SHORT COMMUNICATION

Symbionts of Alder Nodules in New Zealand

Introduction

Interest in nodules and their endophytes on the roots of non-leguminous plants dates back at least as far as interest in those of legumes. However, whereas the isolation and identification of the legume nodule endophyte has been recorded for many years, isolation of non-legume nodule endophytes has never led to unequivocal identification since no one has been able to repeatedly form effective nodules with such isolates. Work on non-legume endophytes has been extensively reviewed [1 4 5 12 13 14] in recent years. Actinomycetes are almost certainly the causative organisms and since cross inoculation successes have been confined to intrageneric ones, except in the Elaeagnaceae, there are probably different groups of nodule organisms that invade roots of non-legumes as in the case of the legumes. Recent work points to differences even within genera in that nodules formed by intrageneric cross inoculation may be physiologically ineffective [6 8 11]. This further endorses the view that endophyte strain differences occur in non-legumes as in legumes.

In New Zealand the green alder, *Alnus viridis*, has shown considerable success in revegetation trials on eroded mountain slopes. New Zealand flora has no indigenous alders and only two nodulated non-legume genera, namely *Discaria* and *Coriaria* [7 9]. It was decided to carry out some preliminary investigations to discover how widespread the alder nodule endophyte is in New Zealand soils away from its host (*A. viridis*), and whether different alders acquire the same endophyte.

Materials and methods

Unnodulated seedlings of green alder were produced by sowing surface-sterilised seed (10 minutes in 0.1% Hg Cl_2) on autoclaved sand in the spring (August, 1967). Seedlings were given several applications of a complete-nutrient solution (including 25 ppm N) to ensure that they were healthy and vigorous prior to the inoculation.

In October, potted seedlings were used to test whether nodulation could be achieved by applying inocula of crushed-nodule suspensions produced from nodules of *Alnus viridis*, *A. glutinosa*, *A. nepalensis* and *A. sinuata*, or by using a layer of soil from a number of sources. Two seedlings were planted into autoclaved sand in each sterilised pot. About $\frac{1}{4}''$ of the appropriate inoculant

soil was incorporated in the sand ¾" below the surface. Pots with control plants and plants that received nodule suspensions had ¼" of autoclaved nursery soil incorporated in the sand.

Nodules were excised from plants of the alder species listed above, all growing in a lowland tree nursery. Nodules were washed clean, rinsed quickly in 98% ethanol followed by 10 minutes in 0.1% Hg Cl_2 solution, with periodic shaking. After 10 rinses in distilled water, nodules were crushed into a fine suspension, and 25 ml of the suspension were applied to each pot.

Each treatment was represented by 12 pots (24 plants), half of which were lifted and assessed for presence of nodules 4 weeks after inoculation (November, 1967), and the remaining half, 7 weeks after inoculation (December 1967).

Results and discussion

TABLE 1

Nodulation of *Alnus viridis* seedlings. 1st assessment 4 weeks and 2nd assessment 7 weeks after inoculation with surface-sterilised nodule suspensions (Treatments 1–4) and soils (Treatments 5–14)

Treatment	1st assessment			2nd assessment		
	No. of plants harvested Nov. 1967	No. plants with nodules	Mean No. nodules per nodulated plant	No. of plants harvested Dec. 1967	No. plants with nodules	Mean No. nodules per nodulated plant
1. *A. viridis* nodule suspension	11	5	1.2	12	11	5.6
2. *A. glutinosa* nodule suspension	12	0	—	12	1	1.0
3. *A. nepalensis* nodule suspension	12	0	—	12	1	1.0
4. *A. sinuata* nodule suspension	12	0	—	12	3	1.0
5. Rangiora nursery soil, 120 ft	12	12	2.3	12	12	6.5
6. Sterilised Rangiora nursery soil	12	0	—	12	0	—
7. Forest topsoil, 2500 ft	12	1	1.0	12	1	1.0
8. Grassland soil, 2500 ft	12	12	6.7	12	12	6.3
9. Grassland soil, 4200 ft	12	10	1.6	12	12	2.8
10. Grassland soil, 5800 ft	12	11	2.2	12	11	3.6
11. Subsoil, 3000 ft	12	8	1.6	12	7	1.9
12. Subsoil from *A. viridis*, 3000 ft	12	2	2.0	12	5	1.2
13. Scree, 4200 ft	12	0	—	12	0	—
14. Scree from *A. viridis*, 4200 ft	12	0	—	12	1	1.0

Note: Treatment 5. Nursery soil known to induce nodulation of alders. 6. Sterilised soil control. 7. Beech forest *Nothofagus solandri* var. *cliff.*) topsoil. 8. Hard tussock (*Festuca novae-zelandiae*) grassland topsoil. 9. Snow tussock (*Chionochloa pallens*) grassland topsoil. 10. Snow tussock (*Ch. pallens, Poa colensoi, Celmisia viscosa*) topsoil. 11. Exposed subsoil in hard tussock grassland area. 12. Exposed subsoil in grassland close to planted green alder. 13. Material from a bare scree. 14. Material from a bare scree close to planted green alder.

The distinct difficulty in obtaining nodule suspensions, other than those from *A. viridis* nodules, to yield nodules on unnodulated *A. viridis* seedlings suggests that the endophytes in nodules of *A. nepalensis*, *A. glutinosa* and *A. sinuata* are different from the endophyte in nodules of *A. viridis*. Because

pure-culture methods cannot be employed, the occasional lone nodule produced by nodule suspensions from species other than *A. viridis* must be viewed as probable contaminants in the surface-sterilised donor nodules. Such lone nodules cannot be considered evidence that endophytes from *A. nepalensis*, *A. sinuata* and *A. glutinosa* have induced nodule formation on *A. viridis* seedlings.

Spontaneous contamination during the course of the experiment seems highly unlikely since sterile-soil controls remained totally free of nodules. The possibility that autoclaving of the soil produced substances inhibitory to nodulation, thereby invalidating the controls, must also be discarded since seedlings inoculated with *A. viridis* nodule suspension were also planted in autoclaved soil and sand.

The use of Rangiora nursery soil, a heavy silt loam, resulted in rapid nodulation. This was to be expected since no difficulty had ever been experienced in eventually obtaining nodulation of *A. viridis*, *A. nepalensis*, *A. glutinosa*, *A. incana*, *A. sinuata*, *A. crispa* and *A. tenuifolia* when these were raised from seed in the nursery, which is situated several miles from the nearest alder (*A. glutinosa*). All provenances of *A. crispa* and one high-altitude provenance of *A. tenuifolia*, however, nodulated more slowly than the rest under similar conditions. Stewart [13] remarked that the endophyte is not carried on the seed coat and this was also the writer's experience since unnodulated seedlings were readily produced with seed which was not surface sterilised. Therefore, the endophytes, of *A. viridis* and *A. glutinosa* at least, must be very common organisms in the local nursery soil because nodulation in large beds is neither slow nor sporadic. Among thousands of young seedlings no unnodulated ones have ever been found and other trials have shown nodules on these plants effectively fix nitrogen.

Nodulation obtained from tussock grassland top soils was impressively rapid. The grassland at 2500 ft included introduced species such as browntop (*Agrostis tenuis*), sweet vernal (*Anthoxanthum odoratum*) and cat's ear (*Hypochaeris radicata*), so the site cannot be considered as truly indigenous. Soil taken from grassland at 4200 ft and 5800 ft, however, came from sites occupied solely by native plants. It is remarkable that an old isolated pedestal topsoil at 5800 ft should have rapidly produced numerous nodules, since the chances of the natural alder endophytes occurring there, or at 4200 ft, are remote. One can only conclude that native soil micro-organisms, perhaps actinomycetes, which form effective nodules on *Alnus viridis* and other alders are widely distributed in New Zealand's native grassland soils. Also, there are probably a number of different organisms suited for symbiosis with different alder species. Whether such endophytes are as effective in nitrogen fixation as organisms which have evolved with alders in their native habitat is something that needs to be investigated.

The formation of nodules was considerably less effective in subsoils than in grassland topsoils. The subsoils used were from small areas within hard-tussock grassland (*Festuca novae-zelandiae*). The low fertility of subsoils must influence the numbers of endophytes present in the free-living stage and this

probably accounts for the decreased nodulation in these soils. However, small quantities of new grassland topsoil regularly become incorporated into the subsoils tested. Rock screes appear to form such a poor medium for the free-living endophytes that nodulation cannot be expected, even in scree material close to alder roots. The extreme infertility, particularly the lack of organic carbon, means this is not surprising. Beech forest topsoil seems to have a microbial population that does not regularly include nodule-forming organisms.

It is also interesting to note that though nodulation of *A. viridis* undoubtedly takes place most readily in spring and early summer, nodulation was also achieved in a similar trial in the autumn. In fact, no difficulty has ever occurred in obtaining nodulated green-alder seedlings at any time during the growing season. The autumn trial also resulted in zero nodulation of *A. viridis* seedlings when nodules of *A. nepalensis* and *A. sinuata* were used as inoculant. In addition, nodules of the native *Coriaria sarmentosa* were found to be ineffective for the nodulation of *A. viridis*.

Roberg [10] tested the ability of seven different soils to nodulate *Alnus glutinosa, Elaeagnus angustifolia* and *Hippophae rhamnoides*, including soils away from the natural habitat of these species. He was able to conclude that the nodule endophytes are well distributed in soils and are able to live and multiply outside the nodule. It would now seem unwise to ignore results such as Roberg's on the grounds of dubious experimental methods. In fact, Becking's view [3] that the alder endophyte may be an obligate symbiont cannot be true in the light of the experiments described here and, as for Coriaria [2], the endophyte must have a free-living stage in the soil which is extraordinarily ubiquitous in distribution. From the practical point of view it can be expected that organisms native to New Zealand will ensure no real difficulty in obtaining effective nodulation of *Alnus viridis*, and most probably other species of alders, on New Zealand's mountain slopes except where very infertile sites extend over large areas. Trials are now under way to compare nitrogen fixing effectiveness of *A. viridis* nodules produced by native habitat (Austria) organisms with those produced by New Zealand soil organisms.

Summary

Micro-organisms capable of forming effective nodules on roots of *Alnus viridis* have been found to be present in New Zealand soils. It is concluded that endemic soil organisms suitable for nodulation of *Alnus viridis* occur, and cross inoculation trials have indicated that there are probably differences between organisms forming nodules with various species of alders.

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U. BENECKE *
Lincoln College, Canterbury, New Zealand

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* Present Address: Forest & Range Experiment Station, Rangoira, New Zealand.