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LIGHT LANDS IN WESTERN AUSTRALIA

3. MICROBIOLOGICAL PROBLEMS IN THE ESTABLISHMENT OF LEGUMES ON LIGHT LANDS

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Experience has shown that the establishment of leguminous pastures is essential for successful economic development of Western Australian light lands. Subterranean clover is the preferred legume in most light land areas, with lupins holding an important place in the more northerly districts, and on the deeper sands.

A serious disease of subterranean clover which occurred on many of these newly cleared soils jeopardised the successful establishment of clover pasture. It was often known as "seedling mortality," and it was prevalent from Esperance in the south to Enneabba in the north.

The disease was characterised by reddening and purpling of the cotyledons, petioles and leaves of the young seedlings, which were commonly stunted and usually bore no root nodules.

Although the disease was most serious on the poorest sandy soils, investigations by the Department of Agriculture showed that it was not prevented by the addition to the soil of nutrient elements, nor was it overcome by inoculation.

The affected soils were usually only slightly acid in reaction, so that soil acidity did not appear to be the cause of the disease.

Importance of Land Preparation

Further work by officers of the Department of Agriculture, in particular Dr. T. C. Dunne and Mr. F. L. Shier, showed that ageing of the soils by means of a long bare fallow (or by cereal cropping), after careful clearing of the virgin scrub, greatly improved clover establishment.

Opinion around 1953 was that toxic factors, derived either from the original vegetation or from soil micro-organisms, were present in newly cleared soils and were destroyed during the ageing process.

Because of the consistent lack of response to inoculation it was widely thought that the problem was essentially a non-microbiological one, especially as clover could by then be established on the better sands with little difficulty.

From 1953 to 1956 few microbiological experiments were carried out, and it was widely accepted that if proper cultural practices were adhered to no serious nodulation problems were encountered.

Large areas of clover pasture were successfully established throughout the more southerly parts of the agricultural region. This was largely due to Department of Agriculture research at the Esperance Downs Research Station and elsewhere.

Esperance Experiments

However, in 1956, serious losses of clover seedlings were still to be seen in newly sown paddocks in the Esperance district, sometimes even where the land had been carefully cleared and fallowed. The losses were worst on the poorest soils, and varied from 100 per cent. mortality in one paddock to about 20 per cent. on some of the better gravelly soils. On two to three-year-old "pastures" which had established badly in the first year, there was a tendency for the clover to grow in clumps, which contained vigorous plants in the centres and reddened stunted plants on the edges. This was considered to be due to a favourable change in the microbial
population under the established clover, a view which was strengthened by the report (Hely, Bergersen and Brockwell, 1956) that microbial competition around the roots of subterranean clover plants had caused nodulation failure at Yarrowyck in New South Wales.

Experimental plots were laid down on two sites at Esperance in 1957, to re-examine the value of seed inoculation. The clover seeds were heavily inoculated with nodule bacteria isolated from an Esperance soil the previous year.

At the first site, about 40 miles east of Esperance and many miles from the nearest established pasture, there was a dramatic response to inoculation. The sterilised un-inoculated seeds produced seedlings which were completely unnodulated, whereas inoculation gave 53 per cent. nodulation.

Nodulation was further increased to 87 per cent. when the bacteria was suspended in a 2 per cent. sucrose (table sugar) solution instead of in water.

The second experiment was on deep sand near Gibson's Soak, north-west of Esperance, not far from pastures of two to three years standing. Here there was 44 per cent. nodulation from nodule bacteria already present in the soil, but inoculation increased this to 91 per cent. Some colonisation of the soil by nodule bacteria had obviously occurred in this older part of the Esperance district.

The results from these two experiments at Esperance proved that “seedling mortality” was a microbiological problem, and suggested that it involved some difficulty which was not understood, in the establishment of the nodule bacteria on the roots of the clover seedlings.

Soil Sterilisation

In the following year, Cass Smith and Holland (1958) obtained a spectacular response to chemical sterilisation of a virgin soil at Wongan Hills. The subterranean clover seedlings on the untreated soil showed all the usual symptoms associated with nodulation failure, whereas the seedlings on the sterilised soil were well nodulated, green and vigorous.

This result provided the first direct evidence of microbial intervention in the nodulation of clover, and further defined the problem as one which involved “antagonism” by the existing soil microflora.

Bacterial Cultures

A complicating factor in the assessment of field experiments over this period was the change taking place in bacterial cultures. It is fairly certain that the rapid death of the bacteria once inoculated on to the seed limited the practical value of the agar cultures used until 1953 (Vincent, 1958; Parker, unpublished.)

The use of neutralised peat as a bacterial carrier greatly improved the survival of nodule bacteria on seeds (Vincent, 1958), but it was not until 1958 that the manufacture of commercial peat cultures was fully controlled and the cultures could be relied upon (Vincent, 1958a). Neither farmers nor scientists were likely to obtain much benefit in the field from inoculation with clover bacteria up to about 1954, while from 1954 to 1958 their results would have been inconsistent because of the variation within the commercial peat cultures.

Methods of Inoculation

These changes caused confusion about the process of inoculation, and much of the improvement credited to various methods of inoculation was more likely to have been due to the improvement in the inoculum.

Nevertheless, improvements were being made in the methods of inoculation. Lime pelleting improved nodulation on acid soils (Loneragan and others, 1955) or where the inoculated seed was in contact with acid superphosphate (Cass Smith and Goss, 1958) whilst Bergersen, Brockwell and Thompson (1958) showed that pelleting clover seed with a mixture of blood and bentonite could increase the numbers of nodule bacteria around the roots of the young seedlings, and give greatly increased nodulation. Incorporation of gum arabic and sucrose into the suspending fluid has increased the survival and longevity of the inoculated bacteria on the seed (Vincent and others, 1962; Parker, unpublished) so that a delay in germination does not mean the death of all the rhizobia before they can colonise the young roots.
The combination of improved cultures and methods of inoculation has meant that since 1958 it has been possible to establish clover on a wide range of soils. Despite the advances made in practical methods, no clear idea of the nature of the original problem had emerged.

**CURRENT RESEARCH**

A study of the microbiology of newly cleared soils at Wongan Hills was begun by Holland in 1958, and is still in progress. His findings are—

1. A large number of fungal species (mostly *Penicillium*) are present in the newly cleared soils, nourished by the decaying organic debris remaining after removal of the original scrub.

2. Some of these fungi could inhibit nodulation and growth of clover seedlings, and produce the same plant symptoms in the laboratory as were observed in the field.

3. If the soil is sterilised, nodulation is normal and plant growth is healthy.

4. The initial population tends to change slowly, so that four to five years after clearing subterranean clover can be successfully established.

5. Where clover has become well established a new soil microflora is evident which is compatible with subterranean clover plants and nodule bacteria. The species composition of this population is very similar over a wide range of soils.

Successful establishment of first-year clover on former problem soils has now brought a further problem into prominence—that of nodulation failure in the second and later years after sowing the inoculated seed. This appears to be more common in the warmer and drier regions of the State and on the deeper sands. On present knowledge little can be done to preserve such clover pastures, and more information is urgently needed on the microbiology of these soils.

The microbial sequence shown at Wongan Hills could still apply to many newly cleared soils, but there are other soils where nodulation failure occurs 10 or more years after clearing. The symptoms observed in the field are similar to those on newly cleared soils and, furthermore, when clover seedlings on these soils do become nodulated they make normal growth. We therefore believe that this too is a microbiological problem, and further work has been initiated in the Midlands area.

Soils have been partially sterilised by aerated steam (Baker, 1962), and also by chemical sterilisation, to see if differences in the nodulation of subterranean clover will occur. It is planned to investigate the ability of several different kinds of nodule bacteria to colonise these soils, and further work on the question of competition and antagonism from existing soil microbes will be undertaken.

There is evidence that the clover nodule bacteria are dying out of some soils in the Midlands. This is not likely to be due to high summer temperatures, as Sanderson (1962) has shown that clover bacteria in dry soil are able to withstand far higher temperatures than ever occur in the field. Again, we suspect that their lack of survival is due either to antagonism or competition from other soil micro-organisms.

**Lupins**

An interesting field observation is that lupins may thrive and spread on soils where clover dies out. Work reported by Lange (1961) and by Lange and Parker (1961) on the roles of the nodule bacteria of our native legumes in the establishment of lupins, and the investigation by Graham (unpublished) of the relationships and characteristics of the various groups of nodule bacteria, suggest a fairly logical explanation of this. The lupin-serradella type bacteria are closely related to the native bacteria found in our wild legumes, and also there is a very pronounced difference between the clover bacteria and the others. The lupins can successfully colonise soils where clover fails because the lupin bacteria can colonise these soils.

The reasons for this difference are not known, but Graham (1962) has shown that the lupin bacteria are less sensitive to antibiotics than the clover bacteria and have very different nutritional requirements—observations which may have some bearing on the subject.
The basic knowledge that we have gained about the clover and lupin bacteria may provide us with a solution. Should it prove impossible to ameliorate the problem soils so that clover can be grown successfully, it may well be possible to find agronomically suitable legumes which have the lupin-type bacteria in their nodules.

Serradella (*Ornithopus sativus*) is just such a legume, and it is already showing promise in this State. Many other legumes with similar bacteria warrant investigation as pasture plants. Some likely genera are: *Onobrychis; Astragalus; Coronilla; Psoralea; Scorpiurus; Securigera; Hedysarium; Ornithopus; Lotus; Hosackia; Cytisus*.

The answer to the question of how to grow legumes on problem soils may thus have to be found by the agronomists, although microbiological considerations may dictate the kinds of legumes which can be grown.

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