

Towards ecologically based weed management systems in vineyards

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Summary 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 threat of herbicide resistance, and a current drive towards lowering chemical inputs for grape production, provides an opportunity for the development of novel approaches for weed management. The aim of this project is to isolate deleterious rhizosphere inhabiting bacteria with the potential to develop commercial products for weed control. Weeds targeted in this project include annual ryegrass (*Lolium rigidum* Gaudin), wild radish (*Raphanus raphanistrum* L.) and capeweed (*Arctotheca calendula* L.). 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 two have been characterised in detail and identified as *Pseudomonas fluorescens* and *Alcaligenes xylooxidans*. The third unidentified isolate 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 (e.g. *Trifolium* spp.) and vine rootlings.

Keywords Deleterious rhizobacteria (DRB), wild radish, ryegrass, capeweed.

INTRODUCTION

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 (The National Weeds Strategy 1997). Winter weeds such as wild radish, ryegrass and capeweed emerge in autumn and early winter and are a significant problem in many vineyards. Weeds growing under the vine canopy are difficult to control by either mowing or cultivation, and herbicides are the method of control frequently employed. While herbicides are a very effective weed management tool, several factors have emerged which limit their potential to continue as a dominant weed control practice. Firstly, in respect of viticultural production, there is a current drive towards lowering chemical inputs for grape production.

Secondly, the increase in resistance of weed species to herbicides is altering the way weeds can be controlled (Alstrom 1987). In recent years some weed populations have developed multiple herbicide resistances, causing major concerns throughout Australia.

Using soil micro-organisms to control weeds is a promising alternative method to reduce grape and wine production costs, decrease dependence on chemical herbicides and increase the use of environmentally sound practices. One group of micro-organisms overlooked as biological control agents of weeds are the deleterious rhizobacteria (DRB), characterised as non-parasitic bacteria (exopathogens) colonising plant root surfaces and being able to suppress plant growth (Kremer and Kennedy 1996). Many DRB are plant specific (Cherrington and Elliott 1987, Elliott and Lynch 1985, Suslow and Schroth 1982) thus, their existence on weeds and their potential as biological control agents has only recently been investigated. DRB with potential as biological control agents were first described on downy brome (*Bromus tectorum* L.) 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). As an example, biological control of downy brome in winter wheat by *Pseudomonas* spp. isolated from downy brome roots has been demonstrated under field conditions (Kennedy *et al.* 1991). Two isolates of *Pseudomonas* spp. consistently reduced downy brome density, growth and seed production. The bacteria did not affect winter wheat densities and grain yield was significantly increased. The increase of wheat yields was primarily due to the growth suppressive effects of the applied bacteria on downy brome, which allowed the wheat to be more competitive (Schroth and Hancock 1982). A study carried by Begonia *et al.* (1994) demonstrated that certain rhizobacteria isolated from velvetleaf (*Abutilon theophrasti* Medik.) are potentially useful in suppressing weed growth. Norman *et al.* (1994) also evaluated the rhizobacteria and their phytotoxins as weed control agents in cranberry vines.

The objectives of this research are to isolate, identify and characterise potential DRB from weed species frequently found under vine canopies in Western Australia, and then investigate the phytopathogenecities of these bacteria on growth of weeds and vine plants.

Isolates that inhibit weeds, but not vines, will be used to develop an effective bacteria based weed management control method for ecologically based weed management systems in Australia.

MATERIAL AND METHODS

Sampling of the weed plants Weeds targeted in this project includes annual ryegrass (*Lolium rigidum* Gaudin), wild radish (*Raphanus raphanistrum* L.) and capeweed (*Arctotheca calendula* L.). Seedlings and mature plants of annual ryegrass, wild radish and capeweed were collected from Henley Park Vineyard, Jane Brook Vineyard and Lamont Vineyard in the Swan Valley, Western Australia during October/November 2000. Samples of weeds for isolation of rhizobacteria from the rhizosphere (soil), rhizoplane (roots) and endorhizosphere were collected between vine rows and under the canopy of vine plants within a row. Three samples were collected of each species from each vineyard. Weed plants were store in sterile plastic bags at 4°C until processed in the laboratory.

Isolation of the rhizobacteria from rhizosphere, rhizoplane and endorhizoplane Standard microbiological methods were used to isolate rhizobacteria from rhizosphere, rhizoplane and endorhizoplane of wild radish, ryegrass and capeweed. Bacterial suspensions were separately diluted in 0.1% (w/v) peptone water (1:40) and then plated onto selective (pseudomonas isolation medium, Sands and Rovira medium) and non-selective (tryptic soy and nutrient agar) media. Isolates were purified in culture, and are being stored cryogenically (at -80°C).

Screening of inhibitory effects of rhizobacteria on weed seedling growth under laboratory conditions Seeds of target weed plants were surface sterilised by immersion in 3.25%(v/v) sodium hypochlorite 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. Effectiveness of surface sterilisation was assessed as described (Gealy *et al.* 1996). One day old cultures of each rhizobacterial isolate, grown in glucose minimum salt medium, were centrifuged at 20,000 rpm for 10 minutes and 2 mL of supernatant was added to the surface of 0.9% (w/v) agar plates. Fifteen surface-sterilised seeds of each weed species were then placed on each plate and incubated in the dark at 20°C for five days. Controls used 2 mL of sterile medium. Each isolate was tested in four replicates (Figures 1 and 2). After five days the seedlings were removed, germination recorded, and root lengths measured. Isolates that inhibited the target weed plants under laboratory conditions were

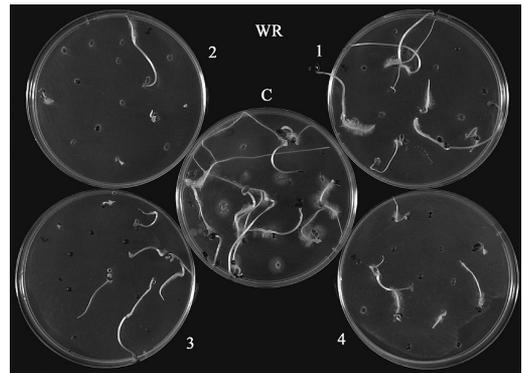


Figure 1. Bioassay of rhizobacterial isolates on wild radish.

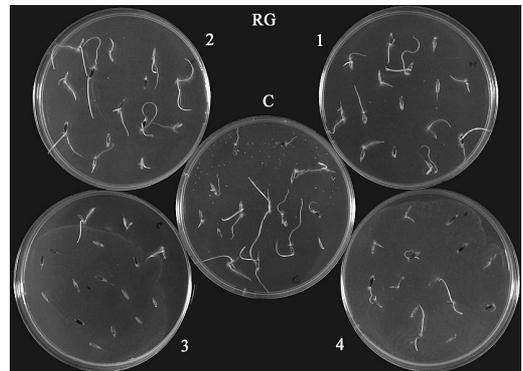


Figure 2. Bioassay of rhizobacterial isolates on ryegrass.

further tested for their effects on plants grown under glasshouse conditions.

Screening for inhibitory effects of rhizobacteria on weed seedling growth under glasshouse conditions Surface sterilised seeds of target weeds were germinated for two days on 0.9% (w/v) water agar and four seedlings were planted into a 110 mm pot containing a sterilised mixture of yellow sand and washed river sand. One day old cultures of rhizobacterial isolates grown on glucose minimal salt medium were used to inoculate the weed seedlings. Each seedling was inoculated with 2 mL of suspension containing 10^8 colony-forming units per mL (CFU mL⁻¹) of either an individual isolate or a combination of isolates. A thin layer of sterilised plastic beads was placed on the surface of each pot to reduce evaporation and airborne contamination (Figure 3). Plants were grown in the glasshouse at 25°C for six weeks. Plants were watered every second day with nutrient solution (CRS)

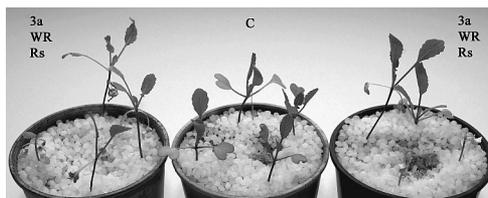


Figure 3. Glasshouse bioassay of wild radish.

containing 0.3% (w/v) KNO_3 . The experimental design was completely randomised with four replicates. After six 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°C for one week and dry weights of shoots and roots were recorded.

Screening for secondary metabolites Production of hydrogen cyanide, (HCN) an inhibitor of plant roots, was assayed by growing isolates on TSA plates supplemented with glycine (Bakker and Schippers 1987).

Characterisation of rhizobacteria Rhizobacteria were characterised using the Biolog System (WA Department of Agriculture). All the data were analysed using analysis of variance (ANOVA) single factor method.

RESULTS AND DISCUSSION

A total of 442 isolates were obtained. To date, 125 of these isolates have been screened individually and in combinations in the laboratory and under glasshouse conditions to investigate deleterious effects on the target weeds. Three of the rhizobacterial isolates (3aWRR, 1'RGRp and 1''RGRp) inhibited the growth of wild radish and ryegrass (Figure 4).

When used individually as inoculum the three isolates significantly reduced ($P < 0.05$) the dry mass of leaves and roots of wild radish (Figure 5). One of these isolates (3aWRRs) produced hydrogen cyanide, an inhibitor of plant roots, as a secondary metabolite (Figure 6).

Two of these isolates have been characterised in detail and identified. Isolate 1''RGRp is *Pseudomonas fluorescens* and isolate 1'RGRp is *Alcaligenes xylosoxidans*.

A range of foliar symptoms was observed in weeds grown in the glasshouse experiments when inoculated with the selected rhizobacterial isolates. The symptoms varied from general growth retardation to various types of leaf chlorosis and distortions. The foliar symptoms were most severe on wild radish. Lateral root development was poor in wild radish and ryegrass inoculated

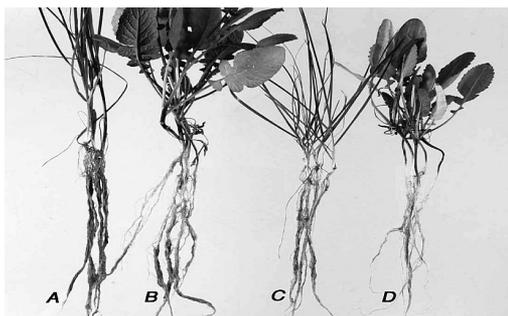


Figure 4. Inhibitory effect of rhizobacteria on ryegrass and wild radish. A – Control ryegrass, B – Control wild radish, C – ryegrass inoculated with combination of isolates 3aWRRs, 1'RGRp and 1''RGRp and D – wild radish inoculated with combination of isolates 3aWRRs, 1'GRp and 1''RGRp.

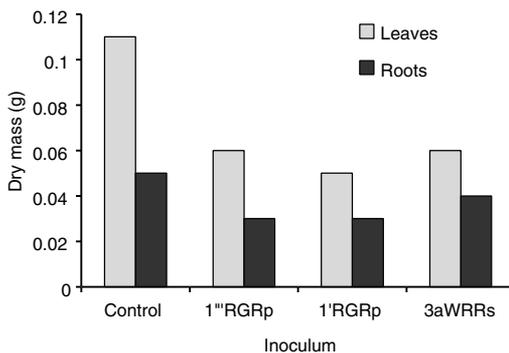


Figure 5. Effect of rhizobacteria on wild radish.

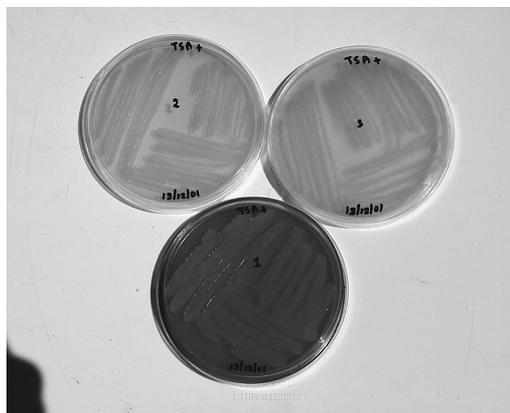


Figure 6. HCN production (dark plate) by isolate 3aWRRs.

with 3aWRR, 1'RGRp and 1''RGRp. These results are very similar to some previous reports (Begonia and Kremer 1994, Kremer and Kennedy 1996). However, most rhizobacteria reduce plant growth without obvious plant cell damage, an effect attributed to rhizobacterially produced toxins that were absorbed by roots (Begonia and Kremer 1994, Cherrington and Elliott 1987, Kremer and Kennedy 1996).

Weed management with DRB does not depend on development of an endemic disease on established weeds. Rather the rhizobacteria strategy is to regulate the development of weeds before or coincident with emergence of crop plants. Therefore DRB do not necessarily eradicate the problem weeds but significantly suppress early growth of weeds and allow the development of crop plants to effectively compete with weakened weed seedlings (Kremer and Kennedy 1996). Therefore, this novel ecologically based weed management option may become a powerful alternative or addition to traditional weed control programs.

ACKNOWLEDGMENTS

Collaboration with the WA Department of Agriculture, Viticulture and Weed Science groups and University of Extremadura, Spain is acknowledged. Authors thank Henley Park, Jane Brook and Lamont Vineyards for permission to collect weed samples.

This project is funded by the Grape and Wine Research and Development Corporation (GWRDC).

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