

Using bacteria to control annual cropping weeds

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Summary Deleterious rhizobacteria (DRB) previously isolated from USA soils and demonstrated to suppress growth of various weeds of USA cropping systems were tested against several important weed and crop species from Western Australia. Some DRB from USA were successful in reducing the growth of some weed species from Australia. They also however, reduced the growth of some major Australian canola varieties. It is recommended that these USA isolates not be used in Western Australia but instead Australian DRB should be isolated and tested for their specificity to Australian weeds, crops and conditions.

Keywords Deleterious rhizobacteria, biological control, cropping weeds.

INTRODUCTION

Deleterious rhizobacteria (DRB) are non-parasitic bacteria that are associated with plant roots and inhibit or reduce plant growth (Kremer and Kennedy 1996). DRB usually inhibit growth by producing phytotoxins but they can also reduce plant growth directly by competing with the plant for nutrients or indirectly by reducing the colonisation of beneficial rhizobia or mycorrhiza. Many DRB are highly specific in their level of plant growth reduction and for this reason can be used as biocontrol agents for weeds. Once a DRB with potential as a weed control agent has been identified, its specificity must be determined against crop species prior to its widespread release.

This study involved selected DRB cultured from Missouri (USA) soils and capable of suppressing seedling growth of selected weeds of USA cropping systems. This paper reports the effects of these DRB on representative weeds and crops of Western Australia cropping systems.

MATERIALS AND METHODS

The effect of deleterious rhizobacteria (DRB) isolated from Missouri, USA soils was tested on Western Australian crop and weed species using three methods. The sterile agar plate method assessed the effect of DRB on germination and early growth, the sterile growth pouch method determined the effect of DRB on actively growing seedlings, while the soil method provided a rigorous evaluation under non-sterile conditions.

For the sterile agar plate method, surface-sterilised seeds were germinated overnight at 20°C on 1.0% water agar then inoculated with a bacterial suspension containing approximately 1×10^9 CFU mL⁻¹. The plates were incubated at 20°C and root length and germination were determined after 48 hours for all species except the lupins, which were incubated for 96 hours. There were three replicate plates for the wheat cultivars and four replicate plates for the other crop and weed species.

The sterile growth pouch technique used was based on one developed by Somasegaran and Hoben (1985) and modified by Kremer *et al.* (1990). Surface-sterilised seed were pre-germinated on 1% water agar for 48 hours then transferred into growth pouches saturated with 50 mL of nutrient solution plus nitrogen (Broughton and Dilworth 1970). The pouches were either inoculated with 1 mL of a suspension of each isolate (approximately 5×10^8 CFU mL⁻¹) or left uninoculated and grown under growth lights for 5–7 days. There were three replicates and root length and shoot fresh weights were measured.

For the non-sterile soil method, seeds of each species were sown directly into small pots of 50% sieved field soil (Mexico silt loam – fine, smectitic, mesic, vertic, epiaqualf) mixed with 50% potting mix. Each pot was either inoculated with 5 mL of a suspension of each isolate (approximately 5×10^8 CFU mL⁻¹) or left uninoculated. Plants were grown under growth lights (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. The experiment was fully randomised with three replicates.

Four DRB isolates were used in this study: LC-19 and G2-11 (both *Pseudomonas fluorescens* isolated from giant foxtail (*Setaria faberi* L.)); D2-11 (*Aeromonas hydrophila* isolated from field bindweed (*Convolvulus arvensis* L.)); and D1 (*P. putida*). The Australian crop cultivars included three spring wheat cultivars (*Triticum aestivum* L. cv. Janz, Camm and Wyalkatchem), three barley cultivars (*Hordeum vulgare* L. cv. Gairdner, Stirling and Yagan), two triazine-tolerant canola cultivars (*Brassica napus* var. *napus*

cv. Karoo and Surpass 501), one narrow-leafed lupin cultivar (*Lupinus angustifolius* L. cv. Merrit) and one yellow lupin cultivar (*Lupinus luteus* L. cv. Wodjil). There were six Australian weed species: rip gut brome (*Bromus diandrus* Roth), red brome (*B. rubens* L.), soft brome (*B. hordaceus* L.), annual ryegrass (*Lolium rigidum* L.), wild radish (*Raphanus raphanistrum* L.) and rat's tail fescue (*Vulpia myuros* L.).

The results were analysed using Genstat Version 6.1.

RESULTS

In sterile agar plate assays, three of four DRB isolates reduced root growth of all crop cultivars and weed species ($P < 0.05$). Two of the rhizobacterial isolates, LC-19 and G2-11, reduced the root growth of all of the crop cultivars and weed species tested. Isolate D1 also reduced root growth of most of the crop cultivars and weed species but to a lesser extent than the other two isolates. Isolate D2-11 had no effect.

The same DRB had a lesser effect on the growth of the crop cultivars and the weed species when grown in sterile growth pouches compared to water agar. Rat's tail fescue and soft brome were the most affected and they were inhibited by either one or both of the isolates LC-19 and G2-11 ($P < 0.05$).

Under non-sterile conditions, in plant-soil bioassays, the growth of canola (Figure 1, $P < 0.05$), rat's tail fescue (Figure 2, $P < 0.05$) and to a lesser extent rip gut brome was inhibited by one or more DRB. The reduction in canola growth occurred after inoculation by isolate LC-19 but not by isolates D1 and G2-11 (Figure 1). All three isolates had previously reduced canola growth in agar assays. Canola (*Brassica napus* cv. Surpass 501) was the only crop with suppressed growth.

Three isolates LC-19, G2-11 and D1 reduced the growth of rat's tail fescue. These same isolates similarly reduced the growth of rat's tail fescue in the growth pouches.

DISCUSSION

This study showed that although the DRB from USA were successful in reducing growth of some of the Australian weeds, especially rat's tail fescue, they also reduced the growth of Surpass canola. This has implications for the application of American DRB in Australian cropping systems: each DRB must be tested rigorously against crop varieties prior to release. The DRB isolates used in this study were selected from USA weeds grown in USA soils and conditions. There is a need to select isolates that target weeds of Western Australian cropping system without damaging local crops and that tolerate the different

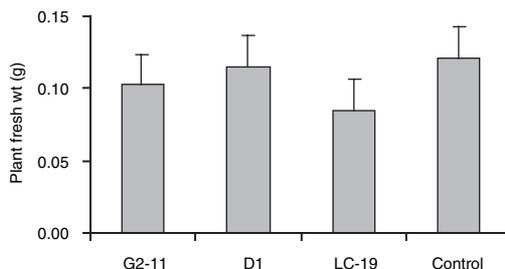


Figure 1. Effect of three deleterious rhizobacteria on the whole plant fresh weights (g) of Surpass 501 canola grown in non-sterile soil (mean of three replicates). Error bars are standard errors of the means.

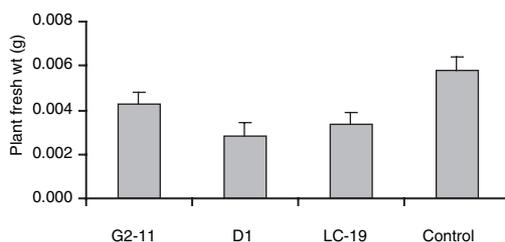


Figure 2. Effect of three rhizobacterial isolates on the whole plant fresh weight (g) of rat's tail fescue (*Vulpia myuros*) grown in non-sterile soil (mean of three replicates). Error bars are standard errors of the means.

soils and conditions that exist in our major cropping zones.

The most probable reason for the reduction in plant growth by these DRB 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. Additional assays of these isolates in the US have confirmed that isolate G2-11 produces considerable amounts of hydrogen cyanide (Kremer and Souissi 2001) and overproduces 3-indoleacetic acid, which can be toxic in high concentrations.

There was no reduction in the growth of all other weed and crop species in non-sterile soil although these same species showed a marked reduction in growth in sterile conditions on agar. Under non-sterile conditions, the DRB must compete with other microorganisms in the rhizosphere. Different plants exude different chemicals into the rhizosphere affecting the type and abundance of associated microorganisms (Nehl

et al. 1996, Kennedy *et al.* 2001). In the case of canola and rat's tail fescue, the DRB successfully competed with other rhizosphere microorganisms reaching high population levels that produced enough phytotoxin to affect plant growth. Conversely, it is likely that the rhizosphere microorganisms associated with the other species out-competed the DRB and no growth reductions occurred. Similarly, in previous US studies, the growth of 47 plant species varied considerably after inoculation with one DRB isolate (Kennedy *et al.* 2001).

DRB isolated recently from Western Australian soils have been found to reduce growth in annual ryegrass and wild radish under glasshouse conditions (G. O'Hara pers. comm.). It is proposed to test these against WA crop cultivars and, if they are found not to be detrimental to crops, test them under field conditions. New application methods developed for rhizobia may be adapted for DRB application, allowing the DRB to survive in Australian soils longer and in greater numbers.

ACKNOWLEDGMENTS

I would like to thank Winston Churchill Memorial Trust, the Swire Group and the Department of Agriculture (WA) for their financial assistance. Special thanks also to the Dr. Kremer's laboratory staff in Columbia, Missouri, USA.

REFERENCES

- Broughton, W.J. and Dilworth, M.J. (1970). Control of leghaemoglobin synthesis in snake beans. *Biochemistry Journal* 12, 1075-80.
- Kennedy, A.C., Johnson, B.N. and Stubbs, T.L. (2001). Host range of a deleterious rhizobacterium for biological control of downy brome. *Weed Science* 49, 792-7.
- Kremer, R.J., Begonia, M.F., Stanley, L. and Lanham, E.T. (1990). Characterisation of rhizobacteria associated with weed seedlings. *Applied Environmental Microbiology* 56, 1649-55.
- Kremer, R.J. and Kennedy, A.C. (1996). Rhizobacteria as biocontrol agents of weeds. *Weed Technology* 10, 601-9.
- Kremer, R.J. and Souissi, T. (2001). Cyanide production by rhizobacteria and potential for suppression of weed seedling growth. *Current Microbiology* 43, 182-6.
- Nehl, D.B., Allen, S.J. and Brown, J.F. (1996). Deleterious rhizobacteria: an integrating perspective. *Applied Soil Ecology* 5, 1-20.
- Somasegaran, P. and Hoben, H.J. (1985). Methods in legume-rhizobium technology. NIFTAL Project, Paia, Hawaii, USA.