

OVERWINTERING OF CLOVER NODULES IN ALPINE CONDITIONS*

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As part of a programme investigating soil conservation in the Snowy Mountains area of New South Wales, trials were conducted in which seedlings of *Trifolium ambiguum* M. Bieb. and *T. repens* L. (Ladino clover) were planted in the spring of 1960 in eroded areas on Carruthers Peak adjacent to Mt. Kosciusko (lat. 36° 24'S., long. 148° 18'E.) at an elevation of about 7000 ft. Seedlings were raised in seed boxes of soil which had been massively inoculated with appropriate strains of nodule bacteria. Effectively nodulated seedlings were transplanted into peat cups filled with soil. This step was necessary because of the extreme host variation with respect to nodule formation and effectiveness in *T. ambiguum* (Hely 1957, 1963). The cups were planted intact together with a complete fertilizer mixture; at that stage all plants were well nodulated and the soil in the cups contained large numbers of nodule bacteria.

In the spring of 1961 plants were lifted at intervals commencing on November 22, 12 days after the snow had melted. The first samples of plants clearly showed that growth had commenced early, possibly under the snow, although for the period of the snow-melt soil temperatures would have been close to 0°C for the whole of the soil profile (Costin, unpublished data). It was also noted that large numbers of nodules from the previous season remained on the roots in a shrivelled condition. Many of these nodules showed signs of regrowth in the form of small white cortical outgrowths at the tips. Plants lifted 1 week later bore old nodules with quite large (1–2 mm) pink tips attached to the shrivelled remnants of the previous season's tissue. Samples of these nodules were detached, fixed in Flemming's solution, dehydrated, and embedded in wax. Microtome sections were then prepared and stained with Heidenhain's haematoxylin and eosin.

The quality of the sections was not good owing to partial drying of the roots during transport to Canberra, but they clearly showed active nodule tissue with active meristems at the tips while the basal tissue consisted of collapsed degenerate cells. However, the vascular elements which traversed the withered basal tissue were healthy, indicating that they remained intact throughout the winter, thus preserving connection with the vascular system of the roots (Plate 1, Figs. 1 and 2). Several Ladino clover nodules showed distinct zones in the central bacterial tissue (Fig. 1) which suggested that regrowth had commenced while the plants were covered with snow, that it was checked during the snow-melt when soil temperatures were close to 0°C, and resumed when soil temperatures again rose.

Plants lifted at the third harvest (21 days) bore copious new roots upon which new nodules were found. Samples of these were fixed and sectioned as before (Plate 1, Figs. 3 and 4) and the nodules were seen to be typical of the species, the Ladino

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Figs. 1 and 2.—Section of a regenerating Ladino clover nodule (Fig. 1) and of a regenerating *T. ambiguum* nodule (Fig. 2) showing withered base (*b*), intact vascular element (*v*), meristem (*m*), and double zone of bacterial tissue (*b.t.*). $\times 25$.

Figs. 3 and 4.—Section of a new Ladino clover nodule (Fig. 3) and of a new *T. ambiguum* nodule (Fig. 4), both from the third harvest. $\times 25$. Labelling as in Figures 1 and 2.

clover nodules maturing earlier than those of *T. ambiguum*. At subsequent samplings the old nodules were seen to disappear and by the end of December were completely replaced by the new nodules on new roots.

T. ambiguum was dormant and had lost all foliage before the first snow fell in the preceding winter, and yet under these conditions the nodules persisted on the roots through the winter period. This plant occurs naturally in elevated areas of the Caucasus region and this mechanism of nodule persistence could be considered to have survival value in the natural habitat of *T. ambiguum*. However, Ladino white clover, which is generally regarded as a short-day, Mediterranean-type clover, was still growing when the first snow fell. On both these clovers a similar overwintering of nodules occurred suggesting that this may be a more common phenomenon than previously thought.

These observations have revealed a mechanism by means of which these two clovers were able to make better use of the relatively short growing season of the Alps. The survival of the vascular system of the nodule appeared to enable tissue capable of nitrogen fixation to be formed at least 2 weeks before any new roots which could be infected and produce new nodules were developed.

Perennial nodules are known to occur on certain tropical woody legumes, on lupin, gorse, and broom (Harris, Allen, and Allen 1949; Pate 1961), and overwintering nodules on winter annual, biennial, and perennial legumes in Northern Ireland have been described by Pate (1958). These latter types, however, differ in several respects from those described herein.

References

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