BENEFICIAL AND DELETERIOUS RHIZOSPHERE BACTERIA IN NEW ZEALAND PASTURES

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SUMMARY

White clover and ryegrass roots were collected from 45 sites in New Zealand to determine occurrence of rhizosphere bacteria with the ability to a) inhibit five root pathogens (four *Pythium* species and *Rhizoctonia solani*), b) inhibit root growth in white clover and ryegrass seedlings and c) produce cyanide. *In vitro* tests showed at least one pathogenic fungus was inhibited by 37-52% of bacterial isolates from collection sites. Root growth of seedlings was inhibited by 0-40% of isolates with at least one inhibitory organism being isolated from 36 of the 45 sites. A consistently high incidence of cyanogenic fluorescent *Pseudomonas* spp. was observed from all sites. There were no statistically significant differences in fungal antagonism, root growth inhibition and cyanogenesis between bacterial isolates from white clover and ryegrass rhizoplanes.

Keywords: antifungal bacteria, cyanogenesis, deleterious rhizobacteria, *Lolium perenne*, *Trifolium repens*

INTRODUCTION

The interactions between rhizosphere bacteria and plant roots can affect plant health in a variety of ways. One of these, plant growth promotion, is thought to result mainly from the suppression of pathogens in the root zone by bacteria (Weller 1988). Several different mechanisms may be responsible for this suppression, including production of antibiotics or siderophores (iron binding compounds), competition for substrate, and niche exclusion (Weller 1988).

Deleterious effects of bacteria on plant growth result from direct invasion of roots by pathogens or the production of toxic metabolites such as low molecular weight toxins (Bolton *et al.* 1989), including cyanide (Alstrom and Burns 1989), which inhibit plant growth. Deleterious rhizobacteria (DRB) can cause symptoms such as reduced seed germination, reduced root elongation, increased infection by root colonising fungi and reduced plant growth (Suslow and Schroth 1982).

With the notable exception of the *Bradyrhizobium* symbiosis, little work has been done with beneficial or deleterious bacteria in white clover (*Trifolium repens* L.) and ryegrass (*Lolium perenne* L.) in New Zealand pastures. About 80% of fluorescent *Pseudomonas* spp. were able to produce cyanide and up to 14% of rhizobacteria were able to inhibit root growth in germinating clover and ryegrass seedlings in a study of pasture roots from the Waikato region of New Zealand (Sarathchandra *et al.* 1993b). Fluorescent *Pseudomonas* spp. isolates from pastures in the Waikato showed antifungal activity against two common fungal root pathogens (Sarathchandra *et al.* 1993a). The growth of *Pythium ultimum* and *Fusarium oxysporum* were inhibited by 17% and 5% of fluorescent *Pseudomonas* spp. isolates respectively.

The aims of the survey reported here were a) to isolate rhizosphere bacteria and fungi from different geographic locations and climatic zones in New Zealand for further testing as potential biological control agents to combat root pathogens, and b) to compare findings from Waikato white clover/ryegrass-based pastures with those from other parts of New Zealand. Some preliminary results of this survey have already been published (Sarathchandra *et al.* 1994).

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METHODS

Turves (approximately 20 cm³) were collected from established pasture from different locations around New Zealand (Fig 1). When there was more than one sample collected from a given area, the collection sites were at least 10 km apart. A total of 45 samples were collected. The turves were processed in the laboratory within 2 days of collection.

Figure 1: Location of sites where turf samples were taken (numbers refer to the number of samples collected from each area).

After washing free of soil, clover and ryegrass roots were separated, and 1 g (wet weight) of root material of each was ground in 9 ml of sterile distilled water in a sterile mortar and pestle. Resulting suspensions were diluted and plated on two different media. Trypticase soy agar (TSA) and King's medium B (KB) were used for isolation of 'total' heterotrophic bacteria and fluorescent *Pseudomonas* spp. respectively.

For each clover and ryegrass root sample, 20 bacterial colonies from TSA plates and 10 colonies of fluorescent *Pseudomonas* spp. from KB plates were randomly selected, checked for purity and stored in 0.1M MgSO₄ for further tests. Thus a total of 60 bacterial isolates were obtained from each turf sample.

Isolates were tested for their ability to inhibit five strains of pathogenic fungi, including a strain each of *Rhizoctonia solani* and *Pythium irregulare* and three other *Pythium* species. *P. irregulare* was tested against bacterial isolates from only 24 turf

samples. Tests were done by growing the fungus and bacteria together on a potato dextrose agar plate and looking for zones of inhibition of fungal growth. Details of the test procedure are outlined elsewhere (Sarathchandra *et al.* 1993a).

Inhibition of germination and root growth of white clover and ryegrass were determined as previously described (Sarathchandra *et al.* 1994). Bacterial suspensions were added to petri dishes containing seeds of white clover or ryegrass and examined for signs of growth inhibition compared to controls. All the isolates of bacteria were initially screened against radish seeds (*Raphanus sativus* cv 'Sparkler') and those showing root growth inhibition of radish were further tested against white clover and ryegrass seedlings.

Cyanide production by all fluorescent *Pseudomonas* isolates was determined using the picric acid- Na_2CO_3 method (Bakker and Schippers 1987).

RESULTS AND DISCUSSION

The proportions of bacterial isolates from different locations which were antagonistic to at least one pathogenic fungus ranged from 37-52% of isolates for both clover and ryegrass (Table 1), and there was considerable variation between sites in the activity of isolates. This suggests that a degree of "natural biological control" already exists in the rhizosphere of many New Zealand pastures. Work is continuing to determine if the bacterial isolates will provide protection for emerging clover and ryegrass seedlings against damping off fungi such as *Pythium* sp. in field soils.

TABLE 1: Percentage of bacterial isolates from white clover and ryegrass rhizoplanes from 45 sites throughout New Zealand with antifungal activity and root-growth-inhibitory activity.

	Total heterotrophic bacteria				Fluorescent <i>Pseudomonas</i>			
	Range		Mean		Range		Mean	
	Clover %	Ryegrass %	Clover %	Ryegrass %	Clover %	Ryegrass %	Clover %	Ryegrass %
Antifungal activity	0-80	0-80	37.5	40	0-100	0-100	52	47
Ryegrass inhibition ¹	0-25	0-35	3.6	2.7	0-40	0-40	3.1	2.6
White clover inhibition ¹	0-15	0-20	4.3	4.2	0-30	0-20	1.6	2.1

¹ Inhibition of root growth of seedlings

The mean percentage of 'total' isolates able to antagonise at least one fungus was similar for isolates from both clover and ryegrass, suggesting that the occurrence of antifungal bacteria on both root systems is similar.

Of those isolates showing inhibition of radish seedling growth, only 4-5% of 'total' bacterial isolates and 35% of fluorescent *Pseudomonas* spp. showed inhibition of clover and ryegrass root growth. The use of radish seedlings provided a quick bioassay to detect inhibitory bacteria. This method was used in preference over other methods (Bolton and Elliot 1989) due to the large number of isolates which needed testing. An assumption was made that radish seedlings were more sensitive to toxins than clover or ryegrass. Our observations that only a small proportion of bacteria which inhibited radish also inhibited clover and ryegrass tend to support this assumption.

There were no inhibitory organisms isolated from some of the sites while from other sites up to 40% of the isolates were inhibitory (Table 1). At least one inhibitory organism was isolated from 36 out of the 45 sites.

TABLE 2: Number of sites, out of 45 sampled throughout New Zealand, with different proportions of cyanogenic fluorescent *Pseudomonas* isolates from rhizoplanes of white clover and ryegrass.

Source	% of cyanogenic isolates						Mean
	0	1-20	21-40	41-60	61-80	81-100	Mean
White clover	1	2	0	13	15	14	72%
Ryegrass	1	3	9	8	10	14	63%

Table 2 shows the number of sites with different proportions of cyanogenic fluorescent *Pseudomonas* spp. from ryegrass and white clover roots. The mean percentage of cyanogenic *Pseudomonas* spp. isolates obtained from clover roots (72%) was not different ($P>0.05$) to the mean percentage of isolates from ryegrass roots (63%). This is contrary to preliminary analysis of data from this survey (Sarathchandra *et al.* 1994) and from the Waikato region (Sarathchandra *et al.* 1993b) where a higher ($P>0.05$) percentage of cyanogenic bacteria were obtained from clover roots compared to ryegrass roots.

This high percentage of cyanogenic bacteria may possibly adversely affect pasture growth in the field, but cyanide may also inhibit the growth of fungal root pathogens (Sarathchandra, unpublished data). Further research is required to determine these interactions.

This survey has been useful in indicating the levels of bacterial populations with potential beneficial and deleterious effects on plant growth in New Zealand pastures. The resulting collection of isolates provides a valuable resource for further research on potential biological control agents active against root pests and pathogens in pastoral soils.

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