POPULATIONS OF RHIZOBIA IN NEW ZEALAND SOILS

R. M. GREENWOOD

Senior Scientific Officer, Plant Chemistry Division, D.S.I.R., Palmerston North

ALTHOUGH LEGUMES are of prime importance in New Zea-, land pastures, the number of species involved is small, and is restricted almost entirely to the genera ***Trifolium*** (the clovers), *Medicago* (lucerne and other medics), and ***Lotus.*** These three genera form nodules with three separate groups of rhizobia (root nodule bacteria) with no effective cross-nodulation between them. Also, within any one of these groups of rhizobia, there are numerous strains. These may differ in a number of respects, but in particular they differ in the range of species with which they are effective in fixing nitrogen. Thus, among clover rhizobia, one strain may be effective on white ***(Trifolium*** *repens* L.) and red clovers ***(T. pratense*** L.) but not on subterranean clover ***(T. subterraneum*** L.), a second strain effective on subterranean, weakly effective on red and ineffective on white clover, and a third strain effective on all three species.

All the species of ***Trifolium,*** *Medicago* and ***Lotus*** now in New Zealand have been introduced from overseas, and they are not closely related to any native leguminous plants. Thus it is very probable that their associated rhizobia are also not native, but were originally introduced by chance during the early days of settlement of the country. Since then they have been spread widely by a number of agencies — for example, as contaminants on seed, the feet of animals, agricultural implements and in dust, flood waters, and agricultural lime. They are now widespread in New Zealand soils, sometimes beyond the range of their host plants, but still are absent from many areas.

The three groups of rhizobia have soil acidity requirements similar to those of their host plants. Thus lotus rhizobia are very tolerant of acid soils, clover rhizobia somewhat less so, and lucerne rhizobia intolerant. Clover rhizobia are, however, by far the most widespread, owing, no doubt, to the much greater range and abundance of their host plants.

To illustrate some characteristics of the populations of rhizobia that have become established in fertile pasture soils, in New Zealand, some results at the Grasslands Division Station, Palmerston North, will be considered. White

clover and red clover have been grown extensively at the station for years, suckling clover (*T. dubium* Sibth.) is present as a volunteer, strawberry clover (*T. fragiferum* L.) has been grown to a small extent, but other species, such as subterranean clover are not commonly grown. Over recent years lotus (*Lotus pendunculatus* Cav., syn. *Lotus major*) has been grown extensively during the current breeding programme on the species. Lucerne (*Medicago sativa* L.) has not been grown to any extent for many years.

Clover rhizobia (counted by a method similar to that described by Brockwell, 1963) are plentiful in the soil throughout the station. Numbers of a few thousand per gram of soil may be found in areas where clover has not been grown for some years, rising to well over a million per gram close to the roots of clover plants. Lotus rhizobia are also present throughout the station, including areas in which lotus plants have never grown. Numbers vary markedly from under one hundred per gram in some samples to many millions per gram in the vicinity of lotus roots. Lucerne rhizobia cannot be detected in the soil, but they are able to multiply in the presence of lucerne roots provided the soil is not acid.

Numerous different strains of clover rhizobia are present in the soil. Most isolates are effective in fixing nitrogen with white and red clovers, many effective with suckling clover and strawberry clover, but with subterranean clover, and other annual species characteristic of dry climates, few isolates are effective. Most isolates cause the formation on these species of small white nodules fixing little or no nitrogen. This agrees with observations on these plants grown at the station. Their roots are found to bear predominantly small white nodules with only occasional large pink effective nodules.

Recently some observations were made on the nodulation of a large collection of *Trifolium* species from overseas grown on the station for the first time. Of 45 species not naturalized in New Zealand, only 4 bore predominantly effective nodules. Most species were similar to subterranean clover in having mostly ineffective nodules but with occasional effective nodules present, but with 10 species, mainly from Kenya, none of the nodules were effective. Should any of these latter species prove worthy of further trial, inoculation with rhizobia obtained from their country of origin would be essential.

Nearly all isolates of lotus rhizobia obtained from *Lotus pedunculatus* nodules have been found effective on *L. pedunculatus*, *L. hispidus* and *L. corniculatus*. However, strains are present which are effective on *L. corniculatus* but ineffective on *L. pedunculatus* and *L. hispidus*. The presence of these latter strains in the soil at Grasslands Division is unexpected, since *L. pedunculatus* is by far the predominant species present, both now and in the past.

Populations of clover rhizobia at least as high as already quoted occur in other pasture soils, even those recently developed from the virgin state — e.g., in pumice lands and Northland gumland. In drier areas where annual clovers are more plentiful or are dominant; a larger proportion of strains is effective or partially effective on subterranean clover and other annual clovers, and ineffective or weakly effective on white and red clovers. In unimproved hill country pastures, clover rhizobia tend to have a very patchy distribution, and may be undetectable under sod-bound browntop for instance. but nlentiful under sheen tracks or sheep camps nearby, and it 'is possible for clover seedlings from oversown uninoculated seed to die from lack of nodulation in the former situations. In South Island tussock country, clover rhizobia, not necessarily very effective strains, are present in small numbers over wide areas where' clover plants cannot be found. Oversowing uninoculated seed may be quite successful, especially if lime is' applied, as this stimulates the multiplication of those rhizobia' already in the soil.

Lotus rhizobia are plentiful wherever lotus is growing, but they are absent or very sparse over large areas where clover rhizobia may be plentiful. It is thus wise to inoculate lotus seed when sowing new areas, especially 'with lotus seed at its present price. Ineffective strains of lotus rhizobia do occur, but do not appear to be very plentiful or important.

Lucerne rhizobia may be quite plentiful in some neutral soils -e.g., over 10,000 per gram have been found under lucerne in the Hakataramea Valley, and under black medic *(Medicago lupulina* L.) in a coastal sand north of Foxton. They will also be present in limestone soils under black medic, and in neutral soils in dry climates under various' medic species. However, over the greater part of New Zealand, lucerne rhizobia are not free-living in the soil, hence the standard procedure of always inoculating lucerne seed. In acid soils lucerne plants will not thrive, but in slightly acid soils it is possible to get a good stand of lucerne

satisfactorily nodulated and yet have very few lucerne rhizobia free in the soil. The lucerne rhizobia survive in and around the nodules and roots of the lucerne plants, and can apparently do so for years. It is thus most important that the initial inoculation of the seed should be done carefully with a good culture. Also, if the stand is ploughed up, and lucerne sown again, this seed too should be inoculated. Rhizobia ineffective on lucerne do occur in New Zealand soils, but as it is recommended that all lucerne seed should be inoculated before sowing, these ineffective rhizobia should not cause difficulties. Some varieties of lucerne require special strains for best effectiveness (Blair and Bennett, 1960), but the commercial inoculants are suitable for all varieties commonly grown in this country.

Various clovers and black medic are often plentiful on limestone soils, and these plants would be the original source of rhizobia that are to be found in samples of agricultural lime. Numbers of rhizobia vary markedly in different samples of lime, even from the same limeworks. They may well be of significance as a source of rhizobia when acid soils are limed. If rhizobia are added to lime, they are found to die out of some samples fairly rapidly, but to survive and even multiply to some extent in other samples. It is of interest that lucerne rhizobia seem to be particularly at home in this environment.

When pasture legumes are sown on land in which they have not grown previously it is most important to ensure that the seedlings become effectively nodulated. In some places this presents no problem, and good nodulation may be obtained readily, even at times from uninoculated seed. Elsewhere it may be very difficult to get most of the plants effectively nodulated. These differences depend on whether the appropriate rhizobia are already present, or, if not, the ease or otherwise of establishing them in the soil. A few examples will illustrate some of the differences that may be obtained.

GUMLAND AT KAIKOHE

This strongly podzolized soil is extremely infertile in its virgin state — very acid, low in plant nutrients, and of poor structure. Lotus and clover rhizobia cannot be detected in the soil before development, but after cultivation lotus rhizobia are able to establish to some extent, and after cultivation and liming both lotus and clover rhiiobia can establish readily and multiply (Greenwood, 1961).

The changes in population of these rhizobia were followed during the development of about 20 acres of this soil to pasture. The initial giant discing did not lead to the introduction of rhizobia, and during the following period of fallow they were found only along tractor tracks made across the area. Further cultivations, with implements brought each day along a roadway past developed pasture, spread small numbers of rhizobia, mainly lotus, over the area. The final sampling taken after liming and just before sowing showed increased numbers of both clover and lotus rhizobia. The numbers varied in different parts of the area, with some samples containing no lotus or no clover rhizobia. However, it so happens that the highest numbers of clover rhizobia (about 100 per gram of soil) were found in the block set aside for sowing uninoculated seed. The lime used contained clover and lotus rhizobia, and apparently the numbers varied considerably in different loads. It was probably an important source of these organisms. Most of the area was sown with inoculated seed, and tests showed that probably about half the nodules were formed with rhizobia from the inoculum. However, seedlings in the uninoculated area nodulated satisfactorily also.

Although in this case a satisfactory pasture might have been obtained if uninoculated seed had been sown, this could not have been guaranteed beforehand, and in a larger-scale sowing on a similar soil not far away much death of clover had occurred because of nodulation failure. Also a high proportion of the rhizobia naturally coming in was ineffective on subterranean clover; and this species, though not important in this case, is a valuable constituent of pastures in drier areas and lighter soils in Northland.

PAKIHI SOILS OF THE SOUTH ISLAND WEST COAST

These soils are similar to the gumland soils. In the virgin state they do not contain clover or lotus rhizobia, but, after liming, clover and lotus rhizobia can establish and multiply readily, and lotus rhizobia can survive in the unlimed soil for long periods. Thus, where small areas are developed adjacent to established pastures, sufficient rhizobia may be carried in to cause nodulation of seedlings from uninoculated seed. It would, however, be unwise to rely on this, and particularly in larger areas the seed should be inoculated. Another factor to consider is that the strains of rhizobia that are carried in may not be of good type. Isolates ineffective on both white and subterranean clover have been obtained in a small trial near Westport.

GLACIAL MORAINE SOILS, TE ANAU

These yellow-brown loams have a high initial phosphate requirement, but are not markedly acid. Nevertheless, lime is important for the survival of rhizobia. In one area clover and lotus rhizobia added to the cultivated soil died out over the summer where lime was not applied, but survived in the presence of lime. Even where lime was applied, there was no evidence of appreciable multiplication or spread of rhizobia in the soil. Inoculation at a high rate was needed to get satisfactory nodulation of sown seedlings. In one unsuccessful sowing where only scattered nodulated clover plants survived, no rhizobia could be detected in the spaces between the plants six months after sowing; and where white clover stems had rooted down, these roots some distance from the primary roots bore no nodules when examined fifteen months after sowing. In another area, investigated by During *et al.* (1963), a small population of clover rhizobia was present in the soil, and definite multiplication occurred where lime was applied, The reason for this difference is not clear, but may be due to the somewhat higher rainfall and consequent leaching, or to the fact that the latter area was not cultivated and the original black peaty topsoil was still in place.

These yellow-brown loams have some similarities to the pumice soils investigated some years ago (Sears *et al.*, 1955). Small resident populations of clover rhizobia occurred in places in the virgin pumice soil and some plants from uninoculated seed formed nodules mainly when rooting into pockets of the black topsoil. However, in order to obtain good nodulation of clover seedlings, high rates of inoculation were found to be necessary.

This initial resistance to the spread of rhizobia appears to be some form of microbial antagonism. Thus, if samples of the limed soil are sterilized by steam and then inoculated with rhizobia, rapid multiplication to very high numbers occurs; yet this is largely prevented by reinoculating with a little unsterilized soil before adding the rhizobia. In pumice soils this resistance to the spread of rhizobia is not permanent. As the amount of clover root tissue increases in the soil, the number of clover rhizobia increases, and at the stage of clover dominance high populations of clover rhizobia are present. The indications are that the same will occur, though more slowly, at Te Anau.

REFERENCES

Blair, I. D.; Bennett, Ann, 1960: *Rhizobium* inoculation of lucerne (***Medicago sativa*** L.). N.Z. ***J. agric. Res., 3:*** 804-19.

Brockwell, J., 1963 : Accuracy of a plant-infection technique for counting populations of ***Rhizobium trifolii.*** *Appl. Microbiol.*, ***11: 377-83.***

During, C. ; Cullen, N. A. ; Mountier, N.S., 1963 : The establishment of pastures on yellow-brown loams near Te Anau. 11. Factors influencing the establishment of white clover (*Trifolium repens* L.) on uncultivated ground. N.Z. ***J. agric. Res., 6:*** 416-31.

Greenwood, R. M., 1961: Pasture establishment on a podzolised soil in, Northland. III. Studies on rhizobial populations and the effects of inoculation. N.Z. ***J. agric. Res., 4: 375-89.***

Sears, P. D.; Hyde, E. 0. C.; Greenwood, R. M., 1955: Pasture establishment on pumice soils. N.Z. J. ***Sci. Tech., A37:*** 110-40.

RELEVANT REVIEWS

Vincent, J. M., 1954: The root-nodule bacteria of pasture legumes. *Proc. **Linn.** Soc.* ***N.S.W.,*** 79: iv-xxxii.

——— 1962: Australian studies of the root-nodule bacteria. A review. *Proc. **Linn.** Soc.* ***N.S.W.,*** *87* : ***8-38.***