

SOME ASPECTS OF THE NODULATION PROBLEM OCCURRING IN THE WITHER HILLS

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Introduction

FOR SOME YEARS NOW, J. P. Beggs, Farm Advisory Officer at Blenheim, has been concerned with problems of establishing legumes in the hill country about Blenheim. The natural cover on this country consists of an almost pure sward made up of *five* species of *Notodanthonia*. Because of the steep nature of the country, oversowing is the only practical method of pasture improvement, and this fails because legumes do not establish adequately. The writer has been interested in this problem for some time and the following is an interim report on some factors which appear to be involved.

Reasons for Establishment Failure

As in all establishment problems, it is necessary to distinguish between climatic and non-climatic factors. To do this, undisturbed turfs of danthonia sward were obtained and placed in the glasshouse at Rukuhia. They were then planted with red clover and lucerne inoculated with approximately 1,000 viable rhizobia per seed and the turfs kept watered to 60% water-holding capacity. Under these conditions, fewer than 1% of the legume seedlings survived. This showed that climatic factors were not the major cause of legume failure.

It had been suggested that soil nitrogen levels might be so low as to prevent establishment, but the addition of nitrogen in pot experiments has given good legume growth until the nitrogen is exhausted, after which the plants disappear. To determine the effect of living danthonia on legume establishment, a small pot experiment was set up, in which Wither-Hill soil was planted with danthonia tillers. After establishment some danthonia was allowed to die from drought, and immediately before planting with inoculated clover seed half the remaining pots had the living danthonia plants removed.

TABLE 1: EFFECT OF LIVING DANTHONIA ON LEGUME ESTABLISHMENT

	% <i>Nodulation</i>	<i>No. Rhizobia per gram Rhizosphere Soil</i>
Live danthonia present	0	0
Live danthonia removed	23	1,670
Dead danthonia	45	14,300

The results (Table 1) suggested nodulation failure due to rhizobial death as the major cause of poor establishment in these soils. In an attempt to find a rhizobial strain which would survive in the area, isolations were made from the soil surrounding some established clover plants in the field. A comparison was made between this strain, WH.1, and one of the commercial strains, NZ.6, in a small pot trial (Table 2).

TABLE 2: EFFECTS OF TWO RHIZOBIAL STRAINS ON NODULATION

<i>Strain</i>	% <i>Nodulation</i>	<i>Ave. No. Nodules per Plant</i>
NZ.6	36	1.4
WH.1	92.5	3.0

This rhizobium seems to have some advantage over those used in commercial inocula, and at present trials are in progress to check its usefulness in the field.

Reason for Death of Rhizobia

The possibility that an inhibitor or toxic material present in the soil was causing the problem was suggested some time ago by Beggs (1961, 1964). On this basis, and on the assumption that any toxic material must be water-soluble, to allow for its apparent widespread dispersion through soil, a number of soil extracts were prepared.

EXTRACTION PROCEDURE — DANTHONIA RHIZOSPHERE

One hundred grams of plant roots and adhering soil were extracted with 100 ml water for 2 hours in an end-over-end shaker. The extract was centrifuged at 30,000 g to remove soil and freeze-dried. The dried residue was redissolved in 1.5 ml water and sterilized by filtration through sintered

glass. Antibiotic activity against rhizobia was checked by a plate assay technique, and a positive result obtained.

SOIL EXTRACTION PROCEDURE

One hundred grams of soil freed from danthonia was extracted as before, but using 100 ml $N(NH_4)_2SO_4$. After centrifugation, $Ba(OH)_2$ was added to remove sulphate and any excess barium was removed as $BaCO_3$. The extract was then evaporated to dryness from ice and treated as above. This, too, gave a zone of inhibition when checked against *Rh. trifolii* by plate assay.

It has not always proved possible to extract this material, but of six attempts four have been successful.

SUSCEPTIBILITY OF TWO STRAINS OF RHIZOBIA TO TOXIN

Susceptibility of strains NZ.6 and WH.1 to this toxin should be different if it is the agent involved in the field problem, and this has been checked by a plate assay (Table 3).

TABLE 3 : SUSCEPTIBILITY OF TWO RHIZOBIAL STRAINS TO TOXIN

Toxic Extract (ml)	Diameter of Inhibition Zone (cm)							
	NZ.6				WH.1			
0.05	2.7	2.7	2.5	2.4	1.8	1.8	1.8	1.7
0.10	2.9	2.9	2.8	2.7	1.9	1.9	1.9	2.0

The heat stability of the toxic material has been measured and it was found that only a small loss in activity occurred after 25 minutes at 80°C (Table 4).

TABLE 4: HEAT STABILITY OF TOXIC MATERIAL

Time at 80°C in minutes	Zone Diameter (Ave. of 3 readings)
0	1.7
2	1.5
5	1.5
10	1.2
20	1.2
25	1.2

Origin of Toxic Material

It is possible that the toxin extracted from this soil is produced either by the danthonia itself or by an organism associated with the danthonia. Attempts to extract toxic material from ground-up danthonia roots have not been successful. Pieces of danthonia root washed exhaustively through 24 changes of sterile distilled water, each shaken for 5 minutes on a wrist-action shaker and then plated on yeast mannitol agar containing a heavy culture of rhizobia NZ.6, gave rise to a considerable number of colonies of bacteria which were antagonistic to the rhizobia. When grown in artificial culture, these organisms produce a toxin with some properties similar to those of the material extracted from soil. Work is progressing on these lines at present.

Conclusion

It seems that failure to establish legumes on Wither Hill soils may be due to a toxic material killing the rhizobia. The presence of this material, which can be extracted from soil and is fairly heat stable, appears to be associated with living danthonia. The source of the toxin may well be organisms associated with danthonia roots.

REFERENCES

- Beggs, J. P., 1961: *N.Z. J. Agric.*, 105: 325.
——— 1964: *N.Z. J. Agric.*, 108: 529.
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JOINT DISCUSSION

Why are some types of pellets, such as Gafsa and dolomite, better than others?

MR HASTINGS: Some pelleting materials are better than others because of the chemical nature of the materials. Dolomite contains 37% magnesium and 60% calcium, Gafsa phosphate approximately 27% phosphoric acid, 46% calcium, with traces of potash, magnesium, iron, sulphur, sodium, fluorine and other elements. It is possible that the rhizobia obtain benefit from some of these materials. Work by Norris in Australia has shown the importance of magnesium.

Has any work been done on different strains of lucerne rhizobia as well as with different lucerne varieties?

MR HASTINGS: I have tested many strains of lucerne rhizobia on the standard Marlborough variety, and Dr Blair at Lincoln College has done some work on strain effects on various varieties of lucerne. I believe this work is being continued.

Is your Division doing any work on the problem as related to strains of lucerne?

MR HASTINGS: Not at present.

Would it be worth while developing inoculants for particular soils?

MR HASTINGS: In general, soil type does not appear to influence the effectiveness of a rhizobium strain. For instance, one of the strains at present in use originated in Tasmania and has worked well on many soil types in New Zealand. A strain that works well in, say, a Northland gumland soil, also works well at Te Anau. For specific problems, where it has been demonstrated that the standard strains are less effective, it may be worth while to search for specific strains which could tolerate better the particular conditions in certain areas. This would possibly be best done in the problem area.

G. A. HOLMES: It seems strange that research has not been done before this on the possibility of death of rhizobia. We have found that sowings in July, August and September are better than November sowings.

Does the micro-climate become critical for rhizobia- has any research been done on this? Do high temperatures affect rhizobia?

DR PARLE: High temperatures cause rapid death of rhizobia, although some species are more resistant than others. *Rhizobium meliloti* will stand temperatures of 41°C for a considerable time, but *Rh. trifolii* usually dies at about 38°C. There will, of course, be some strain variation within a given species. Low temperatures have little or no effect on rhizobial survival.

J. G. WHITE: From observation of trial work in Australia, root development is important where lucerne is being established in acid soils.

How well do rhizobium tolerate wet conditions?

DR PARLE: Wet conditions have very little effect on rhizobial survival. Very dry conditions will cause death very slowly in soil.

Would it be obligatory to inoculate all clovers to get better rhizobia strains in soils?

DR PARLE: Yes. However, in soils with an already high population of a particular rhizobium, it would be almost impossible to effectively introduce a different strain,

What does pelleting actually do? What is its long-term effect on lucerne?

DR PARLE: Pelleting helps rhizobial survival until nodulation has taken place. Once this has occurred, I do not think that pelleting has any effect on the rhizobia.

Can anything be done after a lucerne crop has been sown when inoculation has failed?

DR PARLE: This depends on why it failed. If soil conditions are favourable for rhizobial survival! oversowing with inoculated seed might produce foci from which infection would spread to unnodulated plants.

Would grazing be of any benefit if nodulation failed? Would grazing stimulate root growth?

DR PARLE: Stimulation of root growth would assist if the appropriate rhizobia were present in infection foci.

Are nodules on different parts of the roots of lucerne plants of equal efficiency?

DR PARLE: Yes, provided they all contain effective organisms.

Because of good nitrogen responses at Te Anau, why is it considered uneconomical to use nitrogen? Have rates lower than 50 lb nitrogen been used in pasture establishment?

MR CULLEN: Despite a good initial pasture response to nitrogen, the effect is short-lived and clover growth is affected adversely. However, it is considered nitrogen fertilizers could prove economic on turnip crops in the mutual development phase. Rates as low as 10 lb nitrogen have been tried on pasture.

Can rhizobia pass through stock and remain viable?

DR PARLE: I do not know. As the pH of the lower bowel is about 1.5, many rhizobia would very likely die; some few might survive.

On the area where ryegrass did not grow well at Te Anau, was root formation checked for abnormalities? Ryegrass has been affected by fungus at Gisborne.

MR CULLEN: No detailed check was made on the roots of ryegrass for abnormalities, but cursory examination indicated that cocksfoot had a deeper rooting system.

Have the toxic effects of rhizobia on clover establishment been observed elsewhere in New Zealand?

DR PARLE: I believe that the effect may be more widespread than is realized. There is some suggestion that it occurs on Banks Peninsula and it may occur to some extent wherever danthonia is a dominant species.

Are ineffective native strains of rhizobia present in the soil, and can such populations be changed by introducing the effective strains?

MR GREENWOOD: I have no further information on the occurrence of ineffective strains in New Zealand soils than that given in my paper. It may prove difficult to establish a new legume in a situation where rhizobia ineffective on it predominate. It depends on whether the introduced effective strain can establish itself and colonize the soil in competition with the ineffective strains already present. The presence of roots of the new host should give the introduced strain some advantage, but this may not be enough. Alteration of the soil environment by liming and fertilizing may help also.