SOME FACTORS AFFECTING THE SURVIVAL OF *RHIZOBIUM TRIFOLII* ON WHITE CLOVER

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Abstract

Survival of *Rhizobium trifolii* on white clover seed before sowing is adversely affected by both drying and a water-soluble toxin which diffuses from the seed coat during the inoculation process. Survival of rhizobia is increased by removal of the toxin by seed washing or the suppression of its inhibitory effect by treatment of seed with phenolic adsorbents. Survival of rhizobia introduced into tbe soil on seed before the rhizosphere is capable of supporting growth and multiplication may be affected by the toxic diffusate and competition and/or antagonism from naturally occurring populations of soil micro-organisms, Selection of effective strains of rhizobia on criteria such as survival and competition abilities, as well as on the ability to nodulate and fix nitrogen, is considered to be extremely important.

INTRODUCTION

SUCCESSFUL ESTABLISHMENT of *Trifolium repens* requires the presence of sufficient numbers of an effective strain of *Rhizobium trifolii* to promote nodulation. Three distinct phases are involved:

(1) Inoculant quality and survival of rhizobia in culture.

(2) Survival of rhizobia on seed before sowing.

(3) Survival and multiplication on or near seed before nodulation.

Some of the factors influencing the survival of *R, trifolii* during these three phases are considered in this paper.

INOCULANT QUALITY AND SURVIVAL OF BACTERIA IN CULTURE

tnoculant quality refers to the number of cells of an effective *Rhizobium* strain in an inoculant which are viable and hence determines the number of rhizobia applied to the seed at the time of inoculation. Of the factors which affect the quality of peat inoculants, incubation and storage temperature, moisture content of the peat and sterility of the peat used for inoculant preparation are considered to be the most important (Roughley and Vincent, 1967).

Quality control is at present the responsibility of the manufacturer. However, a scheme to improve and control the quality of legume inoculants similar to that operated in Australia (Gemmell and Roughley, 1975) is likely to be set up in New Zealand in the near future and will be available to manufacturers on a voluntary basis.

SURVIVAL OF RHIZOBIA ON SEED BEFORE SOWING

The survival of *R. trifolii* on inoculated white clover may well be as important as the number of rhizobia applied at the time of inoculation if immediate nodulation and good establishment are to be achieved.

During Inoculation and Drying

The number of viable rhizobia surviving the inoculation and drying process, determined by: a plate count technique (Vincent, 1970), declined rapidly (Table 1) . Storage at room temperature for 24h after inoculation further reduced the number of surviving rhizobia to approximately 0.3% of those applied.

TABLE 1: SURVIVAL OF *RHIZOBIUM TRIFOLII* ON WHITE CLOVER SEED AFTER INOCULATION

Time after Inoculation	*% Surviving*
0	100
5 min	**45.8**
15 min	**30.0**
30 min	12.9
l h	**7.5**
2 h	**5.3**
4 h	**3.8**
6 h	**2.9**
24 h	**0.3**

Effect of Washing Seed on the Survival of Rhizobia

The number of *R. trifolii* cells surviving on white clover seed which had been washed in sterile distilled water and rapidly dried prior to inoculation was 4.0% after 24 h storage subsequent to inoculation (Table 2).

Antibiotic Activty of White Clover Seed Diffusates

The diffusates from white clover seed were shown to have marked antibiotic activity to all but two isolates of *Rhizobium*

TABLE 2: EFFECT OF SEED TREATMENTS ON SURVIVAL OF *RHIZOBIUM TRIFOLII*

Treatment	*% Surviving 24 h after Inoculation*
Unwashed seed	0.3 a
Unwashed seed + PVP	2.1 b
Unwashed seed + activated charcoal	2.8 b
Washed seed	4.0 c

Analysis of variance on angular transformation of data. Figures with no letter in common have significantly different means at the 1% level.

TABLE 3: EFFECT OF PHENOLIC ADSORBENTS ON THE ANTIBOTIC ACTIVITY OF SEED DIFFUSATES

Treatment	*Diameter of Inhibition Zone* (mm)*
Diffusate	17.7
Diffusate + PVP	0
Diffusate + activated charcoal	0
Diffusate + skim milk	0
Diffusate + casein	15.9
Diffusate + gelatin	18.2

*Includes filter paper disc (12.7 mm)

TABLE 4: ANTIBIOTIC ACTIVITY OF DIFFUSATES FROM WHITE CLOVER SEED

Species	*Host*	*PDDCC**	*Diameter of Inhibition Zone† (mm)*
Rhizobium trifolii	White clover	3165	20.7
Rhizobium trifolii		2153	17.7
Rhizobium trifolii		2666	16.7
Rhizobium trifolii		4130	14.7
Rhizobium trifolii	Red clover	2940	16.3
Rhizobium trifolii	Subterranean clover	2939	16.2
Rhizobium trifolii		4240	19.4
Rhizobium leguminosarum	Pea	3666	20.2
Rhizobium sp.	Lotus	1326	20.2
Rhizobium japonicurn	Soybean	3163	19.7
Rhizobium phaseoli	Dwarf bean	3.305	14.7
Rhizobium meliloti	Sweet clover	3261	15.7
Rhizobium meliloti		2750	‡
Rhizobium meliloti	Lucerne	4135	‡

*Plant Diseases Division Culture Collection.
†Includes filter paper disc (12.7 mm).
‡No inhibition but reduced colony size around discs.

species tested using a filter paper disc assay (Table 4). Filter paper discs were saturated with white clover seed diffusate, dried and placed on yeast mannitol agar (YMA) plates seeded with isolates of *Rhizobium* species and incubated at 28" C for 48 h.

CHARACTERIZATION OF THE "TOXIN"

Thompson (1960) and Bowen (1961) have reported that seeds of some *Trifolium* species are toxic to rhizobia. The flavonol myricetin and tannins isolated from white clover seed by Masterson (1965) also showed antibiotic activity towards *R. leguminosarum* and *R. trifolii*. Preliminary experiments have shown that flavonols, although present in white clover seed diffusates, are toxic to *R. trifolli* only at very high concentrations in our bioassays and tannins are considered to be the more likely cause of toxicity.

EFFECT OF PHENOLIC ADSORBENTS ON THE "TOXIN"

The antibiotic activity of the seed diffusate was suppressed when the diffusate was mixed with 5% w/v insoluble polyvinyl pyrrolidone (PVP), activated charcoal or skim milk powder, whereas casein and gelatin had no effect (Table 3). The survival of rhizobia on seed was increased ($P < 0.01$) when suspensions of either PVP or activated charcoal were used to treat seed prior to inoculation (Table 2).

SURVIVAL AND MULTIPLICATION OF RHIZOBIA BEFORE NODULATION

The period between sowing and the formation of a rhizosphere capable of supporting multiplication of rhizobia is critical. During this time the population of rhizobia introduced on the seed surface may be subjected to the deleterious effects of:

(1) Antibacterial compounds in the seed coat or in the soil.

(2) Unfavourable climatic conditions.

(3) Unfavourable soil physical and chemical conditions.

(4) Competitive, antagonistic and predatory activities of soil microflora (including naturally occurring rhizobia) .

Any-one-or more of these factors may affect the viability of rhizobia, resulting in very few remaining to colonize the rhizosphere when the radicle emerges.

The competitive ability of strains of rhizobia and their ability to survive in inoculants, on seed (including coated seed), and in the rhizosphere are criteria which are at present being studied.

Preliminary trials in some soils from Northland and Poverty Bay have shown that the populations of *R. trifolii* occurring naturally are less effective for nitrogen fixation but more competitive for nodule formation than the recommended strains used for the inoculation of white clover. A similar situation may exist in other areas of New Zealand and consequently it is important to know as much as possible about the competitive ability of naturally occurring populations of rhizobia (if any) in a particular area before recommending strains for inoculation.

CONCLUSIONS

Many factors affect the survival of *R. trifolii* on white clover seed before sowing and in the soil before the rhizosphere is capable of supporting multiplication of cells.

(1) The most rapid death- of rhizobia takes place during the period the applied inoculum is drying and in the immediate post-drying period.

(2) The toxic compounds associated with the seed coat also reduce the numbers of rhizobia which survive after inoculation. However, the inhibitory effects of the seed diffusates from white clover may be suppressed either by washing seed or by treating seed with phenolic adsorbents.

(3) The selection of effective strains of rhizobia on criteria such as survival, nodulation, nitrogen fixing and competitive abilities for areas where introduced strains are likely to be challenged by well-adapted but less effective strains is of the utmost importance.

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