grapevines. This project was undertaken with a view to provision of natural strains of weed-inhibiting bacteria for potential development of commercial products for weed control in vineyards.

In this project 442 strains of bacteria were isolated from the rhizosphere (the region of soil under the influence of plant roots), the rhizoplane (the root surface) and endorhizosphere (the intercellular spaces within root tissues) of seedlings and mature plants of three important vineyard weeds, wild radish (*Raphanus raphanistrum*), annual ryegrass (*Lolium rigidum*) and capeweed (*Arctotheca calendula*), growing in three vineyards (Henley Park, Jane Brook and Lamont) in the Swan Valley in Western Australia. A majority (81.5%) of the strains was obtained from either the rhizosphere or rhizoplane of weed seedlings and mature plants, whereas only 18.5% of strains were isolated from the endorhizosphere of weeds. Rapid laboratory bioassays and glasshouse screening techniques were developed to evaluate the effects of the isolated bacteria on plants. Seventy-four strains significantly reduced the root length of wild radish and ryegrass seedlings in laboratory tests. These 74 strains were characteristically common aerobic soil bacteria with an oxidative metabolism; 71 of the strains were motile bacteria, and 64 were Gram-negative bacteria.

The 74 strains that inhibited weeds in the laboratory bioassay were screened in the glasshouse for effects on weed species, grapevine rootlings (*Vitis vinifera*) and the legume cover crop

subterranean clover (*Trifolium subterraneum*). Nineteen strains reduced the growth of wild radish and/or ryegrass in repeated glasshouse experiments. None of the tested strains had any deleterious effects on grapevine rootlings and sub clover. However, ten of the weed-inhibiting strains promoted the growth of subterranean clover and some improved nodulation. Three strains that inhibited wild radish and promoted growth of clover were identified using biochemical molecular techniques. Two strains were *Pseudomonas fluorescens* and one was *Alcaligenes xylosoxidans*, both common bacteria found in soil and associated with plants. One strain of *P. fluorescens* produced hydrogen cyanide, an inhibitor of plant roots.

The results of this project have been communicated to industry and the research community through presentations at workshops, technical meetings and conferences; articles published in industry magazines; and manuscripts in refereed scientific publications.

Some of the strains of bacteria isolated in this project have potential for use as biological agents for weed control. This project has shown that strains of bacteria isolated from soils and weeds in vineyards have a capacity to inhibit weeds. However, further work will be required to demonstrate that the weed-inhibitory effects shown in the glasshouse also occur in the field. Challenges that need to be overcome to develop these strains as biological control agents for weeds include determination of the best methods for field application and management in vineyards. An intriguing finding of this project was that some of the weed-inhibiting strains of bacteria promoted the growth of the pasture legume subterranean clover. This could be a significant finding with potential for development of plant growth promoting organisms for use in broad-acre agriculture, horticulture and viticulture.

3. Background

Weed control is an important part of vineyard management to help maintain vine vigour and productivity. Winter growing annual weeds such as wild radish (*Raphanus raphanistrum*), annual ryegrass (*Lolium rigidum*) and capeweed (*Arctotheca calendula*) emerge during autumn and early winter, and can subsequently cause significant problems in many vineyards in Australia. When these weeds grow under the grapevine canopy they are often controlled using herbicides because of the difficulties with their removal by either mowing or cultivation without damage to the grapevine plants. However, widespread use of herbicides is an important industry issue, and some weed populations have developed multiple herbicide resistances, causing major concerns throughout Australia (Crump *et al*. 1999; Elliott *et al*. 1996). The increased threat of herbicide resistance in weeds together with public concerns over soil and water contamination by herbicides have increased both industry and community interest in alternate methods such as biological control as components of integrated weed management systems.

One advantage of using microorganisms as bioherbicides for weed control is that these can be more selective than herbicides in the weed species they affect (Bolton and Elliot 1989; Adam and Zdor 2001). A second advantage is the potential for bioherbicides to control weeds by complex inhibition mechanisms thus decreasing chances of simple bioherbicide resistance developing in the target weeds. Furthermore, if the microorganism used as a bioherbicide has evolved in association with the target plant host then additional strains of the microorganism could be available to overcome any resistance as it develops in the host (Crump et al. 1999).

Using soil microorganisms to control weeds in vineyards is therefore a promising alternative to herbicides that may reduce grape and wine production costs, decrease dependence on chemical herbicides and increase the use of environmentally sound practices. One group of microorganisms largely overlooked for their potential as biological control agents of weeds are the deleterious rhizosphere-inhabiting bacteria, characterised as non-parasitic bacteria colonising plant root surfaces and being able to suppress plant growth (Kremer and Kennedy, 1996). Many of these plant-inhibiting bacteria are plant specific (Cherrington and Elliott, 1987; Elliott and Lynch, 1985; Suslow and Schroth, 1982).

Rhizosphere-inhabiting bacteria with potential as biological control agents have been reported on downy brome (*Bromus tectorum*) occurring in winter wheat fields (Cherrington and Elliott, 1987; Kennedy *et al*. 1991; Schippers *et al*. 1987) and on several broadleaf weed seedlings (Elliott and Lynch, 1985). Biological control of downy brome in field-grown winter wheat was reported by Kennedy *et al*. (1991). Two isolates of *Pseudomonas* spp. consistently reduced density, growth and seed production of downy brome but did not affect density of winter wheat. The grain yield of winter wheat was significantly increased and attributed to the growth suppressive effects of the applied bacteria on downy brome, which allowed the wheat to be more competitive (Kennedy *et al*. 1991). A study carried by Adam and Zdor (2001) demonstrated that strains of bacteria isolated from velvetleaf (*Abutilon theophrasti*) potentially suppress weed growth. Norman *et al*. (1994) also evaluated rhizosphere bacteria and their phytotoxins as weed control agents in cranberry vines. A major group of rhizosphere-inhabiting bacteria with potential for biological control are the pseudomonad-like bacteria that commonly inhabit soil and

plant environments. Some rhizosphere-inhabiting pseudomonads produce hydrogen cyanide (HCN), a compound known to inhibit plant metabolism and root growth (Adam and Zdor, 2001).

The principle for weed management using deleterious rhizosphere-inhabiting bacteria is not dependent on the development of an endemic disease on established mature weed plants. Rather the bacteria-based strategy is to inhibit the development of weeds before or coincident with emergence or establishment of vines and crop plants. Therefore application of these bacteria will not immediately eradicate the problem of weeds, but they could significantly suppress early growth of weeds, and therefore have potential to be used as beneficial components in integrated vineyard floor management practices to control weeds.

An attractive aspect of the approach taken in this project was the targeted isolation of indigenous, natural soil bacteria as potential biological control agents for weeds. These bacteria would provide a relatively very low risk option for use as inocula to apply to soil and/or plants in comparison with possible alternative biological control strategies based on using exotic (introduced) or genetically engineered microorganisms. The strategy used in this project was to isolate bacteria that already naturally associate with weeds, so that only natural organisms would be used for development as weed control agents. The objectives of the research described here were to isolate potential deleterious rhizosphere-inhabiting bacteria from major weed species frequently found growing under grapevine canopies in Western Australia, and then investigate the effects of these bacteria on the growth of weeds, grapevines and subterranean clover, a legume sometimes grown between grapevines as a cover crop.

4. Project Aims and Performance Targets

The aim of this project was to isolate strains of bacteria that inhibit important weed species in vineyards, but not grapevines and subterranean clover, for potential development of an effective bacteria-based weed management control method for vineyards.

The project had the following performance targets:

1. To isolate strains of bacteria, which may or may not have a potential inhibition of weed seedling growth, from important weed species that frequently grow under the vine canopies in Western Australia.
2. To screen isolated strains in the laboratory for inhibitory effects on major weed species
3. To characterise and identify potential deleterious rhizobacteria (DRB) that inhibits weed species that frequently grow under the vine canopies in Western Australia.
4. To investigate the phytopathogenecities of potential DRB on weed seedling growth and vine plant growth.
5. To provide strains of bacteria with potential to develop commercial products for weed control.

5. Methods

Collection of target weeds for isolation of bacteria

Seedlings and mature plants of the three target weed species, wild radish (*Raphanus raphanistrum* L.), annual ryegrass (*Lolium rigidum* G.) and capeweed (*Arctotheca calendula* L.), were collected during October 2000 from Henley Park Vineyard, Jane Brook Vineyard and Lamont Vineyard in the Swan Valley, Western Australia ($31^0$50'S and $116^0$00'E). At each vineyard three plants of each weed species were collected from between grapevine rows and under the canopy of grapevine plants within a row. Collected weeds were stored in sterile plastic bags at 4^0C until processing in the laboratory.

Isolation of bacteria from the rhizosphere, rhizoplane and endorhizosphere of weeds

Standard microbiological methods were used to isolate bacteria from the rhizosphere (the region of soil under the influence of roots), the rhizoplane (the root surface) and the endorhizosphere (the intercellular spaces within root tissues) of collected weeds (Bakker and Schippers 1987; Collins and Lyne, 1980; Curl and Truelove, 1986). Bacterial suspensions were separately diluted in 0.1% (w/v) peptone water (1:40) and then plated onto two selective media, pseudomonas isolation medium and Sands and Rovira medium (Sands and Rovira, 1970), and two non-selective media, tryptic soy agar (TSA) and nutrient agar (NA). After incubation at 28^0C for 48 hours isolated colonies were sub-cultured onto non-selective media (TSA and NA), using morphological colony characteristics to distinguish different strains. Isolated strains were purified in culture, and stored cryogenically at -80^0C (Adam and Zdor, 2001; Rovira and Davey, 1974; Kennedy *et al*.1991; Kloepper and Schroth, 1981).

Laboratory screening for inhibitory effects of bacteria on germination and growth of weeds

Seeds of the three target weed species (radish, ryegrass and capeweed) were surface sterilised by immersion in 3.25% (v/v) sodium hypochlorite (NaOCl) for 1 min, followed by 70% (v/v) ethanol for 1 min, rinsed five times in sterile distilled water and blotted on sterilised filter paper. The effectiveness of surface sterilization was assessed as described by Gealy *et al*. (1996). Cultures of each isolated strain of bacteria, grown for one day at 28^0C in glucose minimum salts medium (Brown and Dilworth, 1975), were centrifuged at 20,000rpm for 10 min and 2ml of supernatant was added to the surface of 0.9% (w/v) water agar plates. Fifteen surface-sterilised seeds of each weed species were then placed on each plate and incubated in the dark at 20^0C for five days. Controls were inoculated with 2 ml of sterile medium. Each isolate was tested in four replicates. After five days the seedlings were removed, germination recorded, and root lengths measured.

Glasshouse screening of bacteria for inhibitory effects on weeds

Surface sterilised seeds of target weeds were germinated for 2 days on 0.9% (w/v) water agar and four seedlings were planted into a 110mm pot containing a pasteurized mixture (1:1) of yellow sand and washed river sand. Bacterial cultures were grown as described for the laboratory screening. The four seedlings in each pot were inoculated with 2ml of bacterial suspension containing approximately 1 x 10^8 colony-forming units per ml (CFU/ml) of either an individual isolated strain or in some cases a combination of strains. Controls were inoculated with 2 ml of sterile medium. Following inoculation a thin layer of sterilised plastic beads was placed on the surface of each pot to reduce evaporation and help control airborne contamination of

microorganisms. Plants were grown in a temperature-controlled glasshouse at 25^0C, and watered every second day through a PVC tube with nutrient solution containing 0.3% (w/v) KNO_3. There were eight replicates of each treatment. After 6 weeks the plants were harvested, roots were washed free of sand, and shoot and root lengths measured. Shoots were separated from roots, oven dried at 60^0C for one week and dry weights of shoots and roots were recorded. Experiments testing the effect of individual strains or combinations of strains were repeated three times.

Glasshouse screening of weed-inhibiting bacteria on grape-vine plants

Grapevine (*Vitis vinifera*) cuttings were prepared for rooting as follows. Grape-vine cuttings approximately 15 cm long (3 buds) were sterilized in 3% (v/v) NaOCl for 10 minutes, washed five times with sterile distilled water and blotted on sterile filter paper. The cuttings were placed in plastic containers (40 x 65 x 25 cm) with a pasteurized mixture of yellow sand and washed river sand. The required high humidity (70-80%) for vine cuttings was obtained by covering the containers with plastic covers (as recommended by Vinitech nursery, Western Australia). After four weeks grapevine plants with leaves and roots were transplanted into 150 mm diameter pots containing the pasteurized sand mixture. The grapevine plants were inoculated with 10ml of suspension containing 10^8 colony-forming units per ml (CFU/ml) of the bacterial strain being tested. Controls were inoculated with 10 ml of sterile medium. Following inoculation a thin layer of sterilised plastic beads was placed on the surface of each pot. Plants were grown in the glasshouse at 25^0C for 8 weeks and were watered every second day through a PVC tube with nutrient solution containing 0.3% (w/v) KNO_3. After 8 weeks the plants were harvested, examined for disease symptoms, roots were washed free of sand, and shoot and root lengths measured. Leaves were separated from roots, leaf area was measured, leaves and roots were oven

dried at 60^0C for one week and dry mass was recorded. Each experimental design was completely randomised with four replicates and each experiment was repeated three times.

Glasshouse screening of weed-inhibiting bacteria on subterranean clover

Surface sterilised seeds of subterranean clover (*Trifolium subterraneum*) were germinated at room temperature for two days on 0.9% (w/v) water agar. Eight seedlings were planted into a 150mm diameter pot containing a pasteurized mixture of yellow sand and washed river sand. Seedlings of subterranean clover were inoculated with a suspension (2ml per plant) of commercial peat inoculum (Bio-Care Technology Pty, Ltd) containing a strain of root nodule bacteria (*Rhizobium leguminosarum* bv *trifolii* WSM 409) effective in nitrogen fixation on sub clover. One-day-old cultures of weed-inhibiting strains of bacteria grown at 28^0C on glucose minimal salt medium were used to inoculate the seedlings. Each seedling was inoculated with 1ml of suspension containing 10^8 colony-forming units per ml (CFU/ml). Control plants were inoculated with 1 ml of sterile medium Plants were grown in the glasshouse as described above. After 6 weeks the plants were harvested, examined for diseases symptoms and scored for nodulation, roots were washed and shoot and root lengths measured. Leaves were separated from roots, oven dried at 60^0C for one week and dry mass of leaves and roots were recorded. Each experimental design was completely randomised with four replicates and testing of each strain was repeated three times.

Microbiological characterisation of weed-inhibiting strains

Strains of isolated bacteria that inhibited the target weed plants under laboratory conditions were characterised using standard microbiological methods for Gram-reaction, motility, catalase and oxidase production (Prescott *et al*. 1993).

Screening for production of secondary metabolites

Strains were assessed for their ability to synthesize hydrogen cyanide (HCN) on TSA plates supplemented with glycine (Bakker and Schippers 1987).

Phenotypic characterisation and presumptive identification of strains

Strains were metabolically characterised using the Biolog system, which is based on the differential utilisation of a large number of organic compounds (Bochner, 1989). Strains were presumptively identified on the basis of their patterns of utilisation of 95 substrates using the Biolog Microlog software (Biolog, Hayward, California).

Molecular identification of selected isolates

Sequencing of the 16S rRNA gene is used as a means of identification of bacteria to genus and species level. The 16S rRNA gene analysis for strains 3aRsWR, 1'"RpRG and 1'RpRG was carried out using the method described by Yanagi and Yamasato (1993). The 16S rRNA gene of each strain was amplified using PCR primers SeraF (5' GATTGAACGCTGGCGGCAGG 3'), and SeraR (5' CTTCACCCCAGTCATGAATC 3'). The PCR reactions were done in a final volume of 25µL comprising 4mM $MgCl_2$, 200 mM dNTPs, 10 pmol of primers, 1X PCR buffer, and 0.5-1.0 unit *Taq* DNA polymerase. PCR cycling conditions were as an initial denaturing at

94^0C for 3 min followed by 35 cycles of denaturing at 95^0C for 30sec, annealing at 55^0C for 30sec and extension at 72^0C for 30sec. Both strands of the product were sequenced using an Applied Biosystems 377 DNA sequencer. The 16S rRNA sequences were analysed using the gapped BLASTn search algorithm (http://www.ncbi.nlm.nih.gov/) and aligned to *Pseudomonas fluorescens* (strain CHAO AJ 278812.1, strain ATCC AF 094726.1, and strain MM-B16 AY 196702.1).

Data analysis

Data from laboratory and glasshouse experiments were analysed separately for each experiment using software of the Microsoft Data Analysis System. Significance of the data was analysed by one-way analysis of variance (ANOVA) to compare the biocontrol efficacy of rhizobacterial strains on root and shoot growth of weed plants, grapevine plants and subterranean clover.

6. Results and Discussion

Isolation of bacteria from target weed species

A total of 442 bacterial strains were isolated into pure culture from the rhizosphere, rhizoplane and endorhizosphere of seedlings and mature plants of wild radish, annual ryegrass and capeweed sampled from three vineyards in the Swan Valley, Western Australia during October/ November 2000. A majority (81.5%) of these strains were obtained from either rhizosphere or rhizoplane of the weed plants (Table 1). While only 18.5% of strains originated from the endorhizosphere of these weed species. The highest number of strains was recorded from weeds collected from the Henley Park vineyard compared to the two other vineyards sampled (Table 1).

Table 1. Number of strains of bacteria isolated from the rhizosphere and roots of three weed species collected in three vineyards in the Swan Valley, Western Australia.

Vineyard	Source of isolated bacteria									Total number of strains
	Wild radish (*R. raphanistrum*)			Annual ryegrass (*L. rigidum*)			Cape weed (*A. calendula*)			
	Number of strains			Number of strains			Number of strains			
	Rs[1]	Rp	Endo	Rs	Rp	Endo	Rs	Rp	Endo	
Henley Park, WA	23	18	12	19	19	10	22	20	8	151
Jane Brook, WA	21	23	6	23	14	11	23	18	6	145
Lamont, WA	18	20	10	24	17	9	18	20	10	146
Total number of strains	62	61	28	66	50	30	63	58	24	442

[1]Note:Rs = rhizosphere, Rp- = rhizoplane, Endo = endorhizosphere

All the bacterial strains isolated into pure culture in this project were aerobic or facultative anaerobic organisms, and no attempt was made to specifically isolate anaerobic bacteria. This was a deliberate decision to focus on aerobic bacteria because of the particular challenges of dealing with anaerobic organisms especially in relation to their growth in culture as inocula for glasshouse or field studies, and potential use in commercial products. The isolated strains were relatively fast growing bacteria, with most of them producing single colonies after overnight incubation at 28^0C on the media used in this study. Selected strains that repeatedly demonstrated inhibitory effects on weeds in the glasshouse experiments are stored as lyophilised cultures and under glycerol at -80^0C in the WSM collection of bacteria at the Centre for *Rhizobium* Studies, Murdoch University.

The strains isolated during this project and used for the laboratory bioassays and glasshouse-screening experiments were designated using a code based on three components:

a) the root zone of isolation (Rs = rhizosphere, Rp = rhizoplane, Endo = endorhizosphere)
b) the weed species of isolation (CP = capeweed, RG = annual ryegrass, WR = wild radish)
c) a prefix of two or three characters to indicate the plate number and the colony number.

Inhibitory effects of isolated bacteria on growth of weed seedlings under laboratory conditions

A total of 125 strains were screened on agar plates under laboratory conditions to investigate potential deleterious effects on weed seedlings. This screening was predominantly undertaken using wild radish and annual ryegrass because of availability of seed. Approximately 59% (74 strains) of the isolated bacteria significantly reduced the root length of wild radish and/or annual

ryegrass seedlings compared to the control plants grown on agar and inoculated with sterile medium. Experimental data are shown in Appendix 5 (Table 3).

Characterisation of weed-inhibiting strains

The cultural and morphological characteristics of the 74 strains that repeatedly showed deleterious effects on wild radish and annual ryegrass in laboratory screenings were observed using pure cultures grown on non-selective nutrient agar, fresh slide preparations and stained slide preparations of heat-fixed cells. Gram-positive bacteria comprised only 13.5% of strains (10 out of 74 strains). All 74 strains were catalase and oxidase positive, indicative of aerobic organisms with an oxidative metabolism, rather than the fermentative metabolism of enteric bacteria such as *Escherichia*, *Salmonella* etc. Seventy of the strains were observed to be consistently motile when grown in broth cultures, and only four strains (3RpRG, 2aRpWR, 2aRpRG and 2RpWR) were not observed to be motile in freshly grown broth cultures. Motility is a common feature of many soil and rhizosphere inhabiting bacteria. Data on characterisation of strains is shown in Appendix 5 (Table 4).

Inhibitory effects of isolated bacteria on weeds grown in the glasshouse

Further screening of the 74 strains, that were positive in the laboratory bioassay, on weeds grown in the glasshouse showed 19 strains had deleterious effects on the growth of either wild radish or annual ryegrass, or both (Table 2). Results revealed that 47.3% of the strains (9 strains) were specific for growth inhibition of wild radish, 31.6% of the strains (6 strains) were specific for the growth inhibition of ryegrass, and 21.1% (4 strains) had deleterious effects on both wild radish and ryegrass. Analysis of the data indicated that 12 of these 19 strains had detrimental effects on

Table 2. Deleterious effects of rhizosphere-inhabiting strains of bacteria on wild radish and annual ryegrass grown in the glasshouse

Rhizobacterial Strains	Weed species							
	Wild radish (*Raphanus raphanistrum*)				Annual ryegrass (*Lolium rigidum*)			
	Length[a], cm		Dry mass[a], g		Length[a], cm		Dry mass[a], g	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Control	11.50± 0.81	26.80±2.40	0.090±0.019	0.090±0.035	28.90±1.15	28.50±0.60	0.070±0.001	0.120±0.001
3aRsWR	**6.01± 0.91** [b]	**16.20±3.80** [b]	**0.030±0.030** [b]	**0.021±0.036** [b]	25.8±1.55	27.30±0.07	0.071±0.001	0.109±0.006
1"'RpRG	**5.91± 0.64** [b]	**15.20± 5.80** [b]	**0.040±0.025** [b]	**0.019±0.033** [b]	26.98±0.96	28.37±1.99	0.068±0.012	0.0118±0.054
1'RpRG	**6.50± 0.97** [b]	**20.84± 3.80** [b]	**0.042±0.024** [b]	**0.024±0.005** [b]	**23.84±2.53** [b]	**24.53±0.28** [b]	**0.046±0.001** [b]	**0.083±0.019** [b]
3a'RpWR	**9.28±1.11** [b]	**20.88±2.96** [b]	**0.060±0.015** [b]	**0.080±0.026** [b]	28.90±0.51	27.95±0.82	0.069±0.001	0.119±0.001
2aEndoWR	**7.66±1.92** [b]	**17.88±4.46** [b]	**0.051±0.020** [b]	**0.039±0.026** [b]	27.89±0.20	26.87±1.10	0.071±0.001	0.120±0.001
3RpWR	**7.84±1.83** [b]	**19.47±3.67** [b]	**0.054±0.018** [b]	**0.034±0.028** [b]	28.50±0.20	30.70±0.05	0.720±0.001	0.130±0.005
3b'RsWR	**6.50±2.50** [b]	**17.75±4.50** [b]	**0.054±0.018** [b]	**0.034±0.035** [b]	27.96±0.47	28.60±3.03	0.691±0.013	0.125±0.003
3aRpWR	**8.34±1.58** [b]	**17.75±4.52** [b]	**0.061±0.029** [b]	**0.034±0.036** [b]	**23.13±2.89** [b]	**22.44±0.40** [b]	**0.045±0.001** [b]	**0.082±0.019** [b]
1dRpWR	**6.93±2.29** [b]	**19.89±3.46** [b]	**0.034±0.015** [b]	**0.021±0.034** [b]	28.91±0.01	29.30±0.33	0.070±0.001	0.121±0.001
1cRpWR	**6.08±2.71** [b]	**17.70±4.55** [b]	**0.033±0.028** [b]	**0.019±0.034** [b]	28.69±0.11	27.85±0.15	0.071±0.001	0.023±0.049
1cRsWR	**6.18±2.66** [b]	**17.01±4.44** [b]	**0.034±0.029** [b]	**0.022±0.056** [b]	29.30±0.20	28.20±3.22	0.072±0.019	0.022±0.049
1bRsWR	**6.03±2.74** [b]	**17.93±1.41** [b]	**0.032±0.001** [b]	**0.023±0.002** [b]	**20.19±4.36** [b]	**22.06±2.58** [b]	**0.032±0.014** [b]	**0.089±0.016** [b]
2bRpWR	10.80±0.35	24.91±0.05	0.089±0.001	0.079±0.001	**23.50±2.70** [b]	**23.34±2.66** [b]	**0.043±0.013** [b]	**0.089±0.016** [b]
1aRpRG	11.20±0.15	23.98±1.41	0.091±0.001	0.086±0.001	**23.28±2.81**	**23.19±4.60**	**0.044±0.024**	**0.078±0.021**
2bRpRG	9.89±0.81	26.90±0.05	0.088±0.001	0.092±0.001	**22.75±3.08** [b]	**19.30±5.21** [b]	**0.022±0.010** [b]	**0.080±0.020** [b]
1EndoRG	10.93±0.29	25.30±0.01	0.91±0.002	0.088±0.001	**18.50±5.20** [b]	**18.09±4.80** [b]	**0.050±0.010** [b]	**0.080±0.020** [b]
1RsWR	10.89±0.31	23.98±1.41	0.086±0.026	0.085±0.003	**19.56±4.67** [b]	**18.09±3.17** [b]	**0.050±0.017** [b]	**0.080±0.020** [b]
1bRpRG	**5.56±2.97** [b]	**21.00±2.90** [b]	**0.038±0.001** [b]	**0.030±0.003** [b]	**20.56±4.17** [b]	**22.16±3.72** [b]	**0.037±0.001** [b]	**0.073±0.024** [b]
1aEndoRG	10.79±0.36	25.35±0.73	0.092±0.002	0.089±0.001	**22.13±3.39** [b]	**21.06±3.68** [b]	**0.051±0.008** [b]	**0.108±0.006** [b]

[a] The values are means based on eight replicates

[b] **Significant difference from the control ($P < 0.05$)**

their respective host species of isolation. Eight of these strains were isolated from wild radish and the other four strains were isolated from ryegrass. The diversity of possible interactions between different bacterial strains and weed plants is highlighted by these results where four of the strains tested showed broader spectrum weed inhibition and the other 15 strains had greater specificity of weed inhibition. Growth of weeds was also reduced by inoculation with mixtures of the inhibitory strains (Figure 1).

Figure 1. Inhibition of annual ryegrass (A & C) and wild radish (B & D) by bacteria. Control plants (A ryegrass, B wild radish) on left. Plants on right (C ryegrass, D wild radish) were grown in soil inoculated with three strains of bacteria (3aRsWR, 1'"RpRG and 1'RpRG) previously isolated from the roots of weeds collected from vineyards in Western Australia.

Not all the strains that were observed to reduce growth of weed roots in the laboratory bioassays showed deleterious effects on weeds in the glasshouse experiments. Indeed nearly 75% (55 strains) of these strains did not seem to affect the development of shoots and roots weed species when grown in glasshouse conditions.

Deleterious effects of selected strains on wild radish

Three of the strains shown in the glasshouse experiments as being weed-inhibiting strains (3aRsWR, 1'"RpRG and 1'RpRG) were further screened in several experiments for deleterious effects on wild radish plants. All three significantly reduced dry mass of shoots and roots of wild radish ($P < 0.05$) (Figure 2). In some cases there was a reduction in the length of shoots and roots. A range of foliar symptoms was observed in wild radish grown in the glasshouse experiments when inoculated with these three rhizobacterial strains. The symptoms varied from general growth retardation to various types of leave chlorosis. Lateral root development was poor in inoculated wild radish.

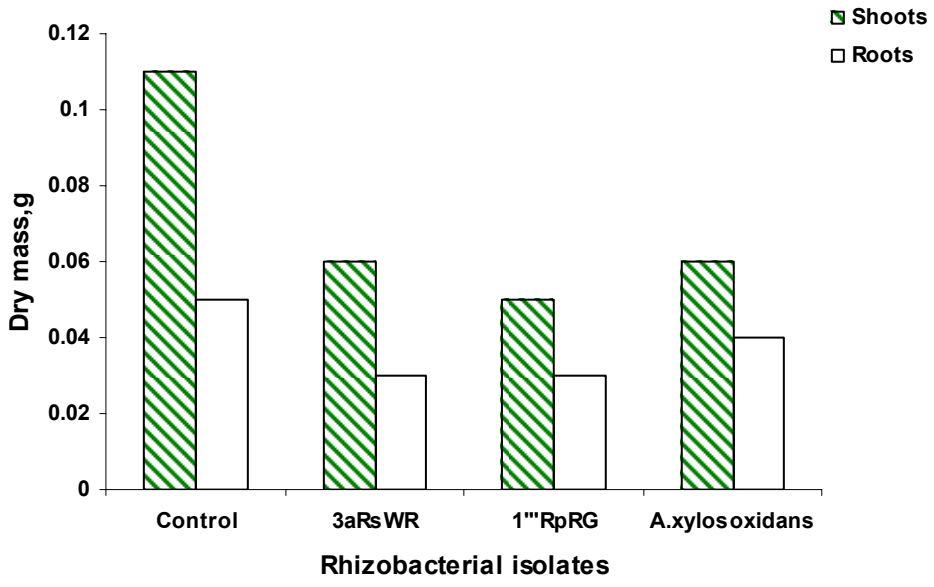

Figure 2. Effect of inoculation with three strains of bacteria on shoots and roots of wild radish grown in the glasshouse. Strains of bacteria were originally isolated from weeds growing in vineyards.

Screening for secondary metabolites

The three strains (3aRsWR, 1'"RpRG and 1'RpRG) were tested for ability to synthesize HCN, an inhibitor of plant roots, and a broad-spectrum antimicrobial compound. The results indicated that strain 3aRsWR produced HCN (Figure 3).

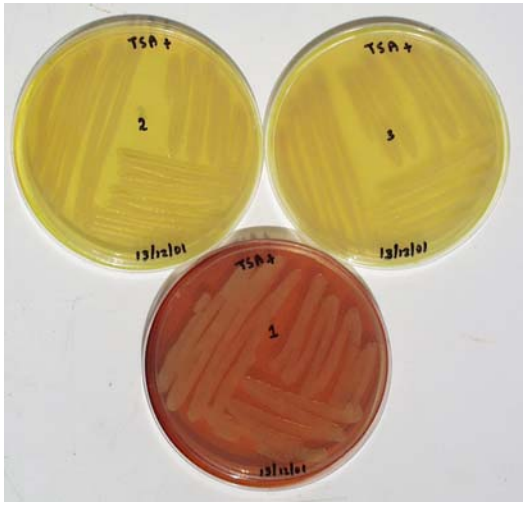

Figure 3. Production of hydrogen cyanide (HCN) (orange-red colour) by weed inhibiting strain 3aRsWR (lower plate). Strains were grown on TSA plates supplemented with glycine. Top plates contain strains not producing HCN.

Identification of selected inhibitory bacteria

The three strains studied in greatest detail in this project were identified using both biochemical and molecular techniques. Using the Biolog system two strains (3aRsWR, 1"'RpRG), were identified as *Pseudomonas fluorescens* and the third strain (1'RpRG) was identified as *Alcaligenes xylosoxidans.* Rhizobacterial strains 3aRsWR, 1"'RpRG were further confirmed by 16SrRNA gene sequence analysis. These two strains exhibited 99% sequence similarity to the four strains (strain CHAO AJ 278812.1, strain ATCC AF 094726.1, and strain MM-B16 AY 196702.1) of *Pseudomonas fluorescens.*

Screening of rhizobacterial strains on grapevine plants

Results from the glasshouse pot trial experiments on grapevine rootlings showed that none of the weed-inhibiting bacterial strains had any detrimental effects on grapevine plants. Representative data are shown from experiments testing the three identified strains that showed strong inhibition of wild radish (Figure 4). The two strains of *P. fluorescens* and the strain of *A. xylosoxidans* had no deleterious effects on the growth of the grapevine plants. Although the strain of *A. xylosoxidans* slightly increased the dry mass of leaves and roots of the grapevine plants the effect was not significant (Figure 4). In general all the inoculated grapevines appeared healthy and there were no signs of any disease symptoms or growth problems.

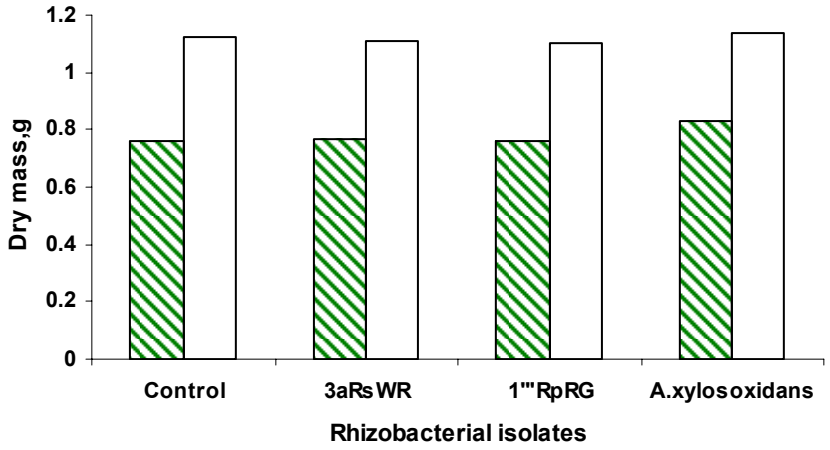

Figure 4. Effect of inoculation with three weed-inhibiting strains of bacteria on dry mass of shoots (stripped columns) and roots (clear columns) of grapevine plants.

Screening of rhizobacterial strains on subterranean clover

By contrast with their effects on weeds and grapevines, some of the weed-inhibitory strains promoted the growth of subterranean clover in glasshouse experiments (Figure 5). The strains identified as *P. fluorescens* (3aRsWR) and *A. xylosoxidans* (1'RpRG) significantly increased

Figure 5 here

accumulation of dry mass of roots of subterranean clover plants (Figure 6). Analysis of the nodulation of subterranean clover revealed that both *P. fluorescens* (3aRsWR) and *A. xylosoxidans* (1'RpRG) increased nodule development on inoculated plants compared to the control plants. Increased root growth of the inoculated subterranean clover may have resulted in increased nodulation because of a greater number of root hairs for infection by the commercial strain of root nodule bacteria (*R. leguminosarum* bv *trifolii* WSM 409) on the roots of plants also inoculated with either *P. fluorescens* (3aRsWR) or *A. xylosoxidans* (1'RpRG). Alternatively the strains of rhizobacteria (*P. fluorescens* (3aRsWR) and *A. xylosoxidans*) may be producing plant growth promoting metabolites. Previous studies on plant growth promoting bacteria have reported that rhizobacteria are potential growth enhancers in different crops like potato, pearl millet, and sorghum (Lazarovitz and Novak, 1997; Umesha et al. 1998; Raju et al. 1999). The other strain of *P. fluorescens* (1"'RpRG) had no significant effects on subterranean clover.

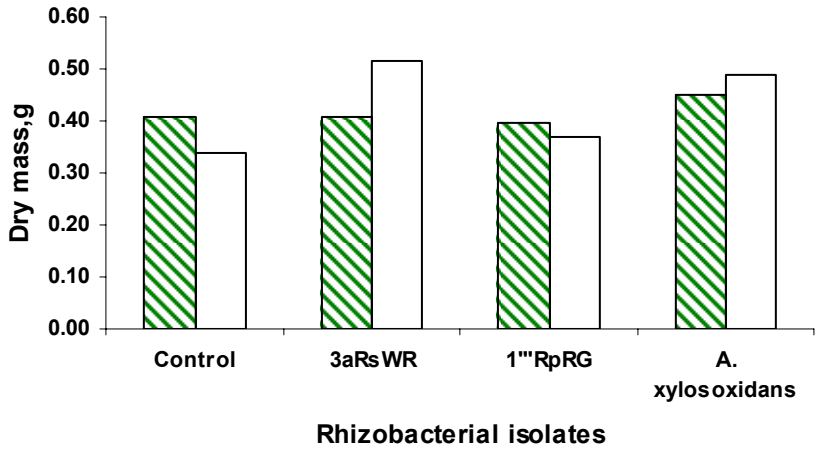

Figure 6. Effect of inoculation with three weed-inhibiting strains of bacteria on shoot (stripped columns) and root (clear columns) dry mass in subterranean clover.

The ability of deleterious rhizosphere inhabiting bacteria to inhibit the growth of various weed plants in different cropping systems has been documented (Bakker and Schippers 1987; Alstrom, 1987; Begonia and Kremer, 1994; Gealy *et al*. 1996; Kremer and Kennedy, 1996; Kremer and Souissi, 2001). The results of this project indicate that large numbers of potential weed-inhibiting strains of bacteria can be isolated from the rhizosphere, rhizoplane and endorhizosphere of seedlings and mature plants of vineyard weed species using standard microbiological methods. The target microorganisms in this study were aerobic bacteria because of their potential easier use for mass culture in commercial volumes without the challenges associated with anaerobic bacteria. Seventy-four of the 125 strains of bacteria screened in the laboratory bioassay for deleterious effects of secondary metabolites on weeds, significantly decreased the root growth of wild radish and ryegrass. These results reported here are consistent with earlier studies and highlight the great potential of many bacteria that inhabit the rhizosphere to interfere with the growth of weed seedlings (Adam and Zdor, 2001; Bakker and Schippers 1987; Begonia and Kremer, 1994; Gealy *et al*. 1996).

The majority of the 74 strains that showed some capacity for inhibition of weeds in the laboratory shared a range of common characteristics, including Gram-negative reaction, testing positive for catalase and oxidase production, and being motile. These results complement and extend previous studies with rhizobacterial isolates (Kremer *et al*.1990). In addition, these results provide evidence to support the proposition that many naturally occurring indigenous bacteria that inhabit soils and plant rhizospheres have the capacity to inhibit weeds.

These results indicated that *P fluorescens* (3aRsWR) has the ability to produce HCN as a secondary metabolite. Bakker and Schippers (1987) reported that cyanide producing rhizobacteria are involved in the reduction of plant development. Most rhizobacteria reduce plant growth without obvious plant cell damage, an effect attributed to rhizobacterially produced metabolites being absorbed by roots (Begonia and Kremer, 1994; Cherrington and Elliott, 1987, Kremer and Kennedy, 1996).

The physiological mechanisms involved in both the deleterious and the growth promoting effects of rhizobacteria on different plant species are not yet clearly understood. However, the research reported here confirms that rhizobacterial isolates can possess a range of diverse properties. Several of the weed-inhibiting strains isolated in this study, including *P. fluorescens* (3aRsWR, 1"'RpRG) and *A. xylosoxidans* (1'RpRG), appear to be both deleterious rhizosphere-inhabiting bacteria for wild radish and plant growth promoting rhizobacteria for subterranean clovers. Furthermore, none of the weed-inhibiting isolates significantly affected the growth of grapevine plants. These results are novel and this is the first report of strains of rhizosphere inhabiting bacteria having such complex interactions with these very different host species. Further studies will be required to determine the specific mechanisms involved in these interactions. The isolation of bacteria such as *P. fluorescens* (3aRsWR) and *A. xylosoxidans* (1'RpRG) provides opportunities for these studies as well as for the development of inocula with capacity for targeted plant growth promotion and weed-inhibiting activity in agricultural, horticultural and viticultural systems.

Weed management with deleterious rhizosphere bacteria does not depend on development of a parasitic infection or an endemic disease on established weeds. Rather the rhizobacteria control strategy is to regulate the development of weeds before or coincident with emergence of crop plants. Therefore this approach will not necessarily eradicate the problem weeds immediately, but is aimed to result in significant suppression of early growth of weeds to allow the development of crop plants to effectively compete with the weakened weed seedlings (Kremer and Kennedy, 1996). The investigations in this project emphasize the potential for manipulating the weed seedling rhizosphere using identified specific rhizosphere-inhabiting bacteria with detrimental activity. However, further studies are required for development of inoculum technologies, and to understanding inoculum response in different cropping and soil systems. In addition multi-site investigations are necessary to determine field responses of inocula in different environments for further development of these weed-inhibiting bacteria as biological control agents in vineyards.

7. Outcomes /Conclusions

This project has achieved its aims in the isolation of strains of bacteria that inhibit important weed species that cause problems in vineyards, but do not adversely affect grapevines and subterranean clover. These strains have potential for further development of effective bacteria-based systems for integration into weed management control method for vineyards.

The project had the following outcomes against its initial performance targets:

1. 442 strains of bacteria were isolated from three important weed species (annual ryegrass, capeweed, wild radish) that frequently grow under the vine canopies in Western Australia.
2. 74 strains showed inhibition of weed seedlings in laboratory bioassays.
3. The weed-inhibiting strains were characteristically Gram-negative, aerobic, motile, oxidative bacteria. Three of the strains showing significant inhibition of weeds were identified as two strains of as *Pseudomonas fluorescens* and a strain of *Alcaligenes xylosoxidans*, both relatively common soil and plant associated bacteria.
4. 19 strains inhibited growth of weeds in the glasshouse. None of the strains tested affected growth of grapevines.
5. The isolated strains of bacteria have potential to provide a basis to develop potential commercial products for weed control. Further work testing the field responses of crop and pasture species to inoculation with these strains is being pursued by the Centre for Rhizobium Studies at Murdoch University.

8. Recommendations

Although 19 of the strains of bacteria isolated in this project have a capacity to inhibit weeds further research needs to be undertaken to assess their potential for weed control in vineyards. In particular further research will be required to assess their efficacy in field situations, and to develop practical methods for their application and management in vineyards. The following are recommended as areas for further research with consideration to the potential development of the strains isolated in this project as biological control agents for weeds.

- field studies to obtain evidence of the effects of strains isolated in this project on weeds growing in vineyards

- field assessment of the effects of the weed-inhibiting strains on other plant species important in viticulture, and on important crop plants, such as cereals, canola and grain legumes

- identification of all strains used in future studies to ensure organisms are non-pathogenic bacteria

- trials to determine efficient methods of inoculation of weeds and soils

- field studies of the plant growth promoting strains to test their potential for increasing growth of productive plant species

9. Appendix 1: Communication

The results of this project have been presented to industry and scientific communities through discussions and seminars with industry personnel, presentations to industry meetings, workshops and scientific conferences, articles in industry journals and refereed publications in scientific journals. Copies of communications reporting results of this project are attached in Appendix 6.

1. Presentations at meetings, workshops and seminars

- Biological control of weeds in vineyards. Quality Factor Seminars, Margaret River, WA. May 2001Wine Industry Field Day, Margaret River WA
- CRS Seminar series, Murdoch University, Perth, WA.
- Inter-Agency Weeds Meetings with researchers from Western Australian Herbicide Resistance Initiative, Weeds CRC, CSIRO, University of Western Australia, Murdoch University, Curtin University, Department of Agriculture WA.

2. Poster presentations

- October 2001 Australian Wine Industry Technical Conference, Adelaide SA
- September 2002 13th Australian Weeds Conference, Perth, WA
- November 2002 Australian Wine Industry Technical Conference, Adelaide SA

3. Publications

- Flores Vargas, R.D., and O'Hara, G.W. (2001). Biological Control of Weeds in Vineyards. 11th Australian Wine Industry Technical Conference, Adelaide SA, pp 85-87

- Flores Vargas, R.D., and O'Hara, G.W. (2001) Biological control of Weeds in Vineyards International Bioherbicide Group News, December 2001 vol 10 no 2
- Flores Vargas R.D. and O'Hara G.W. (2002). Towards Ecologically Based Weed Management Systems in Vineyards, In 13th Australian Weeds Conference papers and proceedings, Perth WA, Edited by Spafford- Jacob H., Dodd J. and Moore J.H. pp. 228-231.
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- Flores Vargas, R, D. and O'Hara, G.W. Isolation and characterization of rhizobacteria with potential for biological control of weeds in vineyards. Manuscript in revision for Journal of Applied Bacteriology.

10. Appendix 2: Intellectual Property

The IP arising from the research is mainly in the form of the isolated strains and knowledge about their characteristics. The strains are being maintained as part of the WSM strain collection at the Centre for Rhizobium Studies, Murdoch University, and are being used for further research by staff and students of the CRS. The IP arising as technical knowledge from the research has been disclosed in the form of communications and publications

11. Appendix 3: References

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12. Appendix 4: Staff

- Dr Graham O'Hara, Centre for Rhizobium Studies, Murdoch University
- Dr Ruben Flores Vargas, Centre for Rhizobium Studies, Murdoch University
- Ms Katrina Wall, Centre for Rhizobium Studies, Murdoch University

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- Mr Ron Yates, Centre for Rhizobium Studies, Murdoch University.
- Dr Gary Bullard, Bio-Care Technology Pty Ltd

13. Appendix 5: Data

Table 3. Effects of strains of bacteria isolated from vineyard weeds on root length of wild radish and annual ryegrass seedlings under laboratory conditions.

Rhizobacterial isolate	Wild radish (*R. raphanistrum*)	Annual ryegrass (*L. rigidum)*
	Root length, cm (X ± SE)	
Control	3.98 ± 0.12	4.12 ± 0.20
3aRsWR	1.05 ± 0.13	1.15 ± 0.13
1’”RpRG	1.12 ± 0.1	1.25 ± 0.14
1’RpRG	1.12 ± 0.14	1.19 ± 0.12
1eRpWR	2.81 ± 0.15	1.69 ± 032
1EndoRG	3.18 ± 0.15	1.91 ± 032
1bRsWR	3.22 ± 0.15	0.05 ± 032
1cRsWR	2.69 ± 0.15	2.06 ± 032
1cRpWR	2.42 ± 0.15	1.82 ± 032
1dRpRG	3.32 ± 0.15	1.91 ± 032
1bRsRG	2.61 ± 0.15	2.81 ± 032
2dRsWR	2.11 ± 0.15	1.42 ± 032
1aRpRG	1.98 ± 0.14	1.38 ± 0.25
1aEndoRG	1.42 ± 0.14	1.51 ± 0.25
1”RpRG	1.64 ± 0.14	0.74 ± 0.25
2bRpRG	1.92 ± 0.14	2.15 ± 0.25
1RsWR	1.40 ± 0.14	0.79 ± 0.25
1bRsWR	0.75 ± 0.14	0.38 ± 0.25
1bRpRG	1.61 ± 0.14	1.36 ± 0.25
1’RpRG	1.25 ± 0.14	2.02 ± 0.25
3a’RpWR	1.38 ± 0.17	1.79 ± 0.20
2aEndoWR	1.27 ± 0.17	1.59 ± 0.20
2bRpWR	0.87 ± 0.17	0.18 ± 0.20
3aRpWR	0.96 ± 0.17	1.45 ± 0.20
3a”RpWR	1.36 ± 0.17	1.90 ± 0.20
2RsWR	1.46 ± 0.17	2.28 ± 0.20
3b’RsWR	1.29 ± 0.17	1.96 ± 0.20
3RpWR	0.83 ± 0.17	1.93 ± 0.20
1eRpWR	1.43 ± 0.54	1.31 ± 0.23
1dRpRG	2.46 ± 0.54	1.75 ± 0.23
3eRsRG	1.71 ± 0.54	0.92 ± 0.23
3RsWR	2.79 ± 0.54	1.64 ± 0.23
2dRsCW	1.16 ± 0.54	2.19 ± 0.23

3dRsCW	2.05 ± 0.54	2.78 ± 0.23
1cRpWR	2.17 ± 0.24	2.73 ± 0.16
2dRpWR	0.84 ± 0.24	2.63 ± 0.16
3dRsCW	2.51 ± 0.24	3.04 ± 0.16
3eRsRG	1.76 ± 0.24	2.11 ± 0.16
1eRsRG	2.53 ± 0.24	2.43 ± 0.16
1dEndoWR	1.84 ±0.24	3.34 ± 0.16
1eRGRp	3.01 ± 0.33	2.69 ± 0.27
3dRpCW	2.52 ± 0.33	2.63 ± 0.27
3eRsWR	1.89 ± 0.33	0.99 ± 0.27
2DRsCW	2.08 ± 0.33	2.80 ± 0.27
1ERpCW	0.61 ± 0.33	1.88 ± 0.27
2ERpRG	1.00 ± 0.33	2.36 ± 0.27
2EndoWR	0.67 ± 0.51	1.39 ± 0.24
3RpWR	2.60 ± 0.51	1.49 ± 0.24
1e Endo RG	2.11 ± 0.51	2.12 ± 0.24
1EndoRG	2.80 ± 0.75	2.49 ± 0.33
1e'EndoRG	1.37 ± 0.75	1.41 ± 0.33
2RpRG	1.84 ± 0.75	2.15 ± 0.33
3RpRG	1.94 ± 0.75	2.40 ± 0.33
2aRpWR	3.27 ± 0.32	3.24 ± 0.22
3cEndoRG	2.97 ± 0.32	2.80 ± 0.22
3cRsWR	2.10 ± 0.32	1.91 ± 0.22
2RsWRht	3.62 ± 0.32	2.57 ± 0.22
2aRpWRht	1.37 ± 0.23	2.26 ± 0.23
2RsWRht	1.25 ± 0.23	2.61 ± 0.23
2bRsWRht	1.39 ± 0.23	1.82 ± 0.23
2RsWRht	1.48 ± 0.23	1.48 ± 0.23
RsWR	1.07 ± 0.25	2.07 ± 0.48
3aRsWR	2.05 ± 0.25	3.79 ± 0.48
RpWR	1.26 ± 0.25	1.12 ± 0.48
2aRpWR	1.37 ± 0.25	2.46 ± 0.48
RpRG	2.05 ± 0.25	2.51 ± 0.48
2RsWR	1.96 ± 0.26	3.03 ± 0.21
3RpWR	1.96 ± 0.26	2.10 ± 0.21
3RsRG	2.59 ± 0.26	3.08 ± 0.21
3RpWRflow	2.74 ± 0.26	2.61 ± 0.21
3aEndoWRht	1.50 ± 0.26	2.64 ± 0.21
2aRsWRht	1.70 ± 0.24	1.80 ± 0.23
2aRpRGht	2.10 ± 0.24	2.10 ± 0.23
2RpWRht	3.01 ± 0.24	2.60 ± 0.23
3aRpRGht	2.10 ± 0.24	2.10 ± 0.23

2RsRGht	2.20 ± 0.24	1.80 ± 0.23

TABLE 4. Characteristics of strains of bacteria isolated from rhizospheres, rhizoplanes and endorhizospheres of three weed species (annual ryegrass, capeweed and wild radish) growing in vineyards in Western Australia.

Strain	Gram reaction	Catalase production	Oxidase production	Motility
3aRsWR	-	+	+	+
1’”RpRG	-	+	+	+
1’RpRG	-	+	+	+
1eRpWR	-	+	+	+
1EndoRG	-	+	+	+
1bRsWR	-	+	+	+
1cRsWR	-	+	+	+
1cRpWR	-	+	+	+
1dRpRG	-	+	+	+
1bRsRG	-	+	+	+
2dRsWR	-	+	+	+
1aRpRG	-	+	+	+
1aEndoRG	-	+	+	+
1”RpRG	-	+	+	+
2bRpRG	-	+	+	+
1RsWR	-	+	+	+
1bRsWR	-	+	+	+
1bRpRG	-	+	+	+
1’RpRG	-	+	+	+
3a’RpWR	-	+	+	+
2aEndoWR	-	+	+	+
2bRpWR	-	+	+	+
3aRpWR	-	+	+	+
3a”RpWR	-	+	+	+
2RsWR	-	+	+	+
3b’RsWR	-	+	+	-
3RpWR	-	+	+	+
1ErpWR	-	+	+	+
1dRpRG	-	+	+	+
3eRsRG	-	+	+	+
3RsWR	-	+	+	+
2dRsCW	-	+	+	+
3dRsCW	-	+	+	+
1cRpWR	-	+	+	+
2dRpWR	-	+	+	+
3dRsCW	-	+	+	+

3eRsRG	-	+	+	+
1eRsRG	-	+	+	+
1dEendoWR	-	+	+	+
1eRpRG	-	+	+	+
3dRpCW	-	+	+	+
3eRsWR	-	+	+	+
2DRsCW	-	+	+	+
1eRpCW	-	+	+	+
2eRpRG	-	+	+	+
2EndoWR	-	+	+	+
3RpWR	-	+	+	+
1e Endo RG	-	+	+	+
1EndoRG	-	+	+	+
1e'EndoRG	-	+	+	+
2RpRG	-	+	+	+
3RpRG	-	+	+	-
2aRpWR	-	+	+	-
3cEndoRG	-	+	+	+
3cRsWR	-	+	+	+
2RsWRht	+	+	+	+
2aRpWR ht	+	+	+	+
2RsWR ht	+	+	+	+
2bRsWRht	+	+	+	+
2RsWR ht	+	+	+	+
RsWR	-	+	+	+
3aRsWR	-	+	+	+
RpWR	-	+	+	+
2aRpWR	-	+	+	+
RpRG	-	+	+	+
2RsWR	-	+	+	+
3RpWR	-	+	+	+
3RsRG	-	+	+	+
3RpWRflow	-	+	+	+
3aEndoWR	-	+	+	+
2aRsWRht	+	+	+	+
2aRpRGht	+	+	+	-
2RpWRht	+	+	+	-
3aRpRGht	+	+	+	+
2RsRGht	+	+	+	+

14. Appendix 6: Abstracts, Posters, Articles and Papers

Biological control of weeds in vineyards.

Dr Graham O'Hara, Dr Ruben Flores Vargas

Centre for *Rhizobium* Studies, School of Biological Sciences and Biotechnology, Division of Science and Engineering, Murdoch University, Perth, WA 6150.

Weeds are among the most serious threats to Australia's primary production and natural environment. They reduce farm and forest productivity, displace native species and contribute significantly to land degradation. Winter weeds such as wild radish (*Raphanus raphanistrum* Linneo), ryegrass (*Lolium rigidum* Gaudi*n*) and capeweed (*Artotheca calendula* Linneo) emerge in autumn and early winter and are a significant problem in many vineyards. Weed control is important in vineyard management to maintain vine plant vigour and productivity. Weeds under the vine canopy are usually controlled using herbicides. The increasing threats of herbicide resistance weed plants, and a current drive towards lowering chemical inputs for grape production, provide an opportunity for the development of novel approaches, for weed management. The project aims to isolate deleterious rhizosphere inhabiting bacteria (DRB) with the ability to inhibit the establishment and development of economic important weed species in vineyards.

In collaboration with scientists in the viticulture and weed science groups of Western Australia Department of Agriculture (WADA) we have established links with 25 vineyards in the Swan Valley, Margaret River, Manjimup and Mt Barker/Albany regions and obtained permission for weed sampling.

A total of 442 rhizosphere bacteria have been isolated using selective (Pseudomonas, Sands and Rovira) and non-selective (tryptic soy and nutrient agar) media. To date, 125 have been screened for effects on weeds individually, and in combination, in the laboratory and glasshouse.

Three isolates have deleterious effects on weeds and using physiological and molecular techniques for identification have been characterised in detail and identified as *Pseudomonas fluorescens* (1 and 2) and one as *Alcaligenes xylosoxidans. P. flourescens* 1 produces hydrogen cyanide (HCN), an inhibitor of plant roots. These isolates are being screened for effects on other weed species, annual species commonly sown in vineyards as cover crops (eg. *Trifolium* spp.) and vine rootlings.

The results of screening these three isolates in vineyard plants and subterranean clover (*Trifoliun subterranean*) under environmental controlled conditions so far showed no deleterious effects of the rhizobacterial isolates on growth and development of vine plants and subterranean clover.

Dr Flores Vargas is funded by Grape and Wine Research and Development Corporation (GWRDC) Components of this project involve collaboration with Western Australia Department of Agriculture (WADA) and the University of Extremadura, Spain.

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