COMPETITION BETWEEN STRAINS OF *RHIZOBIUM TRIFOLII* IN THE ESTABLISHMENT OF WHITE CLOVER

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Abstract

The satisfactory establishment of white clover in redevelopment areas already colonized by ***Rhizobium trifolii*** depends upon (a) the effectiveness of the naturalized population, (b) the ability to introduce more effective strains, and (c) the persistence of introduced strains in a competitive situation.

The effectiveness of naturalized populations of ***R. trifolii*** isolated from pasture soils on the Central Plateau is inferior when compared under standard conditions with strains used for inoculation of white clover. However, the superior competitive ability of naturalized populations prevents the satisfactory introduction of inoculant strains using conventional inoculation techniques.

Alternative inoculation methods have been used to increase the proportion of nodules formed from the inoculant strain. Techniques are now being sought to improve the persistence of introduced strains of ***R. trifolii*** in competitive situations.

INTRODUCTION

FOR THE satisfactory establishment of white clover (*Trifolium repens* L.) in areas of New Zealand devoid of a naturalized population of *Rhizobium trifolii,* inoculation of seed is absolutely necessary (Hastings *et* al., 1966; Vincent, 1974). However, the satisfactory establishment of white clover in redevelopment areas which are already colonized by *R. trifolii* depends upon several factors, such as (a) the effectiveness of the naturalized population of rhizobia in terms of both nodulating and nitrogen fixing ability; (b) the ability of introduced strains to compete with well-adapted naturalized strains; and (c) the persistence of more effective strains when introduced into a soil environment.

In many areas of New Zealand which are being redeveloped and are already colonized by *R. trifolii* there is little or no response to inoculation of white clover seed. Establishment is usually satisfactory, but often the initial pasture production is not maintained.

Studies in Australia have shown that, when tested under controlled environmental conditions. the relative effectiveness of naturalized populations of *R. trifolii* from many areas ranged from 73 to 93% of the standard inoculant strain (Rergersen *et al.,*

1971). Bonish (1979) has found that *R. frifolii* strains isolated from several New Zealand soils vary immensely in their relative effectiveness, from totally ineffective to 90% of the standard strain (TA1).

When inoculated white clover seed is sown, the inoculant strain is present in relatively large numbers around the seed. However, by the time the seed germinates and produces a rhizosphere cap able of supporting multiplication of the introduced rhizobia, the numbers arelikely to have diminished.As a result, the naturally occurring population of rhizobia is likely to be present in greater numbers, and consequently may nodulate much of the root system.

This paper deals with some aspects of the competition between introduced and naturalized populations of *R. frifolii* in soils from the Central Plateau.

MATERIALS AND METHODS

The techniques used to follow the fate of introduced strains of *Rhizobium* spp. in the presence of naturalized populations in soil are well documented — e.g., serology (Gibson *et al.*, 1976), immunofluorescence (Bohlool and Schmidt, 1970), and antibiotic-resistant markers (Brockwell *et al.*, 1977). In this study an antibiotic-resistant strain of *R. trifolii* was used, as this method is generally considered to be most reliable for studying the ecological relationships between introduced and naturalized populations of *Rhizobium* spp. in soil.

ANTIBIOTIC-RESISTANT MUTANTS

The *R. frifolii* strain used (PDDCC* 4644) was resistant to 200 μg/ml streptomycin sulphate (Glaxo Laboratories Ltd), effective for nitrogen fixation and not streptomycin-dependent after subculture and lyophilization. Effectiveness and stability checks were carried out regularly during the experimental work. "Rhizocote"† peat-based inoculants of the above mutant. were prepared by Fruitgrowers' Chemical Co. Ltd, Port Mapua, Nelson.

TEST SOILS

Soils from four pastures on the Central Plateau in which growth of clover was considered to be a problem were used. The pastures

* Plant Diseases Division Culture Collection.
† Registered trade mark.

ranged from 1 to 10 years in age. Fertilizer in the form of potassic superphosphate was added to the soils at the sates recommended for pasture production in each soil type.

The number of *R. trifolii* occurring naturally in each soil as determined using the most-probable-number method described by Brockwell (1963) was high (Table 1).

TABLE 1: DETAILS OF PASTURE SOILS USED IN COMPETITION STUDIES

Age of Pasture bears)	*pH 0-20 cm*	*No. of Viable* R. trifolii/ *gram soil*
1	5.3	1.3 x 10^3
3	5.4	1.4 × 10^3
4	5.1	6.3 × 10^4
10	5.5	4.2 x 10^3

SEEDLING AGAR TUBE CULTURE

Pure cultures of *R. trifolii* isolated from each soil were compared for effectiveness, in terms of nodulation and dry weight of white clover foliage produced, with a strain of *R. trifolii* (PDDCC 2153) currently recommended for inoculant manufacture and the streptomycin-resistant strain (PDDCC 4644). Comparisons were made on white clover plants grown for 4 weeks after inoculation using the seedling agar tube method (Vincent, 1970). Tubes were incubated at 21°C ± 1°C with a 16 h light period. The foliage dry weight was determined on 10 replicate plants for each culture.

GLASSHOUSE TRIALS

(1) *Seed Inoculation*

Seed of white clover ('Grasslands Huia') was inoculated with the commercially prepared mutant at the recommended rate (c.10^3 rhizobia per seed after drying). Seed was air-dried, and 10 seeds sown in the test soils containing naturalized populations of *R. trifolii* in 5 cm × 5 cm plastic pots.

(2) *Soil Inoculation*

Peat inoculant was suspended in sterile distilled water and 5 ml of suspension was added to each pot in the order to introduce approximately 10^4 rhizobia of the inoculant strain. Ten white clover seeds were then sown in the inoculated soil.

(3) Granular Application of Inoculum

"Rhizocote granules" carrying appoximately 10^2 rhizobia per granule were prepared using the mutant strain. Ten white clover seeds were sown in each pot of test soil together with 100 granules.

Non-inoculated controls were set up for each test soil. All pots were held in a glasshouse at **21°C** ± 1°C, watered daily with sterile distilled water, and the germinated seedlings thinned to four per pot. Plants were harvested from each treatment after 6 weeks. Shoot dry weight was determined while roots were washed, and nodulation rated on number, size, colour and distribution on the roots *(i.e.,* 5 = good nodulation, 0 = no nodulation). Isolations were then made from each nodule using the method described by Franco and Vincent (1976) to isolate the streptomycin-resistant strain of ***R. trifolii.***

FIELD PLOT TRIALS (CENTRAL PLATEAU)

Seed of white clover ('Grasslands Huia') was sown in **1** m × 0.5 m plots in each of the four pasture soils using the three inoculation methods described for glasshouse trials. Approximately 10^6 rhizobia were introduced into each plot by seed inoculation, liquid inoculation of the soil or application of granules. Clover plants from each plot were sampled 4, 8 and 12 months after sowing, and the nodules on each plant were tested for the presence of the streptomycin-resistant strain of *R. trifolii.*

RESULTS

Details of each of the pasture soils used are given in Table 1.

SEEDLING AGAR TUBE CULTURE (at 21°C)

The results of comparative tests of effectiveness of pure cultures of *R. trifolii* from each of the test soils are presented in Table 2.

The cultures isolated from each of the pasture soils were significantly ($P < 0.05$) less effective than the recommended inoculant strain PDDCC 2153 when compared in seedling agar tube tests. There was no significant difference in effectiveness in terms of foliage dry weight between the recommended strain and the antibiotic-resistant marker strain used in this study.

GLASSHOUSE TRIALS (21°C)

Results of these experiments are given in Table 3. In each of the pasture soils the use of either soil inoculation or granular

TABLE. 2: COMPARISON OF EFFECTIVENESS OF CULTURES OF *R. TRIFOLII* FROM CENTRAL PLATEAU PASTURES

Age Of Pasture (years)	No. of Cultures Tested	Mean Foliage Dry Weight (mg/plant)	% of PDDCC 2153
1	5	6.1	80.3
3	8	5.2	68.4
4	7	6.0	78.9
10	4	5.3	69.7
	PDDCC 2153	7.6	100
	PDDCC 4644	7.3	96.1

application of inoculum resulted in a significant ($P < 0.01$) increase in nodulation rating, foliage dry weight of plants, and the proportion of nodules, containing the inoculum strain of *R. trifolii* when compared with either seed inoculation or non-inoculated controls.

TABLE 3: EFFECT OF TYPE OF INOCULATION ON NODULATION. DRY WEIGHT OF FOLIAGE AND RECOVERY OF AN ANTIBIOTIC-RESISTANT STRAIN OF *R. TRIFOLII* FROM WHITE CLOVER GROWN IN FOUR PASTURE SOILS

Age of Pasture (years)	Inoculation Type	Nodulation Rating (O-5)	Fol Dry Weight (mg/plant)	% Nodules Cont.4644
1	Seed	2.7	20.7	12.3
	Soil	3.8	28.3	56.6
	Granular	3.6	27.7	41.6
	None	2.7	17.2	—
3	Seed	2.0	22.7	3.0
	Soil	3.2	32.4	29.6
	Granular	3.0	29.6	28.1
	None	1.4	23.2	—
4	Seed	2.5	17.4	18.6
	Soil	3.3	26.7	49.2
	Granular	3.3	22.7	40.2
	None	2.6	16.9	—
10	Seed	1.8	12.6	17.2
	Soil	2.9	20.3	51.7
	Granular	2.6	16.1	37.0
	None	2.0	11.4	—

FIELD PLOT TRIALS

Soil inoculation and granular application of inoculum markedly increased the percentage of nodules containing the antibiotic-resistant inoculum strain of *R. trifolii* at each site 4 months after sowing (Table 4). However, after 8 months the inoculum strain was not recovered from any nodules on plants growing in plots

TABLE 4: EFFECT OF TYPE OF INOCULATION ON THE PERSISTENCE OF THE INOCULUM STRAIN IN FOUR PASTURE SOILS

Age of Pasture (years)	Inoculation Type	% Nodules Containing 4644 Time after sowing (months)		
		4	8	12
1	Seed	6.2	0	0
	Soil	26.1	17.6	9.7
	Granular	12.2	6.0	0.7
3	Seed	2.4	0	0
	Soil	18.3	8.1	2.4
	Granular	14.1	2.0	0
4	Seed	1.2	NT*	NT
	Soil	8.4	N T	N T
	Granular	9.3	N T	N T
10	Seed	8.6	0	0
	Soil	27.4	10.2	9.5
	Granular	21.0	4.1	0

* Not tested.

where seed was inoculated using conventional methods. There was a decline in the persistence of the inoculum strain when both soil inoculation and granular application were used. However, after 12 months the antibiotic-resistant strain of *R. trifolii* was still recovered from some clover plants at each site when soil inoculation was used.

DISCUSSION

The presence of a naturalized population of *R. trifolii* which is less effective but more competitive than an introduced strain is an important factor to be considered when recommending inoculation in a particular soil.

The results of this study show that cultures of *R. trifolii* isolated from four pasture soils on the Central Plateau are less effective when tested under controlled environmental conditions than strains of R. *trifolii* currently recommended for the manufacture of commercial inoculants. It has been shown (Lowe and Holding, 1970; Sherwood and Masterson, 1974) in effectiveness tests that isolates of *R. trifolii* from indigenous *T. repens* populations may be more effective with field ecotypes than with a commercial seed line. Consequently it is possible that naturally occurring populations have a host × strain specificity in symbiotic effectiveness as suggested by Vincent (1954). However, *T. repens* *cv.* 'Grasslands Huia' was used in this particular study because of its typical response with a range of *R. trifolii* strains and because of its widespread use in New Zealand.

Using an antibiotic-resistant strain of *R. trifolii,* it was shown that when seed was inoculated by conventional methods and sown in soils containing naturalized populations there was no significant improvement in either nodulation or foliage dry weight when compared with non-inoculated controls. The majority of nodules formed on inoculated white clover plants in the four pasture soils contained the naturalized population rather than the inoculum strain.

Hale (1977) and Hale and Mathers (1977) showed that the rapid death of *R. trifolii* on white clover seed during the period the applied inoculum is drying and in the immediate post-drying period, together with the inhibitory effects of seed diffusates from white clover, can markedly reduce the number of rhizobia remaining on the seed to effect nodulation. The number of naturalized rhizobia present in the soil, together with their greater adaptation to the environmental conditions encountered, are likely to be important factors affecting the competitive ability of the naturalized population. Consequently, conventional seed inoculation techniques which introduce only relatively small numbers of less well-adapted rhizobia into these soils are unlikely to improve nodulation or dry matter production.

However, there was a marked improvement in both nodulation and foliage dry weight when the inoculum strain was incorporated directly into the soils either as a liquid or as granules. The results suggest that the increases are due to the formation of a greater proportion of nodules by the more effective inoculum strain. By introducing the inoculum as either a liquid or in the form of granules, the adverse effects of drying and toxic seed diffusates are lessened. Consequently the inoculum strain is likely to be present in greater numbers during the formation of a rhizosphere capable of supporting multiplication of rhizobia. This provides more opportunity for nodulation to be caused by the inoculum strain.

Soil incorporation of inoculum showed some promise in field plots, but it is important that the persistence of introduced strains of *R, trifolii* is improved before significant increases in production due to inoculation can be maintained from season to season.

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