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Investigations into Lotus species

by

Mary Elizabeth Wedderburn

A thesis submitted to the University of Glasgow

for the degree of

Doctor of Philosophy in the Faculty of Science

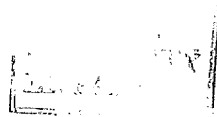
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2. ABSTRACT

The work described here was carried out as part of an extensive study by the Botany Department of the West of Scotland Agricultural College into the suitability of legumes, especially members of the genus Lotus, for the large scale improvement of low grade pasture. This study has involved lowland (44.7 metres a.s.l.) and hill land (206 metres a.s.l.) areas of south-west Scotland. Previous experimental work had shown that Lotus uliginosus Schkur cv. Maku (syn. L. pedunculatus Cav.), a cultivar of marsh birdsfoot trefoil bred in New Zealand, had promise for hill land areas and it was this cultivar which was used for all Lotus studies in this thesis.

The main aims of this study were to investigate the conditions required for the improved establishment, growth and continued persistence of L. uliginosus cv. Maku under hill pasture conditions. To this end experiments were carried out in controlled environmental chambers, a glasshouse and in lowland and upland field conditions. Where possible, comparisons were made with Trifolium repens cvs. S184 or Huia. It was anticipated that the data accumulated could lead to the formulation of guidelines for the preparation and maintenance of land and other management practices, thus allowing good establishment, growth and persistence of this cultivar, or others, leading ultimately to improvement of the pasture and its grazing potential.

The indirect method of measuring N_2 fixation by acetylene reduction was used in modified forms for laboratory and field studies. The exact relationship between acetylene reduction ($C_2H_2 \rightarrow C_2H_4$) and N_2 fixation ($N_2 \rightarrow 2NH_3$) was determined for the cultivar Maku under laboratory conditions and found to be 2.98 moles of C_2H_2 reduced to every 1 mole of N_2 fixed.

The most reliable results when measuring acetylene reduction by cv. Maku in the field were obtained using a sample consisting of seven soil cores (5 cm diameter by 7.5 cm depth) with plant material, placed in a 4.6 litre glass incubation jar.

Growth and N_2 fixation by Lotus cv. Maku and T. repens cv. S184 were studied in a glasshouse pot experiment using a Perlite medium, with pH levels 3.5 to 5.5, three phosphate levels (0, 15 and 30 kg P/ha) and presence and absence of trace elements. The addition of phosphate had no beneficial effect on Lotus when added at pH's 4.5 and 5.5. S184 had a greater response to 30 kg P/ha than Lotus. Increasing pH benefitted both species. The addition of trace elements two days after germination increased growth and effective nodulation of clover and effective nodulation of Lotus.

The development of a Rhizobium - legume symbiotic association can be a key factor in establishment and growth and is affected by combined N. Three sources of combined N (NH_4NO_3 , $NaNO_3$ and KNO_3) at various rates (0, 30, 70, 120 and 180 mg N/pot) were applied in solution to a Perlite medium into which cv. Maku and cv. S184 were transplanted. Supplies of NH_4NO_3 and KNO_3 at 30, 70 and 120 mg N/pot increased shoot growth of Lotus without adversely affecting fixation but only addition of KNO_3 increased total plant weight of both Lotus and clover. $NaNO_3$ did not significantly affect growth or fixation by either Lotus or clover.

To investigate the effect of combined nitrogen on nodule number, effective nodule weight and nitrogen fixation measurements, two levels of KNO_3 (30 or 70 mg N/pot) were applied in solution to a Perlite medium in which cv. Maku and cv. S184 plants were growing. The nitrogen treatment was given at either 0, 6, 12, 18, 24 or 30 days after transplanting. Addition of nitrogen at time of or six days after transplanting improved the growth of both species without inhibiting fixation.

The effect of a commercial compound fertiliser containing slow-release N ("Enmag") added to cvs. Maku and S184 grown in a Perlite medium was studied under growth cabinet conditions. "Enmag" increased shoot growth and length of S184 when added at 30 and 70 mg N/pot but fixation was inhibited on addition of 120 mg N/pot. Addition of "Enmag" had no significant effect on Lotus plants.

Studies were set up to simulate grazing by cutting and to measure the subsequent rate of recovery by cv. Maku to the level of N_2 fixation prior to cutting, in a pot experiment under glasshouse conditions. A lag phase in acetylene reduction after cutting lasted eleven days. Cutting also resulted in a gradual loss of effective nodules and a decrease in root weight.

Maku and Huia grown in peat under controlled environmental conditions were subjected to a combination of day length (12 hr or 16 hr) and day and night temperatures (21/18°C or 18/15°C) and a three-cut regime with cuts taken at intervals of twenty one days. Long day conditions compensated for the lower temperature regime and N_2 fixation was greatest under the long day higher temperature regime and least under the shorter day lower temperature regime.

Seasonal variation in N_2 fixation by cv. Maku plants in the field, when no management practices were applied, was measured in order to interpret any future work on the effect of various treatments on N_2 fixation. Fixation by Maku was recorded during 1978 and 1979 at an upland site. Fixation commenced in June which coincided with increasing soil temperature and rose to a maximum in mid July. The subsequent decline was associated with increasing rainfall, decreasing soil temperature and the onset of rhizome production.

The importance of rhizomes in the establishment and persistence of Maku was recognised and thus management practices promoting production of rhizomes were required. Seasonal development of rhizomes by cv. Maku

was therefore studied in an upland site over two years. Second and third year plants exhibited marked seasonality of growth with aerial shoot production giving way in August to rhizome production which reached a maximum during late September or October, depending on weather conditions. Renewed aerial shoot growth in spring was initiated mainly from nodes on rhizomes formed in the previous autumn.

A study was conducted, utilising 15 cm depth x 5 cm diameter soil cores removed from an upland site in which cv. Maku was growing, to determine the root depth and soil conditions under which cv. Maku could optimally fix N_2 . Increasing fixation rate was found to be correlated with increasing pH and increasing phosphate level. Almost 50% of the total fixation by the soil core occurred in the top 2.5 cm, 95% of the total fixation in the top 7.5 cm, and fixation was detected down to 12.5 cm.

Previous information on nodule distribution and development for Maku was negligible, therefore a study on these aspects was initiated at a lowland site. On plants sown in May (1978) or June (1979) the first nodules [both effective (pink) and non-effective (white)] were detected in mid August. Nodules were found mainly on lateral roots. Throughout both study periods, number and weight of effective and non-effective nodules and the rate of N_2 fixation per plant increased with time.

The morphological variability naturally present within the cultivar Maku was studied using spaced plants grown on a plot under lowland conditions. High shoot dry weight of a plant at the end of a growth season was related to early initiation of growth the following spring. Shoot dry weight may therefore be used to detect a tendency towards winter hardiness in this cultivar.

Future work should concentrate on obtaining more detailed information on the role of rhizomes in persistence of the cultivar to enable grazing management plans to be formulated. Further studies on the effect of type, timing and placement of N on Lotus spp. should be

extended to field conditions. Work on other cultivars of L. uliginosus and associated Rhizobium strains should be investigated to give a varied selection of legumes to choose for improving hill land.

In this study L. uliginosus cv. Maku has been shown to grow and fix N_2 under lower pH and phosphate levels than those required for T. repens cv. S184 and establishment of L. uliginosus cv. Maku was improved when small additions of N were applied at transplanting. However, L. uliginosus cv. Maku compared with white clover has shown a slower recovery of N_2 fixation after cutting suggesting that to allow persistence of the cultivar, a controlled grazing system is required with a suitable rest period during August to October for production of rhizomes. It is therefore unlikely that this particular cultivar Maku should be chosen for use in an unrestricted grazing system. Maku should be used in areas where restricted access can be maintained or for conservation purposes and for improving land of low pH and low fertility.

3. INTRODUCTION

In the present economic climate of rising fertiliser N costs and world shortage of protein, legumes have an increasing part to play by contributing to the improvement of natural and artificial grassland which will result in increased primary and secondary food production.

With the inherently low available fertility of upland permanent pasture, the greater fertiliser requirement and the cost and difficulty of applications as compared to lowland areas, interest has been aroused in low-cost input systems for improvement of hill pasture (Joint Consultative Organisation (J.C.O.), 1975).

Under hill conditions the mineralisation of organic residues of plant and animal origin prior to their availability to the plant is a slow process, due mainly to low temperatures and high acidity (Hill Farming Research Organisation (H.F.R.O.), 1977). It has been reported that although hill soils contain large quantities of N (up to 10,000 kg/ha) only a small part (approximately 30 kg/ha) is available for absorption by plants (Floate, 1971). Substantial inputs of fertiliser N are therefore required to improve hill pasture but a possible economically viable alternative is the fixation of N_2 by legumes. Proper management could ensure that maximum N_2 fixation by the legumes will take place allowing transfer of some of the fixed N to the grass while maintaining a good proportion of legume growth in the sward for grazing purposes.

White clover (Trifolium repens) is the main legume species included in seed mixtures to be sown in upland and hill pasture areas in temperate regions of the world but development of other legumes is required to ensure that a range of legume types is available for use in different ecological habitats and to substitute for T. repens in the event of its failure due, for example, to disease. In the past Lotus corniculatus (birdsfoot trefoil) and, more recently, L. uliginosus (marsh birdsfoot trefoil) have been developed as sown legumes for improvement of natural

grasslands in North and South America, Canada, Europe and New Zealand (Seaney & Henson, 1970, Charlton, 1971). Trials carried out in Britain have produced contrasting results (Davies, 1969; Charlton, 1971). Field trials in Wales showed Lotus to have poor winter hardiness and persistence (Davies, 1969) and any advantages that Lotus may have had over white clover were masked by applying high dressings of P, K and lime (Hay, 1979), whereas work carried out in the west of Scotland has shown that there is a potential for Lotus in improving low grade pastures (Charlton, 1971). L. corniculatus was shown to grow in dry soils of low fertility in Scotland while the performance of L. uliginosus grown as an oversown species in Scottish wet, acidic, upland grassland indicated that this species should be further investigated and exploited.

The role of Lotus in natural grassland is not seen as a replacement for white clover. However, although white clover is widespread on well drained, acidic soils, there are still extensive areas of natural grassland where white clover is not a productive species and it is under these conditions that there may be a potential use for some Lotus species.

Particular interest has been aroused in the performance of L. uliginosus arising from the results cited by Charlton (1971) and from work carried out in New Zealand (Lowther, 1976a; Brock, 1973; Brock & Charlton, 1977). L. uliginosus cv. Maku was found to outyield T. repens cv. Huia by 30% in plots with a low level of phosphate (Brock, 1973). When oversown alone or in a mixture on to a low fertility, acid tussock grassland (pH 4.6) L. uliginosus cv. Maku outyielded T. repens cv. Huia, in the third year, in the presence and absence of lime at four levels of phosphate from 7.5-60.0 kg/ha and at seeding rates from 2 to 10 kg/ha (Lowther, 1976a). The development of improved cultivars of L. uliginosus has taken place in New Zealand using Portuguese and New Zealand parent material. The resulting cultivars have various levels of winter survival and can withstand grazing after establishment.

Results from pilot work by Charlton (1971) in Scotland and from work in New Zealand (Armstrong, 1974) indicated that one of the improved cultivars, a tetraploid, L. uliginosus cv. Grasslands 4705 "Maku" had the greatest potential for wet, acidic and low fertility uplands. It was with this cultivar that all the work on Lotus reported in this thesis was carried out.

If the full role of L. uliginosus as a source of protein and soil nitrogen is to be realized, it is necessary to acquire a better understanding of its growth, development and N_2 fixation potential. At the commencement of the work, little or no published material was available on these features of L. uliginosus cv. Maku. The following section reviews what is known at the present.

4. LITERATURE REVIEW

4.1 LOTUS species

The history of the use of the genus Lotus in agriculture has been well reviewed by Robinson (1934) and Macdonald (1946). The following species of Lotus were noted as being of agricultural importance: Lotus corniculatus Linn.; L. uliginosus Schkuhr (synonyms L. major sm., L. pedunculatus Cav.); L. angustissimus Linn., and L. hispidus Desf. (Macdonald, 1946). L. corniculatus (birdsfoot trefoil) and L. uliginosus (marsh birdsfoot trefoil) are the two main species used in agriculture.

According to Robinson (1934), Ellis in 1744 was the first to report upon L. uliginosus as an agricultural plant. It was recommended for use in moist and shady places within Britain. Buckman in 1868 reported favourable results where the plant was used in situations too moist for clover or lucerne. It has been cultivated in France and Germany on newly reclaimed moorland since the mid 1800's. It is not as important as L. corniculatus in America but has become adapted to the acidic coastal soils of the north western United States.

In New Zealand L. uliginosus is of much greater importance than L. corniculatus. Levy (1918) drew attention to the potential value of L. uliginosus in New Zealand grassland where it grew well under conditions of high rainfall. Barclay (1957) and Barclay & Lambert (1970) have greatly improved the characteristics of L. uliginosus by breeding cultivars with better seedling vigour, better winter hardiness and which are more resistant to grazing.

The first useful account of L. corniculatus as a valuable herbage plant in Britain was credited to Ellis (1744), quoted in Robinson (1934). The plant received little attention in France until the end of the nineteenth century but is now used by French farmers more than in other parts of Europe. The Swiss, Germans and Italians began to use L. corniculatus during the middle of the nineteenth century.

L. corniculatus was introduced into Australia and New Zealand in the mid-nineteenth century, but is of little value due to soil factors such as pH and water content and is not used to the same extent as L. uliginosus.

Seed samples of L. corniculatus were introduced to America from Europe between 1885 and 1900 and were tested at various experiment stations. Two forms of the plant were found to occur in the indigenous state. The broad leaved L. corniculatus var. vulgaris occurred for the most part on upland soils and the slender or narrow leaved var. tenuifolius occurred most commonly on the heavier lowland soils.

Research on the agricultural use of L. corniculatus was initiated by Professor Johnstone-Wallace at Cornell University, New York State, prior to 1937. This led to the introduction of cultivars, notably Empire and Viking birdsfoot trefoil and a rapid increase in its use throughout the north east and north west United States and the neighbouring provinces of Canada.

A great deal more information on the agricultural potential of L. corniculatus compared to L. uliginosus has been amassed over the years. Information on L. corniculatus can aid studies into the value of L. uliginosus in agriculture and is thus included in this literature review.

4.2 Morphology

Root

Birdsfoot trefoil L. corniculatus has a long tap root with numerous lateral branches. Main branches from the primary root are quite large in diameter but secondary branches become smaller and form a thick fibrous root system, especially in the upper one to two feet of soil (Macdonald, 1946). L. uliginosus roots do not grow as deeply as L. corniculatus roots but have a more dense distribution in the upper soil region, and rooting of the rhizomes present at and near the soil surface add to this density.

The underground component of L. uliginosus cv. Maku consists of: taproot, lateral roots and rhizomes plus associated adventitious roots. Expansion of the rhizome system appears to be primarily restricted to the post-reproductive period of late summer/autumn (Sheath, 1977). In general, the taproot is the dominating rooting organ being ten to fifteen centimetres in length for one year old plants and increasing to thirty to forty centimetres in older plants. However, forked root systems do exist with lateral roots, generally originating just below the crown, often being of similar size and rooting depth to the primary tap root.

Adventitious roots plus associated nodules can account for 3% to 5% of total plant weight. Since these roots are predominantly associated with rhizomes they tend to be superficially located in the soil horizon, rarely penetrating below 5 cm. These roots are usually associated with new rather than old rhizome material.

Leaf and Stem

There is considerable variation in leaf and stem morphology; within L. corniculatus size, shape, colour and pubescence of stems and leaves vary greatly among different genotypes.

Each leaf consists of five leaflets, three attached to the terminal end of the petiole and two at the base. Leaflets are typically obovate, although shape may vary from rounded to oblanceolate. L. uliginosus leaves are large but similar in shape to the dwarf variety (arvensis) of L. corniculatus with the apex of the terminal leaflet more rounded or obcordate. In L. corniculatus the upper surface of the leaflets is usually nearly glabrous with a few scattered hairs on the lower surface particularly along the midrib and the margins. In L. uliginosus the amount of hairiness varies greatly from almost glabrous to the quite hairy and in general is more hairy than L. corniculatus. Leaves of both species are attached alternately on opposite sides of the stem. During darkness the leaflets close around the petiole and stem similar to the night closing of leaves in white clover (Macdonald, 1946).

Growth habit of stems may be prostrate like L. corniculatus var. arvensis, a dwarf type commonly naturalised throughout the British Isles or erect, e.g. L. corniculatus var. vulgaris, an erect form with larger leaves which is the basis of European and North American commercial birdsfoot trefoil.

Branching always occurs at the leaf axis of main and secondary stems and the amount and symmetry of branching varies.

In the erect broad leaved and narrow leaved forms of L. corniculatus, the stems or branches all arise from the crown above ground. Stems lying in contact with the soil may become covered and this portion takes on a white or stolon-like appearance. Stems may grow underground for several inches before emerging but the underground parts rarely form adventitious roots. The leaves are reduced but still recognisable as foliage leaves.

Branching is common in these underground stems but the amount depends largely upon soil conditions. New aerial shoot growth in L. uliginosus cv. Maku can develop from three separate regions which are the crown, the rhizomes, and the leaf axils. Crown activity is low and as an actual shoot producing organ is insignificant compared with the rhizome system. Terminal rhizome shoots may emerge above ground, become stoloniferous, and then develop into upright aerial shoots. The bulk of aerial shoots of underground origin arise as lateral shoots from the nodes of more recent rhizomes or from enlarged nodes which may have developed on older rhizomes. While underground rhizomes and associated lateral shoots have leaves that are reduced to stunted scale-like organs, normal leaves develop upon emergence from the soil, although the initial ones are generally smaller in size.

The inflorescence of birdsfoot trefoil is a typical umbel consisting of four to eight florets attached by short pedicels to a long peduncle. Each floret consists of a calyx with five united sepals and a typical legume corolla with five petals. Two petals are fused

together to form the keel which is enclosed by two wing petals and the standard. The toothed portion of the calyx is distinctly different in L. uliginosus where the calyx teeth are turned in an outward direction in the bud giving it a star shaped appearance when viewed from above. This curvature is maintained in the flower and in the later stages (Macdonald, 1946). The interstices between the teeth are angular rather than rounded as in L. corniculatus. Scattered hairs along the rib and margin of the toothed portion are more conspicuous in L. uliginosus. Petal colour varies from a light to dark yellow and may be tinted with faint orange or red stripes. Colour at the keel tip can be yellow, brown or red (Buzzell & Wilsie, 1963).

Reproductive parts of the flower consist of ten stamens and a single pistil. One stamen is attached individually to the base of the flower while the other nine stamens have fused filaments which form a tube surrounding the ovary. The fused stamens are of different lengths, five long stamens alternating with four shorter ones (Macdonald, 1946).

4.3 Seedling properties

A study of the development of the seeds and pods of birdsfoot trefoil between pollination and maturity was carried out by Anderson (1955). The seeds could not be separated from pod fragments until the 9th day after full bloom and then they were extremely small and immature. Rapid seed development began at about the 15th day following full bloom and progressed until the 24th day after which further development was limited. It was calculated that morphological maturity of the seed was attained 27 days after full bloom and that the moisture content of the seed at this time was 35-40%. Early set pods were found to produce higher seed yields than late set pods. In the cv. Empire, 2.4 - 4.3 pods were produced per umbel and the number of seeds per pod ranged from 14.48 at early harvest to 8.04 at late harvest. Associated with the development of pods is a colour change. Dark green pods were harvested 19 days

following full bloom, followed by harvest of light green, light brown, dark brown and black pods each at subsequent 4-day intervals.

One of the limiting factors in successful seed production is the tendency for the pods to dehisce when mature. This is accentuated by the indefinite flowering and maturity of birdsfoot trefoil which makes it difficult to judge the proper time for harvest. A number of studies have been carried out by a variety of workers in an attempt to determine the factors affecting pod dehiscence.

Buckovic (1952) studied the anatomical structure of birdsfoot trefoil pods and found that the pod wall is composed of 2 separate layers. The exocarp is made up of epidermal cells, elongated at 45° angles to the vertical plane, and of parenchyma cells below the epidermis which are primarily elongated in the vertical plane. The mesocarp of the pod wall is made up of fibrous, heavily lignified cells elongated at right angles to the epidermal cell. The mesocarp did not extend completely around the pod but terminated at the thin walled dorsal and ventral suture regions. He postulated that the rate of water loss differed in the two tissues resulting in tensions between individual layers of fibres and possibly in their component fibres. Eventually the tension overcomes the cohesion at the sutures and the two valves of the pod separate and twist. The rate of moisture loss affected the rate of dehiscence, since he found that pods dried at a rapid rate had a high rate of dehiscence whereas those dried more slowly did not dehisce even though they had lost as much water.

Metcalf (1957) found that dehiscence in the cv. Empire occurred at a relative humidity of 35% or higher. The moisture equilibrium value for the lower relative humidity was 10.05% and 10.39% for the higher. This suggests a close interdependence of relative humidity, moisture equilibrium and pod splitting. When placed under controlled levels of different relative humidity the moisture content of pods was rapidly reduced and there was a close agreement between the time lag in moisture

content and pod dehiscence. Depending on the cloud sky cover, the air temperature within and at the surface of mature pods varied from the general air temperature by as much as 5.5°C. Higher pod dehiscence obtained under conditions of full sunlight was therefore due to reduced relative humidity at the pod surface. To avoid pod dehiscence Metcalfe (loc. cit.) recommended production in areas where relative humidity at harvest is normally 40%, and harvest when the pod moisture is still high (about 25%).

Anderson (1955) showed that when seeds attain their morphological maturity the pods are light brown in colour and he recommends that harvesting should take place when the pods are light green - light brown rather than when they are more mature. He also showed that light green pods, 24 days after bloom produced seed of similar size and quality to those of more advanced stages, although they did not dehisce as freely under drying conditions.

Attempts have been made to select plants which are resistant to pod shattering (Gershon, 1962). A cycle of selection for shattering resistance carried out by Peacock and Wilsie (1957) reduced pod dehiscence by 17%. They exposed the pods to a relative humidity of 35% for 72 hours to differentiate the degree of resistance to shattering. The results indicated that the degree of pod shattering of a clone might be predicted if data were available on pod shattering of the open pollinated progeny of that clone.

When the seed comes into contact with water it swells due to moisture absorption. The rate of water absorption differs greatly within seed samples and between cultivars. Over a 24 hour period L. corniculatus seed absorbed approximately 117% of its original dry weight whereas L. uliginosus absorbed only about 70% of the original dry weight.

Studies on the factors controlling germination were carried out by Woods (1966). Atmospheres containing 0.3-3.0% CO₂; 20.7-18.0% O₂ and 79% N₂ stimulated germination whereas those containing 15% CO₂; 6% O₂

and 79% N₂ delayed it. Germination was delayed by temperatures below 15°C or above 30°C and by soil moisture stresses above 4.5 Bars.

Empire was found to be the cultivar least affected by high temperatures but most affected by moisture stress.

In field trials most of the variation in emergence could be attributed to differences in sowing depth, or in factors related to it. Jensen et al (1972) measured the relative force exerted during seedling emergence, keeping a continuous record of the vertical force observed for each seedling. The resulting curve reflected the relative force a seedling exerted at a particular time of emergence after germination. The emergence force was positively correlated with seed weight. L. tenuis was found to exert a significantly lower mean weekly emergence force compared to Melilotus sativa (sweet melilot) and Trifolium fragiferum (strawberry clover). The emergence force was found to increase very rapidly for legume seedlings 24-48 hours after transplanting, with a gradual decrease thereafter.

The percentage of birdsfoot trefoil seeds which germinated was maintained at a high level for 5-10 years but later on sharply decreased although that of individual seeds retained their viability up to 37 years (Mikhina, 1967). The germination of 100 year-old L. uliginosus seed after storage in a museum was noted by Youngman (1951).

MacDonald (1946) gives a detailed description of the seed of birdsfoot trefoil. Hard seed in birdsfoot trefoil is a major factor limiting the quick germination of its seed. An investigation of hard seed was carried out by Brown (1955) between the years 1951-53. The hard seed content in mature hand-harvested seed was found to range from 85-95%. There were no consistent differences observed between the many cultivars of birdsfoot trefoil studied.

Hard seed was affected to a considerable extent by the stage of maturity and moisture content of the ripening seed. Its percentage increased with an increase in both these factors. Seeds which are

permitted to ripen and dehisce on the standing plants were found to have a higher percentage of hard seed than seed harvested at a less mature stage (Taylor et al, 1973). The hard seed was found to be high in viability; over 90% of the seed produced normal seedlings following careful treatment to render the seed permeable to water. Most of the hard seed maintained its impermeability during dry storage, and storage for 12-18 months resulted in a decline of only 5-10% in hard seed content. Less than 10% of the hard seed produced seedlings in the field in the seeding year, regardless of the date of seeding. The survival of these seedlings ranged from 60% down to 0% correlated to increasing delay in time of germination and seedling emergence. The period of greatest germination and emergence from hard seed occurred in the spring of the first harvest year following one full winter in the soil, about 25% of the hard seed originally sown producing seedlings at that time. The survival of these seedlings was generally low, ranging from 0-25% depending on the density of the stand into which they were emerged.

Further studies were carried out by Brown (1955) into methods of inducing permeability and germination of hard seed. Treatment with concentrated sulphuric acid for 45 minutes reduced the hard seed content to a low level and resulted in no injury to the seed. 20% of the seed remained hard after dry heat treatments, at temperatures of 80-100°C. Two other methods of hard seed treatment, mechanical scarification and impaction, were equally effective in reducing the amount of hard seed but impaction resulted in less injury to the seed.

Ghisleni (1959) treated both trefoil and ladino clover seed with liquid O_2 for periods from three minutes to three hours. This treatment was effective in reducing the impermeability of the seed and did not lower the viability of such seed.

Several authors have shown that seedling vigour was primarily related to the size of the seed and that the larger the seed the greater

was its vigour. A high correlation for seed size and seedling weight was obtained by Carleton and Cooper (1972), while Twamley (1969) stated that he could discard 90% of his clones in a trefoil seedling vigour breeding programme on the basis of seed size. The effects of seed size on seedling vigour of six varieties and strains of birdsfoot trefoil were examined by Henson & Tayman (1961). There were striking differences in seedling growth due to seed size in plants grown in a glasshouse. Seed size significantly affected all measured characters of all varieties but differences obtained for yields and root weights at second harvest were less pronounced. Seedling plants from large seed produced significantly more top growth than those from smaller seed and basal shoots appeared earlier on plants from the larger seed. The superior growth of seedling plants of the erect varieties with respect to top growth, root growth and percent of plants with basal shoot as compared to Empire and Empire-derived varieties was clearly evident. Differential seedling vigour in Viking and Empire plants from a common seed size was studied as a function of growth rate by Shibles & Macdonald (1962). Although both varieties were found to have similar net photosynthetic rate/unit area of cotyledon or leaf surface, divergence in growth pattern between them occurred about two weeks after emergence. It was thought to be caused by differential rate of photosynthetic area production. It was thought that the assimilation was enhanced in Viking compared to Empire due to a greater partition of photosynthate into photosynthetic area expansion rather than into axis growth. This was demonstrated by the short internode of Viking resulting in an erect growth habit compared to the large internodes and prostrate habit of Empire. A relationship between size of seedlings and number of seedlings was found by Scholl and Brunk (1962) when investigating yields in the year of seeding and the following year under different methods of controlling vegetative competition. Yield in the seeding year and the year following were more closely associated with size of

seedling trefoil plants in the seeding year than the number of seedlings.

Twamley (1969) examined groups of plants superior either in seed production or in seed size. For the 15 plants which provided the top yield of seed the average 100-seed weight was 127 mg; this was identical to the mean of the population. Similarly, 30 large-seeded plants taken at random out of the fraction with a 100-seed weight exceeding 150 mg produced an average of 1.6g of seed whereas the population average was 1.3g. This difference was not significant. Thus, among the better plants, seed yield appeared neither to affect nor to be affected by seed size.

Genetic differences in seedling vigour are known to exist apart from seed size. Shibles & Macdonald (1962) have shown differences in vigour of trefoil seedlings grown from similarly sized seeds of different cultivars. The environmental effect on seed size may be due to the growing conditions of the plant and the number and position of the seeds during development. It has been noted by Henson (cit. Shibles & Macdonald, 1962) that seed weight of different lots of the same variety varies from year to year because of favourable or unfavourable environmental conditions during seed formation and maturation.

Twamley (1969) studied the progenies of sixty plants of the Russian cultivar Morshansk which differed in maturity, seed load, seed size, speed of germination, seedling vigour and rate of tillering. The results indicated that seed load was unlikely to affect greatly the seed size and seedling vigour of the progeny and that late maturing strains with good seedling vigour can be found. For this particular cultivar plants arising from large seed had a tendency to tiller early. It was found that speed of germination may be of considerable value in detecting lines with good seedling vigour in an unselected population but was of limited value for that purpose within the large-seeded fraction of the population.

4.4 Aspects of nodulation

One of the main reasons why Lotus is sown into pasture is the ability of the species to fix atmospheric N_2 . The enzyme system responsible for the process is contained in the nodules formed from the rhizobium-legume symbiosis. Rhizobium can be classified in a number of ways (Greenwood & Pankhurst, 1977); such classifications are based on the ability to form nodules on different groups of legumes and on the rate of speed of growth on media.

Rhizobia are classified according to the range of host legumes they can nodulate, into so-called 'cross-inoculation groups'. In the U.S.A. Erdman & Means (1949) tested isolates from several species of Lotus and from Anthyllis vulneraria (kidney vetch) for their effectiveness on birdsfoot trefoil cultivars and on marsh birdsfoot trefoil. Strains from birdsfoot trefoil were effective on kidney vetch and slender birdsfoot trefoil but not on marsh birdsfoot trefoil. Results showed that marsh birdsfoot trefoil fixed considerably more nitrogen than other Lotus species when each was effectively nodulated. Strains isolated from kidney vetch showed varying degrees of effective nitrogen fixation on the host species but produced ineffective nodules on birdsfoot trefoil.

The Lotus-Lupin cross inoculation group described by Greenwood & Pankhurst (1977) include rhizobia nodulating a more diverse range of host plants including Lotus and Lupinus species, Serradella, Sarothamnus and Ulex. The Lotus hybrid 'Grasslands 471-2' (L. pedunculatus (tetraploid) x L. corniculatus) is somewhat variable in rhizobial response though close to the female parent L. pedunculatus. The recommended inoculant for this hybrid is a mixture of two strains, a slow growing strain effective with L. pedunculatus and a fast growing strain effective with L. corniculatus, moderately effective with L. pedunculatus. There are many strains of Rhizobium which fix N_2 best with L. pedunculatus, Lupinus, Serradella, Sarothamnus and Ulex but the

strains which fix N_2 best with L. pedunculatus are not the best with the crop lupins or Serradella.

The Rhizobium strains are divided into fast and slow growers. The slow growers are more tolerant of acid conditions and produce alkali whereas the fast growers are more tolerant of alkaline conditions and produce acid. According to Norris (1965) the slow growers represent an older type of legume-rhizobium association and the fast growers a more advanced type. He classified L. uliginosus rhizobia as slow growers and L. corniculatus rhizobia as fast growers.

Brockwell (1966) found that the tendency of Lotus rhizobia to produce alkali in culture as the symbiotic capacity with L. uliginosus increased was so marked as to suggest that acid production is a highly reliable indicator of strain ineffectiveness within the species. The trend in the opposite direction with L. corniculatus was less marked and this species fixed N_2 with rhizobia having a wide range of cultural reactions.

Lotus nodules when they are formed vary greatly in size (Macdonald, 1946). On a large number of L. corniculatus plants studied in the young growing stage, a diameter of from 1.5-2.0 mm was found to be most common. In fertile soils during periods of rapid growth, nodules measuring from 5-7 mm were not uncommon. The nodules are often larger in diameter than the rootlets to which they are attached or than the secondary root supporting the rootlet. Effective nodules usually have pink outer appearance and when cut the pink-red colour of the leghaemoglobin present can be observed. Ineffective nodules are smaller and tend to be white or have a light green colouration. Seedlings which have developed from inoculated seed usually have a cluster of nodules on the root close to the crown as at this point the rhizobia present on the seed coat are closest to the developing root.

Nodulation of seedlings of birdsfoot trefoil produced from inoculated seed subjected to various periods of dessication in air-dry

soil was closely related to soil pH (McKee, 1961). In general, at soil pH values of 6.0 or greater, inoculated seed subjected to desiccation in air-dry soil for six weeks nodulated when subsequently watered.

However, inoculated birdsfoot trefoil in the same soil but subjected to sequential rain, drought and rain did not nodulate adequately.

Nodulation by birdsfoot trefoil was good at the three soil moisture levels of 22.6%, 27.6% and 32.6% (Smith, 1955). He concluded that the moisture levels at which birdsfoot trefoil grows will not inhibit nodulation.

The heavy isotope N^{15} was used to determine the effectiveness of nodulation of birdsfoot trefoil at soil temperatures of 6.1°C and 11.1°C by Smith (1955). The lower temperature limit for nodulation was found to be between 7.2° and 10°C. In New York State it is possible for nodulation to be limited by low temperatures in all months except June, July, August and September. This influences early spring sowing of birdsfoot trefoil and partly explains the reason for failures with autumn seedlings. The effect of five root temperatures (9-30°C) on the growth of three cultivars of birdsfoot trefoil was carried out in commercial growth pouches ("di. SPO") for thirty five days after nodule formation (Kunelius & Clark, 1970). Highest weights and N yields/plant were obtained at 18° or 24°C. At 9° and 12°C, N_2 fixation was depressed and growth was poor. At all root temperatures the growth of plants dependent on symbiotic N_2 fixation was inferior to that of plants receiving combined N.

L. corniculatus has been shown to persist on plots with a pH value of 5.5 or above but seedling growth and nodulation were satisfactory only on plots fertilised and limed to a pH value of 6.7 or over (McKee, 1961). Birdsfoot trefoil nodulated between pH 4.5-7.9 but nodulation was only considered adequate between 6.2 and 7.5. Poorest nodulation but best top and root growth occurred where lime plus a complete fertiliser was used.

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Nodulation by L. uliginosus was found to be low in plots, without lime, of pH 4.5 but markedly increased in the presence of lime, i.e. from 5% nodulation with no lime to 62% with lime, although the nodules were small and white. Nodulation was further increased by the addition of micronutrients, i.e. from 62% nodulation to 86% and the size and colour of nodules was also improved. Induced boron deficiency due to application of lime inhibited the normal development of nodules thus explaining the increase in effective nodules on application of the micronutrient solution (Lambert & Boyd, 1974). In experiments by Greenwood (1961) nodulation of L. uliginosus was not so dependent upon the presence of lime as was T. repens and T. subterraneum, 46% or more of the Lotus plants being nodulated in the various unlimed treatments. Lime, however, did increase the percentage of nodulated plants (from 46% to 99%) and micronutrients improved the size and colours of the nodules present.

The performance of L. uliginosus under different pH and phosphate levels was further studied by Gibson et al (1975). Nodulation occurred at all three pH levels of 4.1, 5.2 and 6.2. L. uliginosus fixed more nitrogen in response to increased pH and phosphate than T. repens, particularly so at the lowest pH. At the higher P levels the high pH gave rise to lower N_2 fixation than at the central pH. An increase in P level was shown to compensate for the effects of low pH on nitrogenase activity in T. repens and L. uliginosus. Brock (1973) found that L. uliginosus cv. Maku grew relatively better at low P levels and utilised P more efficiently than T. repens cv. Huia. Maku was relatively less efficient in utilising the additional P in terms of fixing N. The low N content at both P levels was due to the low production of nodules in the establishment year, but once successfully nodulated and established, Lotus was capable of rates of N_2 fixation similar to those of white clover.

Lowther (1976) found that the yield advantage of L. uliginosus increased with increasing levels of P but this result differs from Brock (1973) where L. uliginosus under conditions of low P outyielded T. repens.

The inhibition of nodule formation by combined nitrogen was first observed by Rautenberg and Kuhn twenty years before Hellriegel and Wilforth demonstrated in 1886 the relationship between nodules and N₂ fixation (Nutman, 1960).

Supplying combined nitrogen to legumes generally depresses the development and nitrogen fixing activity of root nodules (Munns, 1968; Oghoghorie and Pate, 1971; Gibson, 1976). There are two main theories which explain the decline in N₂ fixation when combined nitrogen is added to legumes: (1) there is a diminished supply of photosynthate available to the nodules following its utilization in the assimilation of nitrate in the shoots and roots. Oghoghorie and Pate (1971) argued that nitrogen fixing nodules and the nitrate assimilating centres in Pisum arvense must compete with each other for supplies of reductant and carbon skeletons. They found nitrate reductase predominantly in the root in the presence of low levels of nitrate but as the levels of nitrate increased the enzyme was found more in the shoot; (2) nitrate is thought to act directly within the nodules and inhibit N₂ fixation therefore reducing the role of nodules as a sink for photosynthesis.

Nitrate inhibits clover and alfalfa root hair deformation and infection by Rhizobium, indicating that fixed nitrogen effects occur long before onset of nodulation and symbiotic N₂ fixation (Munns, 1968; Raggio, Raggio and Torrey, 1957). Addition of nitrate to Medicago sativa was found to inhibit formation of infection threads to such an extent that nodule number was limited (Munns, 1968). The addition of fixed nitrogen was shown to prevent Rhizobium trifolii from accumulating in high numbers on clover root hair surfaces thus inhibiting infection and therefore nodule numbers (Dazzo and Ball, 1978). Studies using split root

systems showed that inhibition of nodulation by NO_3^- is localised (Van Schreven, 1959). Nodulation was shown to be inhibited on the part of the root system exposed to combined nitrogen but was unaffected on the part growing in nitrogen free conditions. The ammonium ion has been shown to be less inhibiting to nitrogen fixation than nitrate ions (Oghoghorie and Pate, 1971).

The amount and timing of application of combined nitrogen can affect both nodulation and N_2 fixation. A single dose of high concentration (5 and 10 mg N/plant) of NH_4NO_3 stimulated L. pedunculatus growth but suppressed nodulation and nitrogen fixation (Pankhurst and Jones, 1979). In contrast a continual supply of a low concentration (1 mg N/plant) of NH_4NO_3 stimulated nitrogen fixation by up to 500%. This was due to an increase in nodule fresh weight/plant, doubling of nodule nitrogenase activity and lowering of the flavolan content of plant roots which is known to be toxic to rhizobia. Dart and Wildon (1970) working with cow peas and purple vetch showed that after addition of combined nitrogen at sowing, relatively little change in concentration occurs during primary root nodulation but considerable changes occur during the much longer period of secondary root nodulation. The total numbers of nodules on secondary roots were significantly affected by the form of combined nitrogen applied to the leaves and by its effect on nodulation of the primary roots since infection of the primary roots was already well advanced at treatment. Time of application of combined nitrogen therefore appears to be of importance in restricting its inhibition of nodulation. Reduction of nodulation and nitrogen fixation therefore varies with the species of legume, amount and form of combined nitrogen applied and the time of application.

Stimulatory effects of combined nitrogen on nodule development and nitrogen fixation are also known (Allos and Bartholomew, 1955; Pate and Dart, 1961; Gibson, 1976). Small quantities of combined nitrogen supplied to plants at sowing generally increases nodulation and nitrogen

fixation (Gibson, 1976) but considerable variation in this response is encountered with different Rhizobium strains - host plant combinations (Hoglund, 1973; Gibson, 1976). Stimulation of nodulation and nitrogen fixation by small amounts of added nitrogen has been ascribed to more rapid growth and hence production of more infection sites and to the indirect effect of nitrogen benefiting early seedling growth (Dart and Mercer, 1965). Nitrogen at 5 and 10 mg N/plant added to L. pedunculatus at sowing produced a five-fold increase in shoot dry weight and a 2.5-fold increase in total plant nitrogen after six weeks growth. This increase was primarily due to growth from the added nitrogen. Plants supplied with 0.33 and 1.0 mg N/plant also showed an increase in shoot dry weight and total plant nitrogen. Here the increase was primarily due to an increase in the nitrogen fixing activity of the plants caused not only by a substantial increase in total nodule mass/plant but also to a doubling of the specific activity of the nodules.

4.5 Flowering

Work by Joffe (1958) in Pretoria indicated that birdsfoot trefoil is a long day plant requiring photoperiods of at least fifteen hours for development of flowers and sixteen hours or longer photoperiods before profuse flowering is initiated. A few plants bloomed at photoperiods of fourteen hours but flowers were few and many buds were sterile. The production of some abortive inflorescences was characteristic of this species even under apparently optimum photo-inductive conditions. The percentage of defective inflorescences was influenced by night temperature, light conditions and possibly the N level of the plants. L. corniculatus showed virtually no photo-inductive after-effect of long days, the initiation of floral primordia ceasing upon exposure to short days. Plants kept under short day conditions showed a rosette type of prostrate growth compared with the erect flowering long day plants.

Work carried out by McKee (1963) gave results similar to Joffe (1958) and they found that the critical photoperiod for flowering in L. corniculatus lay between 14 and 14.5 hours. At photoperiods of 16 hours and over blooming was profuse and rapid while at photoperiods of 15 hours blooming was slightly retarded and plants from several seed sources did not bloom. Sixteen or more continuous 24 hour photoperiods were required for floral induction and the production of buds which flowered.

The effect of photoperiod on the flowering of L. pedunculatus was investigated by Forde & Thomas (1966). The critical daylength for inflorescence initiation under natural light alone ranged from 14.5 to over 15 hours. The requirement for long days was satisfied in short days by a two hour light period in the middle of the dark period. Strong flowering did occur with a twelve hour main light period but abortion of all inflorescences occurred at ten hours. The results indicated that both initiation and further development of inflorescences are strongly affected by the intensity and total quantity of light received as well as photoperiod.

The effect of light intensity and light break treatments on inflorescence, initiation and flower development were investigated in L. pedunculatus by Forde & Thomas (1967). Treatments consisted of all combinations of 4842, 9684 and 19368 lux. Initiation was more rapid at the highest intensities but rates of inflorescence growth after initiation were found to differ little between the treatments. Abortions were found to be most frequent when plants were transferred from long days to short days at the lowest light intensities. Flower initiation and full development were promoted by 2 hour breaks but not by four $\frac{1}{2}$ hour breaks. There were strong indications that the latter treatment induced a condition within the plants which enabled them to flower more vigorously when subsequently placed in long days.

Natural plant distribution seems to be more or less restricted to latitudes higher than 30°N. This corresponds to the photoperiodic requirement of 15 hours or longer for rapid and profuse blooming in this species. In Colombia, South America, birdsfoot trefoil does not flower or produce seed while white clover, red clover, alsike clover, alfalfa and sweet clover do. Trefoil blooms scantily in Hawaii and only in June. In Australia birdsfoot trefoil has been observed to flower and set seed at Berry (34°S) but apparently not at Kyogel (29°S) or at Armidale (30°S). In Kenya trefoil grows fairly well during the rainy season at Kitale (6,200 ft elevation) but does not flower. However, a local variety L. corniculatus var. eremantlus flowers and produces seed as does an unidentified trefoil from Ethiopia (Seaney & Henson, 1970)

4.6 Pollination

For many years it was believed that Lotus spp. were self sterile but studies by Silow (1934) disproved this theory. From his experiments he concluded that L. uliginosus, while rarely spontaneously self pollinated, is self fertile when artificially self pollinated. He found that L. corniculatus was practically self sterile (McKee, 1949).

Macdonald (1946) carried out an investigation on self fertility in Lotus and his glasshouse studies were essentially similar to the findings of Silow. Under field conditions where the plants were maintained in bee-proof cages and bees were used as pollinating agents a study was carried out on L. corniculatus vars. arvensis and tenuifolius and L. uliginosus. The results for L. uliginosus confirmed the work in the glasshouse but the conclusions drawn for L. corniculatus were contrary to those obtained in the glasshouse and by Silow. The results showed L. corniculatus to be 100% self fertile when insect pollinated. That the bees were entirely responsible for pollination was evidenced by the fact that no fertility occurred when bees were excluded but smaller insects allowed access. It had been suggested by Knuth (1908) that there

was a possibility of the bee removing a covering from the stigma with its abdomen thus making the stigma more receptive than when hand tripping was involved. It was later discovered by McKee (1949) that L. uliginosus and L. corniculatus with the exception of L. corniculatus var. filicaulis will not set seed until the style is forced out through the keel and the stigmatic surface struck against a foreign object. This means that these species are almost entirely dependent on insect visitations for seed development.

Insects of the genus Hymenoptera appear to be the only insects that operate the flower mechanism of birdsfoot trefoil. Since the flowers of L. uliginosus are smaller it is possible that smaller insects will pollinate it. Observations made in Poland in 1961-67 are supplemented by data from Polish and foreign literature and show that L. corniculatus is visited mainly by Bombus terrestris, B. papidorius, B. agrorum and B. ruderarius (Ruszkowski, 1968). Work carried out by Bader & Anderson (1962) showed that visits of pollen-collecting honeybees resulted in more viable seeds per pod than visits of nectar-collecting honeybees. An increase in live seeds per pod was obtained when the number of visits per floret was increased by nectar-pollen collecting bees and by an increase in the time per visit by pollen collecting bees. Results from Ithaca indicated that flowers not visited by bees remain open for 8-10 days but where the bee population is one bee per square yard the flowering period is reduced to 3-4 days (Morse, 1955).

Work carried out to investigate the mechanism of self sterility showed that it was not due to the differential growth rates of foreign or self pollen tubes. Comparative histological studies were carried out on pistils at various time intervals following cross and self pollination. The pollen tubes reached the ovary at about the same time but although self-pollen tubes grew to the base in ample time to effect fertilization only a small percentage of the ovules became fertile. In highly self-sterile plants these self-fertilized ovules increased in size and

stimulated the spongy tissue of the ovary. Eventually the entire ovule aborted beginning 4 days after pollination. Self-sterility is therefore considered to be primarily an incompatibility reaction between the pollen tubes and the ovules (Giles, 1951). Abortion of self fertilized ovules is not thought to be a related factor in self-sterility whereas the presence of a film on the surface of the stigma was confirmed by Giles (1951), and is believed to be a related factor. Spiss & Paolillo (1969) confirmed the findings by Giles, using semi-vitro cultures. Preliminary tests for chemotropic responses of pollen tubes to ovarian tissues failed to reveal how the differential performance is regulated.

4.7 Cytology

Chromosome numbers have been determined for approximately 70 different species of Lotus. About one third of the species have a basic chromosome number of 6 and two thirds a basic chromosome number of 7. Diploid and tetraploid species occur within both groups (Grant, 1965).

L. corniculatus 2 n = 24

L. uliginosus 2 n = 12

L. tenuis 2 n = 12

Dawson (1941) and others suggest that L. corniculatus is an autotetraploid of L. tenuis. This conclusion is based on their morphological characters, the bivalent pairing in L. corniculatus and the tetrasomic inheritance of cyanogenesis. Other evidence by Wernsman et al (1964), indicates that L. tenuis is a progenitor of L. corniculatus. Backcross progenies of the interspecific cross 4 x L. tenuis x L. corniculatus showed bivalent pairing indicating a high degree of homology between chromosomes of these two species.

4.8 Breeding

The breeding of L. pedunculatus is dealt with in detail by Barclay and Lambert (1970). Barclay (1957) selected a superior grassland variety from widespread New Zealand collections. This variety was based on nine elite parents and renamed Grasslands 4701.

Following on from this three new lines of work were undertaken:

1. A colchicine induced tetraploid based on diploid plants of New Zealand origin was produced. This variety, Grasslands 4702, based on thirteen elite parent plants, was derived after three generations of recurrent selection.

2. 4701 was comparatively dormant for a considerable period during the winter whereas a variety from Coimbra, Portugal, was shown to have a vigorous winter growth but a poor summer growth, relative to the New Zealand variety. Hybridization between 4701 and a Portuguese introduction indicated the possibility of breeding a diploid with improved winter growth. The F1's were back crossed to elite plants of either Portuguese or 4701 and the following two hybrid populations were produced.

Population I : (NA x P) x NZ

Population II: (NZ x P) x P

Recurrent selection was then carried out within each back cross which were finally named Grasslands 4703 and Grasslands 4704.

3. A programme was initiated to produce an autotetraploid hybrid between 4702 and the Portuguese material. An induced tetraploid was produced by colchicine treatment of Portuguese, and elite plants were crossed to 4702. After selected F1 plants were back crossed to 4702, seedlings from thirty-one back cross progenies were planted out as spaced plants. After testing and further selection thirteen elite early flowering plants were isolated in 1962 to provide the nucleus isolation seed named Grasslands 4705, now named tetraploid L. pedunculatus Grasslands Maku.

All the above varieties were found to be successful in a series of trials throughout New Zealand. The tetraploids, particularly 4705, performed well especially when oversown and when grazing pressure was low. It was an outstanding legume on cultivated hill sites both on sunny and shady faces. In revegetation studies on high altitude, acid subsoils it was superior to commercial diploid Lotus in field establishment and yield. In both field and pot trials it outyielded commercial Lotus and Huia white clover at low levels of added phosphate (Armstrong, 1974). Under hard grazing the more dense diploids were clearly superior and within these diploids 4703 best combined winter and summer production and had the highest three year mean annual production.

Because of its performance in these trials Maku was placed on the New Zealand list of acceptable herbage cultivars on 1 January 1973.

4.9 Cyanogenesis

Work carried out by Dawson (1941) showed that both cyanogenetic and acyanogenic forms of L. corniculatus exist in Britain. L. tenuis also was found to have acyanogenic and cyanogenic forms whereas L. uliginosus, angustissimus and hispidis are solely acyanogenic.

There are two types of cyanogenic L. corniculatus (Jones, 1977). One type has a stable phenotype whereas the other has the ability to change phenotype in response to environmental stress, particularly temperature, as occurs in Trifolium repens.

There are different frequencies of stable and unstable plants in different populations. Selective grazing is the predominant agent determining the distribution of cyanogenic L. corniculatus on the south west coast of Holy Island (off Anglesey, N. Wales). The situation is markedly different on the island of Birsay, Orkney Islands, where soil moisture stress is the principal factor associated with the distribution of the cyanogenic form. Experimental work predicts that

T. repens plants containing the cyanogenic glucoside should be infrequent in droughty habitats. Foulds (1972) showed that this prediction is upheld for wild populations of T. repens but the polymorphism of cyanogenesis in L. corniculatus is not affected by drought conditions in the same way.

There is good evidence that cyanogenesis acts as a protection against herbivores in some plants but in those polymorphic species which have been studied it is clear that selective grazing of the cyanogenic form is not intense enough for the species to become monomorphic for cyanogenesis. Different plants in different habitats may respond to selection in entirely different ways and therefore contrary explanations of the role of cyanogenesis within and between species are to be expected.

Part of a study carried out by Urbanska (1977) was an investigation into the variation in cyanogenesis in Swiss populations of L. corniculatus, particularly within the alpine material. Distribution of the cyanogenic/acyanogenic varieties of L. corniculatus, and L. alpinus might be considered as a ratio cline across the Alps with the frequency of HCN positive plants decreasing from N-S. Another ratio cline occurred within tetraploid L. alpinus where the frequency of cyanogenic individuals decreased with an increase in altitude; by contrast diploid cyanogenic plants often occurred at very high altitudes.

The distribution of cyanogenic glucoside was investigated in four varieties of birdsfoot trefoil, Skrzyszowicka, Bursztyn, Viking and Empire by Blaum (1976). In all the varieties great differences exist in the content of cyanogenic compounds in various plant parts (flowers, leaves and stems). The greatest quantity of these compounds was found in flowers. The native variety Skrzyszowicka and Viking contained similar quantities of HCN. Bursztyn contained slightly more HCN and Empire contained exceptionally large quantities of it. There were no marked differences in HCN content in whole plants or leaves in different

cuttings but the flowers were found to contain more HCN in the third cutting than in the first or second. Boros et al (1976) examined the cyanogenic glycoside in six cultivars of L. corniculatus from early spring to the end of their vegetative period. The very high early spring (April) values gradually decreased in May then again increased during the period of flowering (June). After cutting a smaller maximum was observed during the state of full flowering followed later by a decrease of HCN content. During the whole vegetation period the lowest values were measured in late autumn and the highest in periods of growing and flowering. There were no significant differences in HCN content between cultivars.

Although the presence of hydrocyanic acid production has been proved for various species of Lotus there appears to be no authentic record of stock poisoning caused by it.

4.10 Ecology

The ecology of birdsfoot trefoil has been adequately covered by Macdonald (1946). Members of the genus Lotus are well distributed throughout the world. The greatest diversity of species is found in the Mediterranean basin, an indication that this area was probably the centre of origin for the Old World species (Seaney & Henson, 1970). Species of Lotus indigenous to North America extend down the west coast from British Columbia to Mexico and lower California. California contains more of the New World species than any other state. Lotus species introduced into South America from Europe have proved to be well adapted to the areas from southern Brazil across to Chile. In tropical environments the trefoils, particularly big trefoil, grow well and are productive legumes for pasturage. Lotus has been seen to grow in Hawaii at above 300 metres and as an indigenous species in the highlands of Costa Rica (Seaney, loc. cit.). L. corniculatus is one of the most important leguminous plants of the European Alps occurring at altitudes over 3,000

metres (Macdonald, 1946).

The region of Iran, Iraq, Afghanistan and the neighbouring countries is important for some groups of Lotus species, especially those of L. corniculatus and L. gebella complexes. It is in this part of the world that L. tenuis reaches the eastern boundary of the continuous area of distribution (Chrtkovazertova, 1967). Robinson (1934) and Macdonald (1946) report birdsfoot trefoil widespread in Europe and Asia, ranging from the Mediterranean to 71° N and from western Europe into Siberia. Chrtkovazertova (1966) states that L. uliginosus is a European species with sub-atlantic character. It is distributed throughout the greater part of Europe and in North West Africa. L. pedunculatus is a west Mediterranean indigenous species limited to east and south Spain and it is probably found in Africa.

Numerous trials carried out during the past century have shown that birdsfoot trefoil is more adapted to soils lower in fertility requirement than other legumes. L. corniculatus occurs for the most part in soils of medium and low fertility status and in areas considered subject to drought and flooding. It is, however, widely adapted to soils of greatly varying nature.

Kumpruianov (1940) considers that L. corniculatus is especially adapted to the heavy clays of the littoral area of the Black Sea and prevents erosion as well as providing food for animals. In Europe birdsfoot trefoil is more productive than most legumes on soils that are imperfectly drained, or subject to drought (Robinson, 1934). In Chile birdsfoot trefoil tolerates acid soils better than lucerne or red clover and in Uruguay it is widely distributed and well adapted to soils too poor for lucerne. L. tenuis is an important constituent in pastures on the heavy soils in the Hudson River Valley of New York and in California it is used extensively on clay soils difficult to drain after irrigation.

Birdsfoot trefoil has greater persistence than other legumes in

poor sandy soils because of its root distribution; roots grow nearly as deep as lucerne yet have more extensive development in the upper soil.

The flooding tolerance of L. corniculatus has been demonstrated by Heinrich (1970). He showed that L. corniculatus cv. Leo tolerated freshwater flooding at a root temperature of 25°C for twenty days. He later showed that it survived equally well under flooding at root zone temperatures of 25°C, 19°C, and 13°C (Heinrich, 1972). L. corniculatus var. tenuifolius has been shown to have a high tolerance to salinity; grown in a fine sandy loam, it was the only legume which gave an appreciable yield in the high saline plots. L. uliginosus was found to be much less salt tolerant than L. corniculatus. Some of the plants were killed by the concentrations present in the high salinity plots and the remaining ones made very little growth. Salinity tolerance at the lower levels was relatively good.

L. corniculatus and Festuca ovina were characteristic species of a plant community growing on lead mine spalse. They grew on soil of pH 6.3-7.4 and of 1900-3500 ppm zinc and 75-1500 ppm lead (Shimwell & Lowrie, 1972). L. corniculatus has also been shown to be resistant to SO₂ pollution. The SO₂ was emitted from a sulphite pulp and paper mill and varying degrees of vegetation injury were recorded. Trifolium species were found to be only partially resistant (Linzon et al., 1973).

L. corniculatus is also used to recolonize land, thus preventing erosion. Given no fertilizer its population oscillated throughout four years but increased in reclamation trials on abandoned calcareous spoil banks from open cast coal mines (Sabo & Sabo, 1973). Its abundance in sand dune associations at sea level has been noted and there are cases where it has been used in seed mixtures for sand stabilisation purposes (Charlton, 1971).

Birdsfoot trefoil is found in grasslands of varying types, notably low grade pastures (Robinson, 1934). On the well drained natural pastures in the west of Scotland major companion grass species include

perennial ryegrass, timothy and cocksfoot. Tall fescue and smooth stalked meadow grass are drought tolerant species and could prove to be good companion species for birdsfoot trefoil under these conditions (Charlton, 1971).

L. uliginosus differs in its adaptation; it occurs naturally in wet soils and in shady situations and is acid tolerant. It is well adapted to the acid coastal soils of the Pacific north-west and grows well on low lying soils that are frequently flooded during the winter months (Seaney & Henson, 1970).

Studies by Zimney (1965) showed that the P content of the soil correlates well with the P content in the plant, with the Ca in the plant, and with the weight of root nodules. Nodule size and habitat conditions were also found to correlate well. The NH_3 content in the soil, particularly where present in large amounts, was found to exert an unfavourable effect on the total N content in the plant. He considered it to be a species of economic value and suitable for introduction into the habitats of periodically moist meadows.

In copper deficient peat soil, pH 3.5-4.0, L. uliginosus survived and nodulated normally without treatment with copper. White clover required 0.9 kg CuSO_4 /ha and red clover 3.6-7.2 kg CuSO_4 /ha for normal growth (Parle, 1958).

Work on the tetraploid L. uliginosus cv. Maku has shown it to be widely adaptable. In revegetation studies on high altitude acid subsoils, in New Zealand, it was superior to commercial diploid Lotus, in both field establishment and yield. It was also shown to be one of the outstanding legumes on a north facing sunny area at 1060 metres above sea level. It established well on a shaded face at 1060 metres above sea level and on a level site at 1310 metres. In the absence of protective tussock cover Lotus plants were subjected to severe frost heaving to the detriment of winter survival (Barclay and Lambert, 1970). Cv. Maku can survive in the hill areas of New Zealand particularly where

soils are too moist, too acid or too infertile for high productivity from white clover. It will establish, flourish and persist in most soil types and sites but is of special value under wet conditions, in shady and hill side situations and on poor, low pH soils (Armstrong, 1974).

4.11 Establishment

One of the features limiting the use of Lotus is its poor establishment. This has been attributed to small seed size, low seedling vigour, poor seed bed preparation, its inability to compete successfully against associated species, and poor nodulation. Attempts to improve establishment of Lotus, by the Grasslands Division of DSIR, New Zealand, resulted in the development of three diploid and two tetraploid selections. Grasslands Maku, a tetraploid, is now available commercially from New Zealand, and its increased seed size gives the greater seedling vigour common to most induced tetraploids. It is of extreme benefit in the establishment stage especially when sown at a slightly higher rate; 3 kg/ha is advocated by Armstrong (1974) to provide a vigorous stand. Increasing the seeding rate has been shown markedly to increase yields of Lotus (Lowther, 1976a). In experiments comparing the growth and establishment of L. pedunculatus cv. Maku and T. repens cv. Huia, fewer Lotus seedlings established than those of white clover at the low seeding rate of 2 kg/ha. At the higher seeding rate of 10 kg/ha Lotus outyielded white clover in both the absence and presence of lime. Increasing the seeding rate also markedly increased the dry matter/m² of Lotus. Brock and Charlton (1977) used high seeding rates (3 to 5 kg/ha) to ensure adequate numbers of seedlings for good establishment.

Early spring sowing is generally most successful in northern regions. The actual date of sowing varies from mid-March to June but Macdonald (1946) showed that sowing later than the first week in June was not advisable. Autumn sowing (because of low seedling vigour) does not

allow the seedling enough time to develop to a stage capable of winter survival (Winch, 1961). The seedlings formed from L. pedunculatus seeds sown in spring and developing under warmer conditions will have 4-5 shoots/crown present. With autumn sowings, however, seedlings developing during subsequent cooler conditions will have fewer shoots/crown (2-3) but with slightly increased numbers of prostrate aerial shoots compared with those sown in spring (Sheath, 1977). Such autumn sown L. pedunculatus was generally poor despite relatively good initial establishment and Brock & Charlton (1977) attributed this to vigorous competition from ryegrass and white clover which rapidly dominated the areas. In contrast spring sown Lotus established satisfactorily because spring sown ryegrass was much less competitive.

Several workers have reported sparse stands and slow establishment of Lotus under conditions of limited seed bed preparation. Williams (1953) designed the following experiments to determine the seed bed condition requisites for successful establishment of L. corniculatus in hill pastures. L. corniculatus cv. Empire, 6 lbs, was sown in a mixture with Phleum pratense 8 lbs but no inoculum was added.

- (a) No tillage - control;
- (b) Spring tooth harrowing twice to a depth of 1.5 inches;
- (c) Field cultivation twice to a depth of four inches;
- (d) Field cultivation six times to a depth of four inches;
- (e) Ploughing to six inches followed by spring tooth harrowing twice.

The population of Lotus plants increased from one plant/sq. yd. for the control up to a maximum of 75 plants/sq. yd. on the ploughed plots. Yield increased progressively with seed-bed tillage intensity and vegetation kill from 800 pounds/ac. for the control to 3,000 pounds/ac. on the ploughed plots. The quality of the forage was enhanced by an increase from 0% legume in the control to 43% legume content in the ploughed treatment.

Precision placement of seed and fertilizers was carried out by Duell (1964) on soils of low fertility. Band seeding did not contribute to the successful establishment of birdsfoot trefoil to the degree that it did with lucerne, and both legumes showed reduced emergence when their seeds were close to concentrations of soluble fertilizers while soil moisture was low. Bryant (cit. Duell) found that more seedlings of birdsfoot trefoil were present where a general fertilizer was broadcast rather than banded. Banding with a phosphate and potassium mixture, however, resulted in seedling weights significantly higher than those which resulted from broadcasting a similar fertilizer. Banding seed in seven inch rows gave significantly better initial stands of birdsfoot trefoil over broadcast seeding but banding did not affect yields either when seeded alone or in mixture (Baylor, 1959). Band placement of fertilizer, however, did not improve yield or winter survival over broadcasting. Forbes (1959) attributed band placement responses to closer positioning of fertilizer in low fertility soils. Higher levels of L. pedunculatus establishment were obtained in mixed pastures by broadcast sowing compared with drilling in early spring (Brock & Charlton, 1977).

Lotus is known to be productive on low fertility hill country where it has low lime and phosphate requirements (Greenwood, 1961; Brock, 1973). Lowther (1976a) found that the addition of lime did not appear to increase growth of Lotus on low fertility grassland tussock soil of pH 4.6 but it did increase the establishment of Lotus seedlings. This may be a reason for a relatively greater dry matter production of Lotus sown at a low seeding rate and with added lime. Unlike Brock's (1973) results where Lotus outyielded white clover only under conditions of low P, Lowther (1976a) found the yield advantage of Lotus increased with increasing P levels. Brock & Charlton (1977) advocate that for mixed pastures on high fertility there should be no fertilizer application at sowing. Nitrogen fertilizer application resulted in a

60% reduction in size of Lotus plants and reduced content of Lotus in the herbage presumably through increased competition from companion grasses. Superphosphate applied at spring sowing increased white clover growth to the detriment of Lotus while autumn applications at sowing benefitted white clover but only in the absence of Lotus. These results on high fertility pastures are the reverse of those of Lowther (1976a) on the acid tussock grasslands.

Any reduction in competition will aid establishment of Lotus. The effect of varying degrees of competition on the number and size of birdsfoot trefoil plants in the seeding year and their performance during the following two years was studied by Scholl & Brunk (1962). Yields of weed-free legume dry matter, produced during the year of seeding, varied from a minimum of 1.1 kg/ha on plots in which the companion crop of oats was harvested for grain, to 416 kg/ha in plots kept free of all other vegetation. Yields of birdsfoot trefoil in the year following seeding varied significantly and were more closely related to the size of the trefoil seedling in the autumn of the seeding year than to the numbers of seedlings at that time.

Weeds are always found to be a problem when birdsfoot trefoil is seeded alone (Baylor, 1959) and these must be controlled either by mowing or by herbicide applications. The methods used to control the weeds have an effect on yields during the first and second years of the sward as shown by Scholl and Brunk (1962). Mowing of weeds also reduced the trefoil plant population (Winch, 1961) so that chemical weed control was deemed to offer most promise. A method of reducing the spread of white clover involves direct drilling of alternate rows of Lotus and white clover separated by a ryegrass row (Brock & Charlton, 1977). White clover can also be suppressed by application of ethofumesate (Brock & Henderson, 1976). Suppression of white clover took place in areas where a mixture of white clover and Lotus was required because a proportion of Lotus gave adequate control against grass grub and

did not cause bloat.

Lack of nodulation or inadequate nodulation may at times be a contributing factor towards poor establishment since the plants are likely to be deficient in N. McKee (1961) found that inadequate nodulation, or inhibition of nodulation was more common in birdsfoot trefoil than in cv. Vernal lucerne or red clover and was associated with the relative shade intolerance of birdsfoot trefoil. It is recommended that seed be inoculated before sowing especially where there is no history of Lotus rhizobia in the soil (Armstrong, 1974). In the absence of inoculations Lotus almost completely failed to establish on hill sites but Lowther (1976b) indicated the need for caution in recommending the use of pelleting on L. pedunculatus. Gum arabic adhesive alone appeared to be the most consistently effective treatment for retaining Rhizobia and increasing the establishment of Lotus although its effect was more marked after fifteen days storage. As a general rule Norris (1965) recommended the use of rock phosphate as a suitable pelleting material for alkali producing rhizobia; Lowther (1976b), however, found that a coating of this type reduced Lotus establishment even with one day's storage and only when mixed with dolomite (50:50) did it appear to be a more suitable coating material. In an acid site where establishment of Lotus had been increased by broadcast lime, coating the seed with lime or Gafsa-phosphate dolomite had no effect on the establishment of Lotus sown one day after inoculation (Lowther, 1976b). It therefore appears that further work is required into inoculation and pelleting of Lotus on acid soils as apart from an effect on establishment, broadcast lime does not increase long-term Lotus growth on this soil. The establishment phase of Lotus extends until adequate effective nodulation, N₂ fixation, tap root and rhizome development have occurred to increase persistency and this could take up to two years from sowing (Brock & Charlton, 1977).

4.12 Persistence

Persistence of Lotus under various management practises has been studied by several workers. Wedin et al (1967) looked at the persistency, yield and suitability for grazing of pastures renovated with birdsfoot trefoil compared with fertilised grass and unimproved pasture. Over the eight year period the pastures renovated and seeded with birdsfoot trefoil were the most productive in terms of beef product. In the second year pastures renovated with birdsfoot trefoil provided excellent grazing but were allowed to set seed between June 12 and July 9, thereby benefitting the stand. These pastures provided 22% more grazing days than the fertilized grass and unimproved pastures over six years respectively.

Persistence of birdsfoot trefoil under grazing management has been shown to be greater under rotational rather than continuous grazing. The amount of birdsfoot trefoil in a mixture with Poa pratense gradually decreased with continuous grazing and was virtually eliminated by the end of the third grazing year (Davis & Bell, 1957). A "fair" to "excellent" stand of trefoil remained with a rotation system of ten to sixteen days grazing and a rest period of twenty-four to thirty days. Davis & Klosterman (1959) maintained a satisfactory stand of European birdsfoot trefoil for four years under rotational grazing by cattle.

Van Keuran & Davis (1968) looked at the effect of grazing management and of Hereford steers and California crossbred lambs in the persistence of Empire and Viking birdsfoot trefoil. A good stand of Empire (a prostrate cultivar) was maintained under rotational grazing during the entire six year period with no significant difference between the effect of cattle or sheep grazing. Empire continuously grazed by cattle had only a "fair" stand for the final three years, apparently leveling off at a low degree of persistence. A "poor" stand was found under continuous grazing by sheep but it persisted at a low level making minor contributions to the forage. Cv. Viking under rotational grazing

behaved in a similar manner to Empire under the same conditions. The Viking stand declined rapidly under continuous grazing by both cattle and sheep, contributing little forage. The stand declined more rapidly under continuous grazing by sheep than by cattle. Rotational grazing provided more total liveweight animal gains/ha for the six years than continuous grazing within each animal species. Empire produced more total gain/ha over all treatments than Viking for the six years and also within each animal species for the six years. The best Lotus establishment (28% of total yield) under sheep grazing on fertile land resulted from infrequent grazing. In the second year, Lotus was virtually eliminated even although resting for a hay crop in summer took place.

It had been suggested by Smetham (1977) that selective grazing would be a major problem for Lotus under low stocking rates and selective grazing may be reduced at higher stocking rates. The high stocking rate in Brock & Charlton's (1977) trial, however, did not reduce selectivity. These authors therefore advocated lenient and infrequent grazings for successful establishment.

Birdsfoot trefoil in parts of America has been shown to increase in pastures from a very low proportion of a sward to where it constitutes approximately half of the herbage in summer (Templeton et al, 1967). This occurred under each of the following spring grazing managements in an area where a decade previously it was generally agreed birdsfoot trefoil had no useful place:

- (1) Grazing initiated from April 10-30;
- (2) Grazing initiated from April 20-May 15;
- (3) Grazing initiated or cut for hay from May 25-June 15.

Grazing during the summer was delayed from 8-10 weeks to permit the birdsfoot trefoil to produce seed. These observations suggest that under a management programme which will permit natural reseeding for a few years birdsfoot trefoil shows promise for improving pasture

production in some areas where it has been thought to have little or no value.

4.13 Effect of cutting

The stage of development that Lotus plants are at when cutting takes place can greatly influence the yield of forage. Kostov (1973) found in field trials that L. corniculatus gave higher yields of crude protein (CP) and dry matter (DM) when it was cut at the flowering stage in the first year and at the bud stage in following years. A low yield and low CP was obtained when the plots were mown in the first year at formation of 1st pods and at full bloom, followed by grazing phases the following years. Pierre & Jackobes (1953) found that cutting L. corniculatus at the full bloom stage produced a higher yield than cutting at pre-bloom, 1/10 bloom or maturity. Langille and Calder (1971) cut L. corniculatus at four different growth stages: vegetative stage four times/year; 10% flowering three times/year; 50% flowering two times/year and 75-100% flowering two times/year. The highest average yields, best regrowth and greatest nodulation were obtained by cutting at the 75-100% flowering stage. Work by Langford (1950) showed that Lotus withstands close mowing in the seedling stage. When the main stem is severed, new shoots arise from axillary buds. Seedling survival was somewhat lower under close and frequent cutting than under more lenient defoliation, whereas established stands of trefoil persisted longer and produced more DM under light infrequent mowing. Old stands deteriorated rapidly under close and frequent clipping. Stands of Lotus cut at early bloom persisted longer than those mowed later.

The height of stubble remaining after cutting and the frequency of cutting is very important to the persistence of trefoil. Work by Grueb & Wedin (1971) showed that a storage capacity for high levels of total nonstructural carbohydrate (TNC) is present in trefoil.

Trefoil failed to accumulate carbohydrate during the major portion of the season although sufficient excess should have been available for storage in the roots; instead it exhibited a strong tendency throughout the summer towards indeterminate growth. Although some small changes occurred in TNC levels there was no increase until during the regrowth period. At this time TNC% increased while flowering occurred and dry matter accumulated above ground. It was thought that a reduction in temperature or a change in photoperiod induced these higher levels of stored TNC. The importance of leaving a stubble height adequate to maintain a level of photosynthesis which will provide the energy for regrowth is therefore emphasised because of this low store of carbohydrates present in the roots throughout the season. Work by Pierre & Jackobs (1953) with plots cut to a height of one inch produced a higher yield than those cut to a height of four inches every three weeks. It was found the following year that there was a significant reduction in the advantage of cutting to one inch. The four inch cut removed less photosynthetic tissue and the plants responded in the second year with enough additional growth to make up for most of the forage left on the field in the higher stubble. In the third year there was a definite response in favour of cutting at four inches instead of one inch. An effective system was to allow plants to grow to heights of four inches rather than two or three inches before cutting to a height of one inch. Smith & Nelson (1967) found that for stands of cv. Empire, yield, forage and protein were in the descending order of 2, 4, 5 and 6 cuts. During the first year highest yields were obtained when least stubble was left after cutting except for the five and six-cut treatments. Again, in the second year a tall stubble was needed to maintain a high forage yield with trefoil, under all cutting frequencies, and this was increasingly apparent as the cutting frequency was intensified.

Langille et al (1968) seeded cv. Empire and Viking with timothy

cv. Climax and subjected them to a cutting management of four cuts at pre-bud stage, three cuts at 10% bloom, two cuts at 50% bloom and two cuts at 75-100% bloom. The latter system produced most forage in both years and the root weights were generally higher for the two cut system. The etiolated regrowth from birdsfoot trefoil did not differ significantly due to the management in either year, nor did the % TNC. Frequent harvesting reduced the forage production and the amount of K removed from the soil by the plant. The effect of frequency of cutting on L. uliginosus wild and cv. Maku type, L. corniculatus cv. Leo and T. repens cv. Huia, was studied in a heated glasshouse by Simpson (1977). Five different cutting regimes, over a period of 16 weeks were used: (1) cut at six weeks and weekly thereafter; (2) cut at six weeks and every two weeks thereafter; (3) cut at six weeks and every four weeks thereafter; (4) cut at six weeks and at end of trials; (5) cut at end of trial. The plants were cut back to 2.5 cm at each cutting. Yields of fresh and dry forage increased successively from treatment 1 to 5 in all cultivars. Fresh and dry forage yields were in the following order for the cultivars: Maku - Wild type - Leo - Huia and the survival ability was in the order: Maku - Leo - Wild type - Huia. The main conclusion to be drawn from the trial was that the Lotus species can be defoliated to a low stubble height provided that the frequency of defoliation is not less than four weeks.

Parsons & Davies (1961) found that the DM producing capacity for a birdsfoot trefoil/orchard grass association was greatest in the spring. The highest hay yields were obtained with those treatments (i) which permitted the longest spring growth period and (ii) which had the largest growth interval between harvests. Birdsfoot trefoil, which started spring growth later than the associated grass, made up a large proportion of the hay, as the first cutting was made later in the spring. Production of birdsfoot trefoil was high at late compared to early first cutting dates. The density of trefoil was found to be less

with those treatments that were last cut the previous autumn. Gasser & Lachance (1969) found that a late cut in October did not adversely affect DM yield on stands of L. corniculatus cvs. Viking and Empire. Three cuts did not affect the Viking stand but reduced the Empire the following year.

Protein in the forage followed inversely the same pattern as that of the DM yields i.e. where intervals between cuts were shortest the protein content was highest. The total available carbohydrate and the N content of the roots were lowest following the treatments which had the shortest intervals between them.

The effect of shading and defoliation on the growth of root and nodules of T. repens, T. pratense and L. uliginosus was studied by Butler et al (1959). These caused a marked depression in the % of pink nodules and no new nodules were formed. Shading brought root growth almost or completely to a halt. Lotus had a rapid loss of nodules and roots under both treatments with only slight regrowth occurring in the defoliated plants. Lotus plants reacted as quickly to the treatments as did white clover but showed a slower capacity for recovery. All three species showed that under recurrent defoliation the root systems underwent a cyclic pattern of decay and renewal.

Mitchell (1956) subjected T. repens, T. subterraneum and L. uliginosus to a treatment with defoliation to approximately 0.5 inches, resembling hard grazing. Defoliation reduced the quantity of tissue formed by plants relatively more at 22°C than at 11°C. There was a relative reduction in the rate of stem initiation and a decrease in stem elongation of Lotus after defoliation. Defoliation and shading both tended to reduce the appearance of leaves and defoliation also reduced the dimensions of subsequent leaves and petiole thickness. At 22°C the shoot/root ratio was increased more by shading than by defoliation. Defoliation at the low temperature increased the proportion of root in white clover but made no difference at harvest to

Lotus. This is in agreement with Butler et al (1959) who found a rapid cyclic pattern with the root systems in white clover after defoliation. On defoliated plants of all three species there was a zone at third to a half way down the root system where nodule development was poorer than either above or below. Nodulation had been occurring in this area of root at the time defoliation took place.

In a closely grazed pasture the growth habit of Lotus militates against its spread. The plant has relatively small leaves set close to stems that often grow well above ground. Grazing will therefore remove not only the leaves but also the stems with their relatively large apical meristems. As Lotus plants form comparatively large numbers of stems there would generally be a considerable number of active meristematic centres remaining after defoliation from grazing but these would probably be on small stems for many of which active internode elongation would not have commenced. They would therefore initially form less tissue than the large apical meristems removed. This suggests that recovery from defoliation would be slower than in subterranean or white clover in which the meristematic centre of tissue formation at the shoot apices are not normally removed during grazing.

4.14 Effect of light

The phytochrome system is thought to be partially responsible for the growth habit of birdsfoot trefoil plants.

The critical daylength of between 14-15½ hours for causing a large percentage of the plants to shift from decumbent to intermediate or intermediate to upright forms appears to be the same as the critical daylength for flowering (Nittler & Kenny, 1965). The long photoperiods of light rich in the red spectrum slow down the production of secondary stems suggesting that this production is partly controlled by the phytochrome system. Nodulation in leguminous plants can also be affected by the phytochrome system. Leguminous plants grown under light

conditions resulting in the production of the same amount of dry matter produce more nodules when the shoot is exposed to red light than when exposed to blue light (Lie, 1969). He observed that exposure of the shoot to far-red light (730 nm) for a few minutes at the end of the photoperiod reduced the number of nodules formed. The inhibitory action of far-red light on nodule formation was partly reduced by subsequent irradiation with red light (660 nm) provided that the same part of the plant was exposed to both far-red and red. When different parts of the plant were exposed to red and far-red light respectively there was no interaction between the two kinds of light on nodulation, suggesting that any effect of the red or far-red light is not translocated. The interaction between red and far-red light in the nodulation process, whether these are applied to the shoots or the roots, demonstrates that nodulation is controlled by the phytochrome system. From experiments where plants were exposed to far-red light at different periods of time from inoculations, it was deduced that far-red light acts directly on the symbiotic system and not indirectly by prior effect on the root system.

Grabbelaar et al (1971), working with beans, found that light immediately before inoculation stimulates the early stages of the nodulating process. Light during the actual infection process appears to be either without effect or slightly stimulatory. During some later stage in the development of nodules but before they become microscopically visible light does permanently arrest the further development of nodules. The effect of light on nodulation depends both on the length of the light period and its time of application.

Further work by Rhykerd et al (1959) showed that the responses of seedlings to a given quantity of light depended upon the manner in which the light was received. A 12 hour photoperiod at a constant light intensity resulted in the production of a greater amount of dry matter than a 12 hour photoperiod with part low, part high light

intensity. Only at a light quantity less than 51548 lux hrs/day was a high intensity for a short period as efficient as a long illumination at a low intensity. Birdsfoot trefoil produced less top and root growth under all light treatments compared to lucerne and red clover.

McKee (1962) found that plant height, form, top and root weight, foliage colour and nodulation in birdsfoot trefoil were all affected by day-length. The root growth was at a maximum under the natural photoperiod of 8 hours and not significantly different under the 11-13 and 15 hour photoperiod. These responses appeared to be associated with daylength rather than with total radiation which was essentially equal for all plants. Under 9-11 hour photoperiod the birdsfoot trefoil plants were stiffly erect, light green in colour and had greatly elongated internodes.

The effect of differing light energy totals on four periods of seedling development in lucerne, red clover and birdsfoot trefoil was studied by Gist & Mott (1958). Top and root growth for all three species was greatly affected by light treatments ranging from 25,824-154,944 lux per day. Growth of birdsfoot trefoil seedlings under all of the light treatments and stages of development was much less than that of lucerne or red clover. During the period from 26-56 days after emergence the top and root growth of birdsfoot trefoil was less than one third that of red clover. During this period the top growth of birdsfoot trefoil under 154,944 lux per day was less than that of lucerne and red clover supplied with only 58,104 lux per day. The top and root growth responses of seedlings to increasing lux per day were linear and the growth responses of seedlings to different quantities of light varied with the stage of development of the seedlings. During the period from 10-40 days after emergence, top and root growth of lucerne slightly exceeded that of red clover and was approximately three times greater than birdsfoot trefoil. There were also important differences among the three species in their growth response to light

during the various period of growth. The percentage decrease in the growth of birdsfoot trefoil seedlings under decreasing quantities of light did not differ greatly from those of lucerne or red clover.

Investigations by Rhykerd et al. (1959) showed the proportion of leaves to stem of birdsfoot tresoil seedlings increases with increasing light intensity. The leaf/stem ratio of birdsfoot trefoil was low at low intensities and became higher with increased intensity.

Mitchell (1956) subjected L. uliginosus, T. repens and T. subterraneum to two forms of shading at two different temperatures i.e. shading at 50% of full light and at 22°C and 11°C. Lotus grown in full light formed not only axillary branches from successive nodes along the elongating stems but also initiated fresh stems every few days from the thickened crown that developed at the cotyledonary node. Particularly under conditions of low temperature or high light intensity, thickened white stems with rudimentary leaves appeared on plants of Lotus. These stems, which generally originated from the crown, grew down into the soil and could travel for several centimetres before reappearing above ground. With severe shading at high temperatures the whole plant became rather spindly with a limited number of relatively thin but long stems. Under moderate shading at cooler temperatures the plant appeared well adapted for competition in a relatively tall plant association. The aerial stems were robust and elongated faster than those of plants in full light. Both at high and low temperatures the proportion of root was reduced considerably by shading. In general the proportion of nodule tissue was reduced by higher temperatures, shade and defoliation; these effects were cumulative. At full light and low temperatures there were numerous large pink nodules present whereas with shade, at 22°C, nodules were fewer in number, small, round and white.

Butler et al. (1959) found that shading to approximately 75% of full light induced a rapid fading of the pink colour of the nodules

present on the roots of T. repens, T. pratense and L. uliginosus. The nodule colour gradually changed to brown and some of the main roots died. No new root growth occurred on plants kept in shaded conditions. Shading also caused a depression in the percentage of pink nodules present and no new nodules were formed. Lotus plants reacted as quickly to the treatments as white clover but showed a slower capacity of recovery. This partial or complete stoppage of root growth, and senescence or decay of root tissues is expected since shading will reduce the carbohydrate supply to the root system. McKee (1962) noted the appearance on birdsfoot trefoil of the first nodules twenty-three days after seeding, but they were small and ineffective. Utilizing nodule colour, size, numbers/plant and mass as criteria, nodulation was closely correlated with photoperiod. On average the largest nodules occurred on plants produced under the natural photoperiod and the smallest on plants grown under conditions of the nine-hour photoperiod. The Empire and Viking cultivars of birdsfoot trefoil have been shown to require at least 25% of natural day light to be nodulated functionally and 50% to be nodulated adequately (Cooper, 1966). He also found that there was a reduction in the number of nodules in birdsfoot trefoil only under a treatment which utilised 95% shade.

The responses of different cultivars to varying quantities and qualities of light can help distinguish between them; Nittler & Kenny (1965) found large varietal differences in growth habit between three cultivars of birdsfoot trefoil: Empire, Viking and European. To obtain maximum varietal differentiation it was necessary to provide plants with short photoperiods and relatively high light intensity with a limited proportion in the red end of the spectrum. In addition relatively warm (16-28°C) light and cold (5°C) dark periods were also required. Maximum differentiation between early and late maturity in stem length occurred when plants were grown with short photoperiod and light limited to the red end of the spectrum. Maximum varietal

differentiation in stem number was obtained with long photoperiods and light at the red end of the spectrum. Relatively large varietal differences in stem colour, stem diameter and leaflet size occurred under a number of environmental conditions enabling early and late maturity types to be distinguished.

During early growth, legume and grass seedlings are frequently aided by small grain companion crops, weeds or other forage plants. It is generally recognised that the amount of light reaching such seedlings is an important factor in their survival and growth. Due to the recumbent growth habit, short internodes and reduced nodulation induced by a decreasing photoperiod, birdsfoot trefoil seedlings initiating growth in the late summer or early autumn might be at a competitive disadvantage. Conversely, under the longer photoperiods of midsummer the ability of birdsfoot trefoil seedlings to compete with taller growing associated species might be enhanced. The low proportion of leaves and stems of birdsfoot trefoil at low light intensities compared to species such as lucerne and red clover may partly explain the lack of competitiveness of birdsfoot trefoil when being established with a companion crop. It therefore appears that the influence of light intensity and quantity on the proportion of leaves to stems may be an important factor in determining the vigour of the seedlings. Another contributory factor to loss of seedlings in the field is the proportional decrease in nodulation because of reduced light intensity.

4.15 Effect of temperature

Qualls and Cooper (1968) studied over two years the speed of germination, elongation and respiration rate of four varieties of birdsfoot trefoil grown in the dark at three temperatures (15.6, 21.2, 26.7°C). Seed size was kept constant so that differences in growth could be attributed to the rate at which reserves were mobilized and

the efficiency with which they were utilised.

Germination and elongation rate both increased with increasing temperature. Rank order of varieties for both parameters was Leo, Tana, Viking and Empire. Respiration rates increased significantly for all varieties with increasing temperature and there were no significant differences in the rate between varieties. The greatest difference in germination rate occurred between 15.6 and 21.1°C. Maximum respiration took place from 4-8 days of age at 15.6°C and also at 2-4 days of age with 21.2 and 26.7°C.

Germination in varieties of L. corniculatus was delayed by temperatures lower than 15°C and delayed and reduced by temperatures of 30°C or higher (Woods and Macdonald, 1971). The germination of the Empire variety was reduced less than that of Viking or Mansfield by temperatures of 30°C or higher. The relatively lower inhibition of large seeds by high temperatures, their overall speed of germination and their higher speed of germination at 30°C would indicate that large seeds would be more likely to succeed in summer sowings compared to small seeds. The germination of small seeds was reduced more than that of large seeds by temperatures of 25°C or higher.

Germination was buffered against temperature and osmotic moisture stress in the region 10-25°C and 0-4 bars. At higher temperatures or higher osmotic moisture stresses both factors delayed and reduced germination with one factor intensifying the effect of the other.

Cold alternating temperatures have been shown to reduce speed of germination and total germination of legume seeds, including seeds of L. corniculatus (McElgunn, 1973). Although cold constant temperatures did not affect total germination it retarded the rate.

In laboratory trials seeds of eight grasses and eight legumes with high, air dry or low moisture contents were exposed to temperatures above and below 0°C for seven hours before, during and one day after imbibition and then germinated at room temperature. Low temperature

delayed germination in all the species studied but final germination was only reduced in white clover cv. Huia and red clover cv. Hamua; L. pedunculatus cv. Maku was one of the species least affected in final germination by freezing temperatures after imbibition.

Mitchell (1956) studied the effect of a range of temperatures (7.2, 13.1, 18.3, 24, 29.4 and 35°C) on the growth of L. uliginosus, white clover and subterranean clover. The optimum temperature for growth of whole shoot was about 24°C for both L. uliginosus and white clover. More detailed studies were carried out by Mitchell (1956) at two temperatures 11°C and 22°C with the same three species grown at full light and 50% shading. A notable feature with L. uliginosus, throughout the trial, was the contrast between the rapid growth of plants given full light and the higher temperature and their slow growth under cooler conditions. At 11°C the young L. uliginosus plant was small and the actual weight of tissue formed in the fifteen-day growth period was only one-sixth the quantity of tissue formed by subterranean clover. It is not surprising therefore that Lotus is considered a slow establishing plant in cooler districts particularly from autumn sowings. At 22°C the rate of stem initiation by undefoliated plants in full light was greatest with Lotus, least with subterranean clover. A decrease in temperature to 11°C without reduction in light intensity most severely reduced rate of stem initiation by Lotus and had least affect on subterranean clover. Defoliation was found to slow down formation of leaf tissue at 22°C but reduced it by only 36% at 11°C.

Gist & Mott (1958) obtained a temperature/light intensity interaction during the growth of seedling plants of lucerne and birdsfoot trefoil at several light intensities (12,912; 6,456 and 2,152 lux) and temperatures (16, 21, 27 and 32°C). Both top and root growth were much greater at 6,456 lux and 16°C than at 12,912 lux and 32°C. The two legumes responded similarly although lucerne produced more dry matter than trefoil.

Nelson & Smith (1969) found that Vernal lucerne and Empire birdsfoot trefoil flowered almost simultaneously in a cool regime (19/10°C). The warm regime (32/34°C) hastened flowering in lucerne (24 days) but delayed it in trefoil (60 days).

Joffe (1958) found that within the temperature range 27°C day/10°C night and 27/18°C there was little effect of temperature on the rate of production of normal inflorescences of L. corniculatus plants.

Smith (1970) grew one year old legume plants, including birdsfoot trefoil, to first flower in low temperature regimes (a) 32°C day/27°C night, (b) 27/21°C, (c) 21/15°C and (d) 15/10°C. Herbage, root and total plant dry matter yields increased as temperatures decreased except for trefoil in (a) where maturity was delayed and yields were as large as in (d). Herbage shoot height changed little with trefoil as the temperature decreased whereas the protein content decreased. Total nonstructural carbohydrate (TNC) percentage, however, increased in trefoil as temperatures decreased.

Hamilton (1970) studied the effect of three soil temperatures (10, 18.3 and 26.7°C) on competition i.e. timothy grown with lucerne or birdsfoot trefoil. A highly significant effect of temperature on yield was established; the shoot to root ratios of timothy when in association with lucerne or trefoil increased with increasing soil temperatures. Nitrogen had a significant effect on trefoil at the highest soil temperature but no significant effect at the lower soil temperatures. With trefoil at a soil temperature of 26.7°C K increased yields irrespective of N Level but the increase was greater when N was applied. Irrespective of soil temperature, shoot and root ratios when N was applied were at a minimum for trefoil. K had a greater effect than N at 10 and 18.3°C but a somewhat similar one at 28.7°C. Shoot and root ratios increased with increasing temperature for trefoil. In the absence of added N and at the lowest temperature, 10°C, the lack of N was such that the yields from the different crop

associations were alike. Once the available N pool was increased either by microbial action as influenced by increased temperature or added nitrogenous fertilizers then differences between the crop associations were apparent. Trefoil did not respond to added N at the lowest temperatures.

The optimum root temperature for symbiotic nitrogen fixation and growth of birdsfoot trefoil was from 18 to 24°C (Kunelius and Clark, 1970). It was found that although N₂ fixation and growth of birdsfoot trefoil were drastically reduced at 9 and 12°C, compared with 18 and 24°C, some symbiotic combinations could fix N₂ up to 1.5 times more than the less effective ones at low root temperatures. Careful selections of strains of Lotus rhizobia could possibly mean improved early growth of birdsfoot trefoil at lower root temperatures.

Rao (1977) transferred intact nodulated plants of L. corniculatus from 15, 20, 25 or 30°C to 4°C. This stopped nitrogenase activity but activity was completely restored within one hour of returning the plants to their original temperature. Wilkins (1967) demonstrated that strains of Lotus rhizobia from Western New South Wales survived higher temperatures than strains from New Zealand which suggested ecological adaptation of the strains.

Rachie (1954) looked at artificial freezing on persistence of birdsfoot trefoil both in the field and glasshouse. The varieties Empire and Viking had good cold hardiness but were not as hardy as lucerne. The varieties Cascade and Granger were intermediate in cold hardiness. Data which Bubar and Lawson (1959) collected on different varieties, strains and ecotypes of birdsfoot trefoil indicated that their ability, as spaced plants, to survive the winter was genetically determined. It appeared that winter survival acted as a genetically dominant character because in general each progeny survived almost as well as a non-winter hardy parent. Since the use of reciprocal crossing had little affect on winter survival it was assumed that the character

was nuclear rather than cytoplasmic.

Surveys were carried out on Prince Edward Island to determine the extent of the damage to forage legumes, grasses and winter wheat incurred by a severe winter (Suzuki, 1972). Soil temperatures reached record lows, being -12.8, -6.7, 0, +2.2°C at depths of 5, 10, 20, 50 and 100 cms respectively. The top 50 cm of soil remained frozen until May, although subject to occasional alternate freezing and thawing in March and April. Birdsfoot trefoil was damaged to a lesser extent than red clover or lucerne. It was noted that damage to the legumes grown in mixtures with grasses was much less than in pure stands of legumes. Birdsfoot trefoil and lucerne plants which survived through February maintained high vitality of crown and root tissues until the third week of March. New shoots of birdsfoot trefoil did not appear above the ground until May.

Mitchell (1956) noted that particularly under conditions of low temperature or high light intensities thickened whitish stems with rudimentary leaves appeared on plants of L. uliginosus. These stems which generally originated from the crown grew down into the soil and could travel for several centimetres before reappearing above ground. Initiation and expansion of these rhizomes generally occurs in late summer to mid autumn (Sheath, 1977). Accumulation of total non-structural carbohydrates (TNC) is centred on the crown, taproot and rhizome system and increases over the autumn period. Since rhizomes store carbohydrates they are important for winter survival of the plant and enough time should be allocated for rhizome production to ensure persistency of the plants (Brock and Charlton, 1977).

4.16 Acetylene reduction technique

The enzyme system responsible for biological N₂ fixation, the reduction of di-nitrogen (N₂) to ammonia (NH₃), is called the nitrogenase system. It consists of two proteins, one containing

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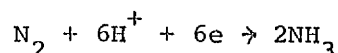
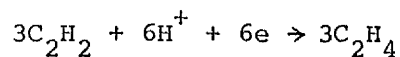
molybdenum-iron and acid labile sulphur (Mo-Fe protein) and the other iron and labile sulphur (Fe protein). Both are essential for activity together with Mg^{2+} and Adenosine triphosphate (A.T.P.). The reduction is always an anaerobic one, in the sense that several of its components are unable to function in the presence of even low partial pressures of oxygen. Both proteins are oxygen sensitive, particularly Fe protein, which is rapidly and irreversibly inactivated (Stanier, 1974). The strict aerobes of the rhizobium group constitute a special case, since they fix N_2 only in the complex environment provided by the root nodules of the host.

The development of root nodules is accompanied by the synthesis of leghaemoglobin, an oxygen binding pigment, of which the spectral properties are modified by oxygenation. Spectroscopic measurements indicate that, in the nodule, leghaemoglobin is largely in the non-oxygenated state (Bergerson, 1962). Since the pigment has a very high affinity for oxygen this suggests that although the rhizobia are strict aerobes the partial pressure of O_2 under the specific conditions that permit effective N_2 fixation is always an extremely low one.

The first evidence that any compound, other than N_2 , could bind to the N_2 fixing site on the enzyme and be reduced was supplied when Mozen and Burris (1954) demonstrated that N_2O was reduced by Azotobacter vinelandii and soyabean nodules. The reduction of azide constituted the next evidence for the low specificity of reduction at the N_2 fixing site.

Shortly thereafter Schollhorn and Burris (1967) also reported the reduction of acetylene (C_2H_2) and its competitive inhibition of N_2 fixation, and Dilworth (1966) independently also observed C_2H_2 reduction and established that the reduction yielded ethylene (C_2H_4). It is now known that the enzyme system can also reduce cyanides, nitrous oxide, methyl isocyanide and some homologues of C_2H_2 , alkyl cyanides and isocyanides.

The knowledge of the non-specificity of the nitrogenase enzyme was utilised by workers in determining a technique for measuring N_2 fixation. C_2H_2 was used as the substrate for this technique because to obtain its product, C_2H_4 , only two electrons had to be reduced i.e. $C_2H_2 - C_2H_4$ compared to say six electrons required to reduce $HCN - CH_3CN$. Further, the detection of the product C_2H_4 is relatively simple by means of gas chromatography. C_2H_4 also inhibits N_2 fixation indicating that it occupies the enzyme site preventing other substrates from binding therefore only C_2H_4 will be produced and this will be directly related to the rate of N_2 fixation. A quantitative relationship between C_2H_2 reduced and N_2 fixed by the nitrogenase enzyme exists, and has been investigated by many workers, notably Bergerson (1970). If the substrate energy supply and reductant supply are not limiting the ratio according to the following reactions is $3C_2H_2$ reduced : 1 N_2 fixed i.e. 3 : 1.



The above ratio is theoretical and in practice is seldom obtained.

Factors which may result in deviations from the above include

(a) differential solubility of the two gases in water, (b) fate of the products of reduction i.e. NH_3 enters a nitrogen pool within the plant, whereas C_2H_4 does not and (c) differences in environmental conditions under which the assays are performed (Hardy et al 1973).

Shubert & Evans (1976) have shown that production of C_2H_4 may replace not only reduction of N_2 but evolution of H_2 therefore raising the $C_2H_2 : N_2$ ratio in some cases to 6 : 1. The acetylene reduction assay is a measurement of activity only for a specific time whereas N_2 fixation as calculated from plant analysis integrates the activity throughout the entire experimental period. To some extent therefore the ratio is a characteristic of the assay rather than an absolute equivalence of C_2H_2 reduced : N_2 fixed. It is therefore advisable to

determine a calibration ratio for the system under test.

Sinclair et al (1978) carried out experiments to determine the accuracy of non-destructive assays and their applicability to different species, conditions and actual plants. The plants were grown in pots containing peat, gravel and vermiculite and raised in a temperature-regulated glasshouse for fifty seven days. The pots were then transferred to controlled environment rooms where temperature and water stress were varied. Molar ratios of C_2H_2 reduced to N_2 fixed were calculated for each species. The ratios obtained ranged from 2.8 : 1 for Huia white clover to 4.3 : 1 for L. pedunculatus cv. 'Grasslands Maku'.

The chemistry of the technique indicated the feasibility of using C_2H_2 as a substrate and measuring the product C_2H_4 . The basic technique is that the enzyme system be incubated in a gas tight container where a certain volume (5%) of air has been removed and the equivalent volume of C_2H_2 gas added. After a given period of time, in which a proportion of the gas is reduced to C_2H_4 , gas is removed and analyzed by gas chromatography. The amount of C_2H_4 recovered will then give a measure of the rate of N_2 fixation under these conditions. The method is applicable to suspensions of free living N_2 fixing bacteria and extracts thereof as well as whole nodulated plants, nodules and bacteroids isolated from nodules and cell free extracts (Hardy et al 1973).

Koch & Evans (1966) published the first data on the application of the assay for symbiotic N_2 fixation having measured N_2 fixation in isolated nodules. First studies utilizing acetylene reduction (AR) as a means of measuring N_2 fixation in symbiotic systems were carried out on root nodules which had been removed from the soil environment and exposed to C_2H_2 when either excised or attached to the root. This use of nodules has several disadvantages in that they are removed from their immediate environment and subjected to various forms of stress

which effects their ability to reduce C_2H_2 , thus giving a false impression of their N_2 fixing capabilities. Nodules must be harvested quickly because their activity declines but they have a tendency to be lost or damaged when being removed from the soil or excised from the root (Moustafa et al 1969). The assay reflects the ability of the nodules to fix N_2 at the time of harvest only and therefore to obtain a quantitative estimate of N_2 fixed an integration of results obtained at several sampling times per day is required. Conducting such tests involves destruction of the plants.

Hardy et al (1969) introduced a sampling method which involved the incubation of roots. Although this technique did not involve whole plants the very fact that soil was still enveloping the roots suggested that tests could be carried out on a complete plant in soil samples. Lie (1971) was the first to follow N_2 fixation activity in undisturbed soil by use of the AR assay. He utilised air tight polythene jars and injected the C_2H_2 into the soil through the bottom and gas samples were drawn off at intervals throughout the test.

The method now widely used in the field for following N_2 fixation was devised by Sinclair (1973). Intact plants growing in soil were placed in air tight transparent plastic containers equipped with a serum stopper in the lid to allow injection of the C_2H_2 gas into the container and removal of the product gas after a period of incubation. He showed that the reduction of C_2H_2 by the nodules when they were surrounded by soil compared to the rate achieved when nodules were directly exposed to C_2H_2 was sufficiently rapid to make the techniques valid provided incubations were not too short. It must be noted, however, that the success of the test depends on rapid movement of gases through the soil and when soil conditions prevent this the assay will fail.

The technique has the following advantages:

- 1) It allows repeated measurements of individual samples over a range

of time scales from hours to seasons. It therefore reduces the number of replicate samples required in experiments where the course of N_2 fixation is being followed.

- 2) The task of having to isolate nodules at the risk of destroying or damaging them and thus affecting the N_2 fixation results is eliminated.
- 3) Since the symbiotic system is not removed from its immediate soil environment there is a greater chance of obtaining conditions in which N_2 fixation would normally occur.
- 4) The equipment used with the assay is easy to handle and easily transportable to field sites. The detection of the C_2H_4 product is also very simple involving use of a gas chromatograph.
- 5) C_2H_2 can act as an internal standard which enables detection of gas leakage from the apparatus.

Since the assay is affected by technical and environmental factors care should be taken both in procedure and interpretation of the results. This therefore entails various precautions when carrying out the assay:

- 1) Controls to measure C_2H_4 production by the plants, incubation chambers and that contained in commercial C_2H_2 gas should be included.
- 2) Preliminary tests should be carried out to ensure that the incubation containers are gas tight.
- 3) Care should be taken to ensure that the temperature within the incubation containers does not fluctuate widely.
- 4) All precautions should be taken to ensure that environmental conditions close to that of the natural system are obtained.

The spatial distribution of N_2 fixation in pastures is likely to be extremely uneven because of irregular distribution of legumes and of urine patches in which legumes probably fix little N_2 (Sinclair et al 1976). Thus a large number of samples is required to provide

good representation of the study area. The use of pasture turfs like those utilized by Sinclair (1975) would therefore be laborious. An alternative is to collect small samples such as the 2.54 cm. diameter cores widely used in soil sampling and bulk a large number of these for each AR assay (Sinclair et al 1976). The same authors showed that assays on cores gave similar relative measures of AR activity to earlier assays on the turfs from which they were obtained although the activity of cores relative to that of whole turfs declined markedly with incubation periods in excess of two hours. This was probably due to damage and severing of the foliage. Twelve, 20 or 40 cores were placed in a five-litre white polythene box with a snap-on air-tight lid and were incubated for two hours with C_2H_2 . The method had a high coefficient of variation (CV) probably arising from the very irregular short distance spatial distribution of N_2 fixation in the pastures. Under good conditions, i.e. apparently an even distribution of white clover in small plots not recently grazed, the CV was 25% on a twelve core sample whereas assays on a large area of continually grazed pasture required a twenty core sample for a similar CV. Clearly the assay requires large numbers of cores per sample to distinguish small treatment effects.

Hoglund & Brock (1978) utilise fourteen 25 mm diameter 75 mm deep soil cores selected randomly from each paddock and bulked in one litre incubation jars. The time course graph of C_2H_2 production against time gave linear rates of C_2H_4 production over two hours, after an initial lag of five minutes. Workers in New Zealand study N_2 fixation by Lotus in the field utilising fourteen 2.5 cm diameter cores per one litre incubation jar but incubations are for only one hour (Charlton, pers comm).

5. THE OBJECTIVES OF THIS WORK

The Literature Review gave the basic background information about the genus Lotus and particularly with regard to L. uliginosus cv. Maku. It emphasised that most of the work on Maku has been carried out in New Zealand where the results have been favourable and indicate that Maku has a potential for improving low grade pasture. The main problems lie in its poor establishment, management requirements for persistence, and the ultimate definition of its actual role in upland pasture as a plant suitable for grazing purposes or only for pasture improvement.

Management practices to improve N_2 fixation, and hence production, by legumes will depend on the application of information derived from laboratory, glasshouse and field experiments such as those described in this thesis.

The two main objectives of this study were, firstly, to record and analyse for L. uliginosus cv. Maku ~~the~~ morphological and physiological responses in the laboratory and glasshouse to varying conditions such as pH, phosphate level, presence and absence of trace elements, variation in the source and timing of nitrogen supply, cutting regimes combined with different levels of day length and temperature and, where possible, to make comparisons with T. repens cv. Huia or S184; secondly, to investigate the growth, development and nitrogen fixation capability of cv. Maku in an upland acidic pasture.

Nothing was known of the pattern of rhizome development, the distribution of nodules on the root system or the genetic variability within this cultivar and, where appropriate, investigations on these aspects were undertaken.

In most cases growth was measured by fresh and dry weight, and measurements of nitrogen fixation were obtained by using the acetylene reduction technique. The results of the laboratory and glasshouse work were used to interpret later field work and vice versa.

Investigations into improving establishment of Maku had to look first at conditions under which it would grow. An experiment was planned to determine the effect of growth and fixation at five pH levels (3.5-5.5), three phosphate levels (0, 15, 30 kg P/ha) and the presence or absence of trace elements to obtain an indication of the best conditions for Maku growth and fixation. Improved establishment can also be obtained by boosting the growth of legume seedlings at an early stage and experiments were planned to investigate the effect of adding combined nitrogen by varying the concentration, the source and the time of application. Three soluble N sources (NH_4NO_3 , NaNO_3 and KNO_3) at five concentrations (0, 30, 70, 120 and 180 mg N/pot) were added to seedlings transplanted two days after germination. KNO_3 was added alone at two concentrations (30 and 70 mg N/pot) on the following days after transplanting (0, 6, 12, 18, 24 or 30). To determine the effect of a slow release compound fertiliser on growth and fixation by Maku plants a commercial fertiliser "Enmag" was added at four concentrations (0, 30, 70 and 120 mg N/pot) on the day the seedlings were transplanted.

Good establishment is usually obtained if nitrogen fixation can occur shortly after germination. A study was designed to give information on the initiation and distribution of nodules and on N_2 fixation by a field study of the seasonal N_2 fixation of Maku in an upland site.

Persistence of Maku was investigated by following the seasonal growth pattern of rhizomes formed by Maku plants and the role which they played in establishment and persistence. An experiment to investigate the effect of cutting, to simulate grazing, under two light regimes (16 hrs day; 12 hrs day) and two temperature regimes (21°C day/18°C night; 18°C day/15°C night) was planned. The results from this could possibly give some indication of how well Maku persisted and recovered under grazing. An investigation into the morphological

characters of the cultivar Maku was designed to obtain information on the range of variation and winter-hardiness.

Interpretation of the results should allow us to define more accurately the role of Maku.

6. TO DETERMINE THE CALIBRATION RATIO FOR THE
REDUCTION OF ACETYLENE (C₂H₂) TO ETHYLENE
(C₂H₄) BY L. ULIGINOSUS CV. MAKU

6.1 Aim

The acetylene reduction (C₂H₂ → C₂H₄) assay is an indirect measurement of N₂ fixation and a conversion factor (derived from the calibration ratio) is used to express the measured C₂H₂ reduction in terms of nitrogen fixed. Although the conversion factor had been established for various legume species, it is strongly recommended (Sprent, 1979) that the factor applicable to each new system be determined. The aim of the experiment was to determine how close was the theoretical ratio of 3C₂H₂ reduced: 1N₂ fixed to the actual ratio for L. uliginosus cv. Maku, using the assay technique for measuring acetylene reduction (App. I : A). Also incorporated was a time course experiment to detect the presence of a lag phase at the start of the incubation, and to determine if acetylene reduction was constant throughout the incubation period.

6.2 Materials and Methods

Sixty pots of four inch diameter were filled with 250 g of damp Perlite, which was used because it is an inert material and would provide a N-free growth medium. The pH of the Perlite medium was adjusted to pH 4.5 by acidifying Dart & Pates (1959) N free nutrient solution, 100 ml of which was added to each pot weekly. Distilled water was added from the bottom when required. Planted in each pot were fifteen seedlings of L. uliginosus cv. Maku, which had been pregerminated on water agar, 2 days earlier, and 25 ml suspension of a rhizobia strain CC814s, in tap water, was added. The rhizobia suspension was added on the third and tenth day after planting. The plants were thinned to nine per pot one week after planting. Three blocks each containing fifteen pots were set up on a glasshouse bench subject to natural day length (c.14 hrs and temperature

of $20^{\circ}\text{C} \pm 5^{\circ}\text{C}$). The pots were rotated within each block in an attempt to cut down on environmental variation due to position effect. One pot from each block was randomly removed commencing one week after planting of the seedlings until week fourteen when the experiment was terminated.

The Perlite was shaken from the roots of the plants and the plants were then transferred to separate 4.6 litre incubation jars. Control jars were also set up (App. I : A). During the incubation period the jars were kept in natural daylight indoors at 21°C ; incubation was terminated after eight hours and gas samples were removed at the following times during the incubation period:

30; 60; 90; 120; 180; 240; 300; 360; 420; 480 minutes.

The gas samples were analysed by use of the GLC (App. I : A).

After incubation the plants were removed, the numbers and fresh weights recorded of effective nodules, i.e. those capable of fixing N_2 and pink in colour, and non-effective nodules, i.e. those not capable of fixing N_2 . The plants plus nodules were then dried in an oven at 100°C for sixteen hours and the root and shoot dry weights recorded. The N content of the plants was determined using the modified Mitchell's technique (App. I : D).

6.3 Calculation of Ratio

The total N_2 fixed in μmoles was calculated by determining the total nitrogen content of the plants at the start of the experimental period and subtracting this figure from the nitrogen content of plants at the end of the experimental period. The final figure was then converted to a per day basis. The C_2H_4 μmoles produced per day were totalled over the experimental period and the μmoles of N_2 fixed was also calculated for the same period. The ratio of C_2H_4 reduced to N_2 fixed was then calculated using this data. Corrections were made to allow for the fact that C_2H_2 reduction was only detected on week three while a measurement for N content was obtained from week one onwards.

TABLE 1

Weekly molar ratios of total accumulated moles C_2H_4 produced per day by *L. uliginosus* cv. Maku and the total accumulated moles N content per day present in *L. uliginosus* cv. Maku. Accumulated moles total N content/day = N, accumulated moles C_2H_4 produced/day = A. Experiment 6

Week	Block I			Block II			Block III		
	A	N	A:N	A	N	A:N	A	N	A:N
3	0.024	1.112		0.024	1.112		0.024	1.010	
5	0.968	1.742	1.50:1	0.488	2.623		0.663	2.062	
6	1.523	1.913	1.87:1	1.806	1.513	1.44:1	1.097	1.785	1.38:1
7	7.506	3.906	2.68:1	3.749	2.128	3.69:1	5.006	2.623	3.09:1
8	9.895	2.806	5.83:1	8.284	3.737	3.15:1	8.931	4.011	2.97:1
9	12.219	3.270	5.66:1	13.855	5.975	2.84:1	20.510	9.433	2.43:1
10	16.961	6.341	3.24:1	16.863	5.760	3.62:1	23.350	7.168	3.79:1
11	24.677	8.998	3.13:1	29.66	17.685	1.79:1	30.621	13.455	2.46:1
12	32.670	12.253	2.93:1	39.07	13.660	3.11:1	36.462	15.391	2.53:1
13	39.520	19.638	2.13:1	42.92	21.950	2.06:1	41.614	21.483	2.03:1
14	46.51	18.170	2.73:1	43.16	25.302	1.78:1	47.706	16.603	3.06:1
Mean Ratio = 3.17 ± 0.45			Mean Ratio = 2.94 ± 0.31			Mean Ratio = 2.64 ± 0.23			

Week	Mean Ratio
6	2.56:1 ± 0.95
7	3.15:1 ± 0.29
8	3.98:1 ± 0.92
9	3.64:1 ± 1.02
10	3.55:1 ± 0.16
11	2.46:1 ± 0.39
12	2.86:1 ± 0.17
13	2.07:1 ± 0.05
14	2.52:1 ± 0.38

Overall Mean Ratio = 2.98:1 ± 0.21

TABLE 2

Student t tests to determine any significant differences between L. uliginosus cv. Maku plants subjected to an acetylene reduction assay. Experiment 6

Week	Dry Matter (mg)		% Nitrogen	
	Control	Assayed	Control	Assayed
1	8.40	9.90	5.55	4.54
2	23.00	20.33	2.18	2.27
3	35.00	36.93	1.91	1.73
5	55.80	67.13	3.60	3.11
6	82.80	107.80	2.50	1.91
7	148.20	165.40	2.02	2.37
8	198.00	245.80	2.19	2.26
9	341.00	452.27	1.79	2.33
	t = 1.98 @ 7 df N.S.		t = 0.34 @ 7 df N.S.	

TABLE 3

Correlation coefficients to establish any relationships among numbers of effective nodules (NE), fresh weight of effective nodules (FWE) and acetylene reduction (AR) by L. uliginosus cv. Maku plants, over fourteen weeks. Experiment 6

Number effective nodules (NE)	1	1.00		
Fresh weight effective nodules (FWE)	2	0.79	1.00	
Acetylene Reduction (AR)	3	0.86	0.92	1.00
		1	2	3

6.4 Results

The molar ratios for each week are given in Table 1. There was no significant difference in plant dry matter and percentage nitrogen between assayed and non assayed plants (Table 2). A good correlation between numbers of effective nodules, fresh weight of effective nodules and acetylene reduction throughout the experimental period was obtained (Table 3). The time course graphs on the production of umoles C_2H_4 at hourly intervals over an eight hour assay period, for each week, are shown in Fig. 1. Data pertaining to number and weight of nodules throughout the experimental period is noted in Appendix I : D : Table 7.

6.5 Discussion

Nitrogen fixation was first detected in week three and as the growth period increased so also did the rate of fixation and the total nitrogen content of the plant. Not until week six was there substantial C_2H_2 reduction to allow a ratio to be calculated. The low ratios obtained in the first weeks are possibly due to the fact that the nodules were in the process of maturing and the assay conditions may have had some inhibitory affect. As shown on the time course graphs there was only a slight increase in acetylene reduction rate, for weeks five and six, during the assay time. The effective nodules at this point in the growth stage were small and during the assay may have dried out resulting in an inhibition of acetylene reduction. Dehydration has been shown to reduce C_2H_4 production (Hardy et al., 1973). This would therefore give an underestimate of fixation, at that time, resulting in a low ratio.

The mean ratio for the total growth period was 2.98:1 which is a close approximation to the theoretical ratio. The calculated ratio is lower than the 4.3:1 ratio obtained for L. uliginosus cv. Maku by Sinclair et al. (1978). Variations in the ratio are to be expected since the ratio is a characteristic of the method of assay and many factors may influence it (Hardy et al., 1973; Schubert & Evans, 1976).

Although variation in the ratio was obtained between weeks, it was not significant. Due to the lack of a measurable ratio during the first few weeks of growth it would be necessary, when measuring both C_2H_2 reduced and N_2 fixed, to quote the age of the plants.

Comparisons of assayed with non assayed plants showed that subjecting plants to the assay does not affect their dry weight or percentage nitrogen.

The time course graphs showed no lag phase except in younger plants where reduction tended to level off. Young plants should therefore only be subjected to short assays of up to four hours as, after this, activity tends to be inhibited. Activity in the older plants continued linearly for eight hours but at weeks nine, ten and eleven there was a slight reduction in activity. This indicated that assays for this age of plant should be conducted with incubation periods of not longer than six hours in order to obtain accurate results using these techniques.

The good correlation obtained between acetylene reduction, number and fresh weight of effective nodules indicated that increased acetylene reduction was dependent on the increase in production and development of effective nodules as plant growth increased.

The assay method used therefore closely relates acetylene reduction to nitrogen fixation and allows monitoring of changes in the N_2 fixation potential of plants subjected to different treatments. Due to the variation obtained in the ratio it cannot be used as an accurate conversion factor for calculating N_2 fixation in absolute terms. Assay times will vary with the age of the plant and only in the very young plants will a lag phase be present.

7. EXPERIMENT INVESTIGATING THREE SOURCES OF N -
NH₄NO₃ : NaNO₃ : KNO₃

7.1 Aim

The experiment was set up to determine the effects of different sources and concentrations of nitrogen applied at the time of transplanting on plant establishment and N₂ fixation. The overall aim was to obtain data which would give an indication of the source and level of nitrogen which would give a combination of good establishment, maximum yield and N₂ fixation by Lotus and clover plants.

7.2 Materials and Methods

Eightyfour, 4" diameter pots were filled with 250 g of damp Perlite and the pH adjusted to 4.5 by adding acidified Dart and Pate's (1959) nutrient solution. To each of fortytwo pots, nine L. uliginosus cv. Maku pregerminated seedlings were planted per pot and to each of the remaining fortytwo pots nine pregerminated seedlings of T. repens cv. S184. The seeds were germinated on water agar and the resulting seedlings transplanted two days after germination. The seedlings were inoculated by adding 25 ml of a rhizobia plus tap water suspension to each pot. Rhizobium strain CC814s was added to Maku seedlings and strain FA6 to seedlings of S184. At four and ten days after transplanting a further 25 ml of the rhizobial suspension were added to each pot. 100 ml of Dart and Pate's (1959) N free nutrient solution were added to the saucers holding the pots weekly throughout the experimental period and distilled water was added as required. The N sources used were NH₄NO₃ and NaNO₃, at the following concentrations of N per pot: 30 mg, 70 mg, 120 mg and 180 mg. These two sources of N were chosen to compare the effect of the presence of the NH₄⁺ ion and the NO₃⁻ ion. A further source of N, KNO₃ was used with the intention to show that any effects from additions of NaNO₃ and KNO₃ could be due solely to the presence of the NO₃⁻ ion. KNO₃ was added at the following concentrations:

30 mg N/pot, 70 mg N/pot and 120 mg N/pot. The different concentrations of combined nitrogen were dissolved in 200 ml of Dart and Pate's (1959) N free nutrient solution and applied at transplanting by watering on to the top of the rooting medium in the pots. A control series of pots received no combined nitrogen.

The pots were labelled with the appropriate treatments and placed in a controlled environmental growth cabinet where cool white fluorescent light and light from a single tungsten filament bulb were given for 16 hours per day. The temperature was set at 21°C during the day and 18°C at night, and a relative humidity of 70% was maintained. The experimental layout took the form of a randomised block design replicated three times. Each block contained twentyeight pots and the pots were rotated daily within each block to minimise environmental variation due to position in the chamber. Analysis of variance was carried out on the recorded measurements.

Eight weeks after transplanting root and shoot dry weight, root and shoot length, fresh weight and number of effective nodules and acetylene reduction were recorded.

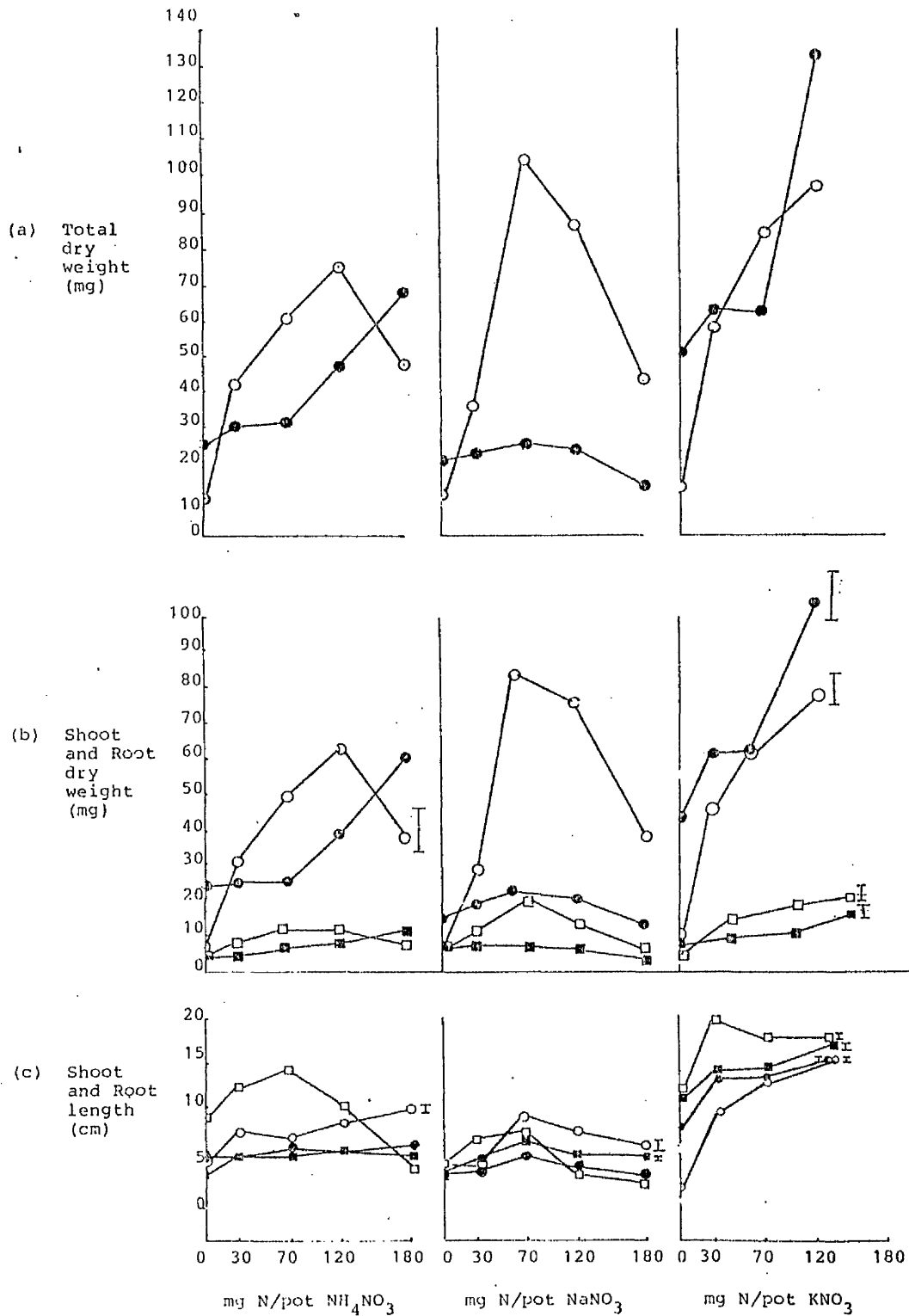
7.3 Results

Total Plant Dry Weight (Fig. 2 a) (App. III : A : Tables 1, 9 and 10)

Analysis of variance revealed that there was no significant difference between the total plant weight of the species. Addition of NH_4NO_3 over the range 30-120 mg N/pot had no effect on the total plant weight of Lotus and clover. However, Lotus significantly ($P < 0.05$) outyielded clover on addition of NaNO_3 . All additions of NaNO_3 had no effect on total growth of Lotus and clover.

There was a significant increase ($P < 0.001$) in total plant weight of Lotus at all levels of KNO_3 with the maximum yield obtained on addition of 120 mg N/pot. Addition of 30 mg N/pot of KNO_3 to clover plants did not increase the total plant weight but further additions of nitrogen

Fig. 2 The effect of varying levels of nitrogen supply (0, 30, 70, 120 or 180 mg N/pot), applied at transplanting as NH_4NO_3 , NaNO_3 or KNO_3 on (a) total dry weight (mg) Lotus \circ , clover \bullet , (b) shoot dry weight (mg) Lotus \circ , clover \bullet , root dry weight (mg) Lotus \square , clover \blacksquare , (c) shoot length (cm) Lotus \circ , clover \bullet , root length (cm) Lotus \square , clover \blacksquare . Plants harvested at ten weeks old. Experiment 7. \bar{x} = SED.



resulted in a significant increase ($P < 0.01$) with the highest yield obtained at a level of 120 mg N/pot.

Dry Weight of Shoot (Fig. 2 b) (App. III : A : Tables 2, 13 and 14)

The general pattern of shoot response for both species appeared to be similar to that of total plant dry weight. There was a significant increase ($P < 0.05$) in Lotus shoot weight at 30, 70 and 120 mg N/pot as NH_4NO_3 with the highest amount of shoot produced at 70 and 120 mg N/pot. Addition of NH_4NO_3 to clover plants had no effect on shoot growth.

Application of nitrogen in the form of NaNO_3 over the concentration range used had no effect on the shoot weight of either clover or Lotus.

Clover showed a significant increase ($P < 0.05$) in shoot weight only at the level of 120 mg N/pot of KNO_3 , while a significant rise ($P < 0.01$) in Lotus shoot weight occurred on addition of 30, 70 and 120 mg N/pot KNO_3 with the maximum effect being achieved at both the 70 and 120 mg N/pot level.

Dry Weight of Root (Fig. 2 b) (App. III : A : Tables 3, 11 and 12)

There was no effect on dry weight of Lotus or clover roots when N in the form of NH_4NO_3 or NaNO_3 were applied. However, Lotus root weight significantly outyielded ($P < 0.01$) clover when NaNO_3 was added at 70 mg N/pot.

Lotus significantly outyielded clover root weight when KNO_3 was applied. All levels of nitrogen significantly increased ($P < 0.01$) the root weight of both species with increasing additions of N resulting in increasing root weights.

Shoot Length (Fig. 2 c) (App. III : A : Tables 4, 17 and 18)

Application of nitrogen in the form of NH_4NO_3 and NaNO_3 over the concentration range used had no effect on the shoot length of clover whereas Lotus shoot length increased significantly ($P < 0.01$) with increasing additions of NH_4NO_3 , the greatest responses occurring on addition of 70 or 120 mg N/pot.

In general the shoot length of both species was significantly increased ($P < 0.01$) with increasing additions of N as KNO_3 ; the maximum

length was obtained on addition of 120 mg N/pot.

Root Length (Fig. 2 c) (App. III : A : Tables 5, 15 and 16)

Addition of NH_4NO_3 had no effect on root length of both species.

However, addition of NaNO_3 significantly increased ($P < 0.05$) the root length of clover plants on addition of 30 mg N/pot but a significant decrease ($P < 0.05$) resulted on addition of 120 mg N/pot. There was no significant effect on root length of Lotus plants with the addition of NaNO_3 .

Clover root length remained unaffected by the various levels of N applied as KNO_3 except at 120 mg N/pot but there was a significant increase ($P < 0.01$) in Lotus root length at all levels of N as KNO_3 with a maximum length of root occurring at 30 mg N/pot of KNO_3 .

Number of Effective Nodules (Fig. 3 a) (App. III : A : Tables 7, 21 and 22)

NH_4NO_3 added to both species had no effect on the numbers of effective nodules.

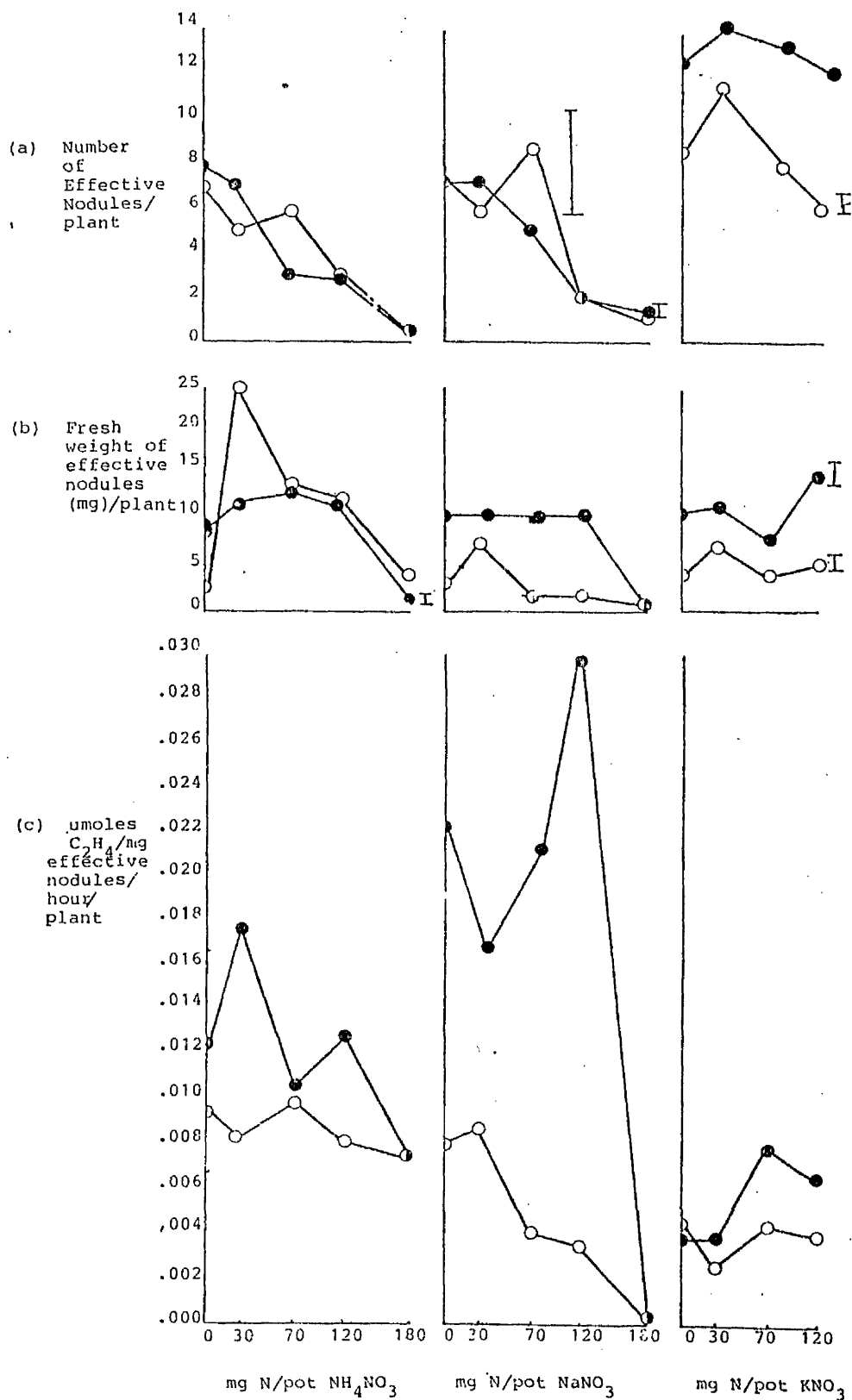
There was no change in the number of Lotus and clover effective nodules on addition of 30 mg N of NaNO_3 but higher levels of 120 and 180 mg N significantly decreased ($P < 0.05$) the number of clover and ($P < 0.01$) of Lotus effective nodules below that of the controls. At a level of 70 mg there was no change in Lotus nodule number but a decrease occurred in the clover nodule number.

Addition of 30 mg N/pot of KNO_3 significantly increased ($P < 0.05$) the number of Lotus effective nodules whereas addition of 120 mg N/pot resulted in a significant decrease ($P < 0.05$) in numbers. It is of interest to note that 70 mg N had no effect on Lotus nodule number as had the addition of all levels of KNO_3 to number of clover nodules.

Fresh Weight of Effective Nodules (Fig. 3 b) (App. III : A : Tables 6, 19 and 20)

No increase in the weight of Lotus effective nodules was observed with the different levels of NH_4NO_3 . There was a significant increase ($P < 0.05$) in the weight of clover nodules when NH_4NO_3 was added with the maximum level obtained at 70 and 120 mg N/pot.

Fig. 3 The effect of varying levels of nitrogen supply (0, 30, 70, 120 or 180 mg N/pot), applied at transplanting, as NH_4NO_3 , NaNO_3 or KNO_3 , on (a) number of effective nodules, (b) fresh weight of effective nodules (mg), (c) umoles C_2H_4 /mg effective nodules/hour. Lotus \circ , clover \bullet . Plants harvested at ten weeks old. Experiment 7. $\text{I} = \text{SED}$.



Increasing additions of NaNO_3 resulted in no change in the weight of Lotus effective nodules while complete inhibition occurred on addition of 180 mg N/pot. The addition of NaNO_3 to clover plants had no effect on weight of effective nodules.

Addition of KNO_3 had no effect on weight of Lotus effective nodules whereas all levels of KNO_3 significantly increased ($P < 0.05$) the weight of clover effective nodules. The maximum weight of clover effective nodules was obtained on addition of 120 mg N/pot.

Acetylene Reduction (Fig. 3 c) (App. III : A : Tables 8, 23 and 24)

All levels of NH_4NO_3 , NaNO_3 and KNO_3 had no effect on acetylene reduction by either Lotus or clover plants but clover significantly ($P < 0.05$) fixed more N_2 than Lotus plants with NaNO_3 addition.

7.4 Discussion

The significant increases in total growth produced by Lotus and clover when nitrogen in the form of KNO_3 was added at different levels was similar to the findings of Allos and Bartholomew (1955). Nitrogen applied at 108 mg/pot over ten weeks stimulated growth of both L. corniculatus and L. uliginosus and it was suggested that symbiotic N_2 fixation may therefore have limited Lotus growth at least under the growth cabinet conditions of their experiment. Fixation was not increased by the addition of N therefore the increased total growth by Lotus during this study was due to the added combined N rather than nitrogen supplied by an increase in N_2 fixation.

A major fact to emerge from the present results was that shoot growth was increased to a greater extent than root growth when NH_4NO_3 and KNO_3 were added to Lotus and clover plants. This phenomenon has also been demonstrated by Barta (1979). This suggests that most of the

extra carbohydrate produced was used for shoot growth and the shoot therefore appears to be a very dominant sink for assimilate even to the detriment of root growth. The addition of all forms of N increased the shoot length of Lotus plants resulting in the production of spindly plants but this did not occur with clover. The increase in shoot yield must therefore have been caused partially by stem growth in Lotus and leaf production in clover. This may result in tall Lotus plants being preferentially grazed before low growing clover plants.

The inhibitory effect on effective nodule number in clover plants on addition of 120 or 180 mg N/pot as NaNO_3 and Lotus plants on addition of 180 mg NaNO_3 and 120 mg KNO_3 tends to indicate that either free living rhizobia in the soil or infection by rhizobia and nodule development are adversely affected. For infection to take place in clover plants root hairs have to preferentially absorb infective Rhizobium trifolii. This involves the specific binding of a carbohydrate - binding protein trifoliin found on root hairs, to unique carbohydrate structures found exclusively on the surface of R. trifolii. The addition of fixed N prevents R. trifolii from accumulating in high numbers on clover root hair surfaces thus possibly reducing infection and therefore nodule numbers. It is not yet known whether fixed N prevents accumulation or formation of trifoliin on the root hair surface or whether trifoliin that is present is somehow modified or masked (Dazzo & Brill, 1978). No evidence of binding sites for infective rhizobia have been quoted for Lotus, but rhizobia which nodulate birdsfoot trefoil are specifically accumulated in the rhizospheres of the host and are stimulated by the hosts root exudates (Summerfield & Bunting, 1980). Addition of fixed N may therefore alter the root exudate resulting in less stimulation of the rhizobia towards the root thus less infections. Infection threads may be affected by fixed N as addition of nitrate to Medicago sativa was found to inhibit formation of infection threads and augment the proportion of arrested infection threads to such an extent that nodule

number was inhibited. (MUNNS, 1968)

The reduction in effective nodule number of Lotus plants when increasing concentrations of NaNO_3 and KNO_3 were added and to clover plants when increasing NaNO_3 was added was similar to the findings of Pankhurst and Jones (1979). When compared with low levels of N (30 mg N/pot) higher additions (70 and 120 mg N/pot) resulted in a decrease of effective nodule numbers. The remaining nodule tissue must have had a higher specific activity because it maintained a level of acetylene reduction activity similar to that of the controls, perhaps through increasing the level of nitrogenase.

Addition of NH_4NO_3 and KNO_3 at 30 mg N stimulated existing nodule fresh weight of clover but did not stimulate production of new nodules. The existing nodules may well have acted as strong sinks for the increased carbohydrate obtained on increased growth of these plants on addition of nitrogen.

The main value of the experiment has been to show that addition of KNO_3 at two days after germination will increase total growth of Lotus and clover plants without affecting N_2 fixation. The results highlighted the beneficial effect to both species of adding KNO_3 compared with the addition of NH_4NO_3 and NaNO_3 and illustrates the point that different sources of N affect species in different ways. KNO_3 should therefore be used in future field work to aid establishment of both Lotus and clover plants. The variation in results between the species on addition of different sources and levels of nitrogen exemplified the complexity of the interaction. The facts emphasised the need for further study in the field to determine the sources and levels of nitrogen required to obtain a combination of maximum yield and fixation rate by the legume. However, caution must be exercised when extrapolating this data to field work as account must be taken of the effect of soil nitrogen and the environmental conditions.

8. EXPERIMENT INVESTIGATING THE EFFECT OF TIMING
OF APPLICATION OF KNO₃

Introduction

After a study of the results from Experiment 7, it was decided to use KNO₃ as the nitrogen source in an experiment dealing with the effect of time of nitrogen application on Lotus and clover. KNO₃ was chosen because it released N quickly and gave the highest increase in growth without affecting nitrogen fixation by both species when added at low levels (30 and 70 mg N/pot).

Work carried out to study the calibration ratio (App. I : D : Table 7) for L. uliginosus cv. Maku entailed weekly removal and examination of Lotus seedlings over a period of fourteen weeks, and a study of the formation of nodules and their nitrogen fixing capabilities was made. These results will therefore give some indication of the stages in the establishment of an efficient symbiosis by the seedling which could be affected by the application of nitrogen.

Nodules first appeared between two and three weeks after addition of rhizobia but no fixation took place at that time. The nitrogen content of the seedlings remained unchanged during the first three weeks of growth when the seedlings were relying for survival on the nitrogen supplied from the embryo. By five weeks the nodules were fixing nitrogen such that the nitrogen content of the seedlings rose accordingly. After five weeks both number and weight of effective nodules increased with a corresponding increase in fixation and total growth of the plant.

Addition of nitrogen at transplanting (when rhizobia were first added) and six days later, may possibly affect the rhizobia in the soil, the infection process, later infections and the development of nodules. Addition of nitrogen at twelve and eighteen days after transplanting may affect nodule development and addition at twenty-four and thirty days may affect fixation.

8.1 Aim

The aim of the experiment was thus to investigate the effect of combined nitrogen applied at various intervals after transplanting on nodule number, nodule fresh weight, N_2 fixation and growth by L. uliginosus cv. Maku and T. repens cv. S184.

8.2 Materials and Methods

Seventy-two pots of 4" diameter were filled with 250 g of damp Perlite. The pH of the medium was adjusted to 4.5 by the addition of acidified Dart and Pate's (1959) nutrient solution, 100 ml of which was added weekly to the saucers holding the pots. Nine pregerminated seedlings of L. uliginosus cv. Maku were planted into each of 36 pots and similarly nine pregerminated seedlings of T. repens cv. S184 were planted into the remaining 36 pots. The procedures for germination of seeds and addition of rhizobia were similar to those described for experiment 7.

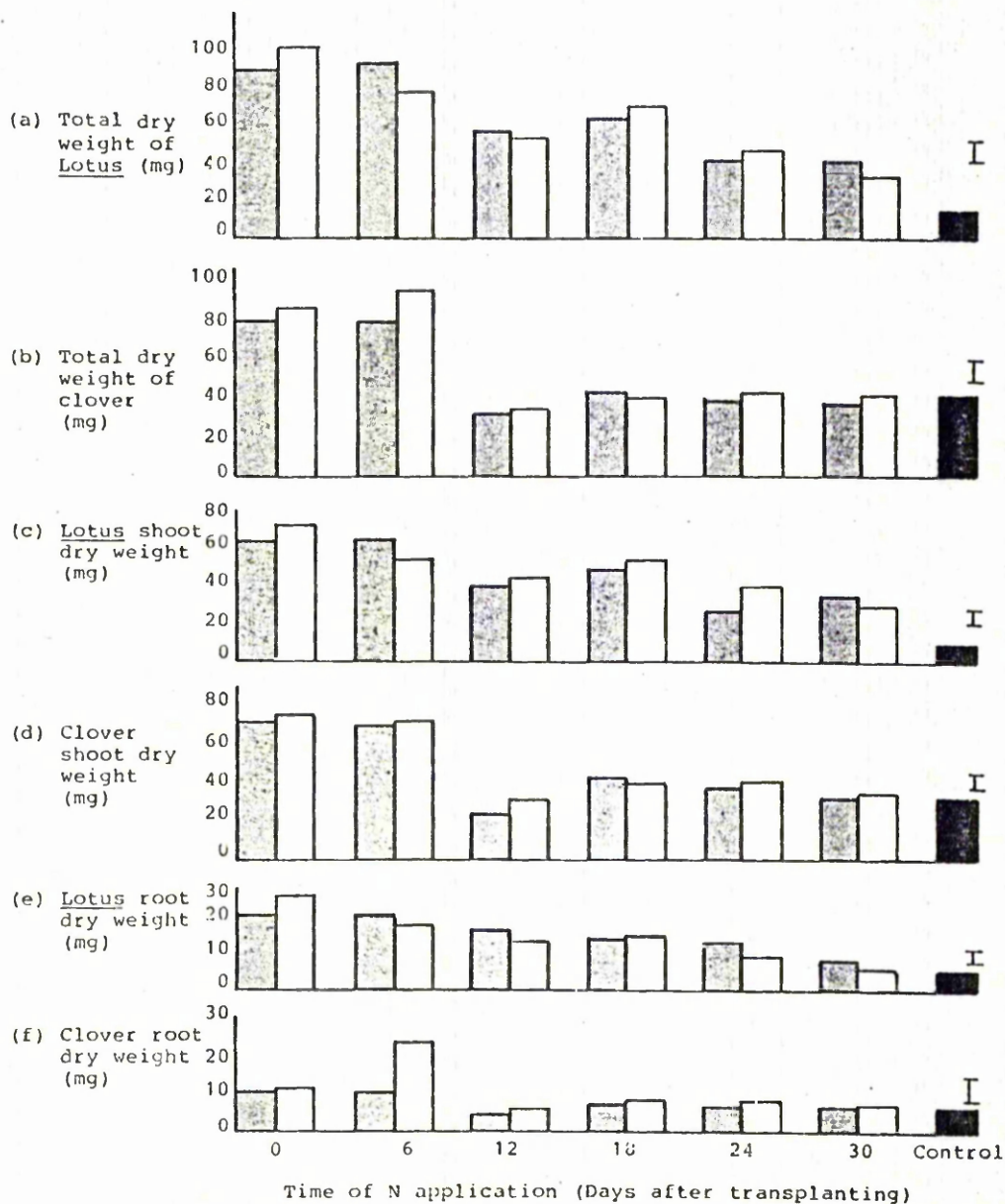
Two levels of KNO_3 (30 and 70 mg N/pot) dissolved in Dart and Pate's (1959) N free nutrient solution were applied at six different stages in seedling growth (0, 6, 12, 18, 24 or 30 days after transplanting). A control series received no combined nitrogen. The experimental layout, the conditions under which the plants were maintained and the measurements recorded were similar to those in experiment 7.

8.3 Results

Total Dry Weight of Plant (Fig. 4 a,b) (App. III : B : Table 1)

Addition of nitrogen at all seedling growth stages significantly increased ($P < 0.001$) the total weight of Lotus plants compared to those plants which received no nitrogen (control). The greatest increase in weight was obtained when nitrogen was added at both the 30 and 70 mg N/pot levels on the day of transplanting or six days later but as the number of

Fig. 4 The effect of KNO_3 at two rates (30 \square or 70 \square mg N/pot) applied at different days after transplanting (0, 6, 12, 18, 24 or 30) on total dry weight (mg) of (a) Lotus, (b) clover, shoot dry weight (mg) of (c) Lotus, (d) clover, root dry weight (mg) of (e) Lotus, (f) clover. Control plants received no nitrogen \blacksquare . All plants harvested at ten weeks old. I = SED.



days increased after transplanting before nitrogen was applied there was a decrease from the maximum. On the other hand, only addition of nitrogen on the day of transplanting or six days later increased the plant weight of clover above that of the control.

Dry Weight of Shoot (Fig. 4 c,d) (App. III : B : Table 2)

When compared with the control plants, all additions of nitrogen significantly ($P < 0.05$) increased the shoot weight of Lotus. The significant increase ($P < 0.05$) in shoot weight for both Lotus and clover was greatest when nitrogen was added at time of transplanting and six days after. Generally, as the delay in N application increased beyond six days after transplanting, the shoot weight of Lotus decreased from the high level reached at both the zero and six days but remained above that of the control whereas a corresponding delay had little effect on clover compared to the controls.

Dry Weight of Root (Fig. 4 e,f) (App. III : B : Table 3)

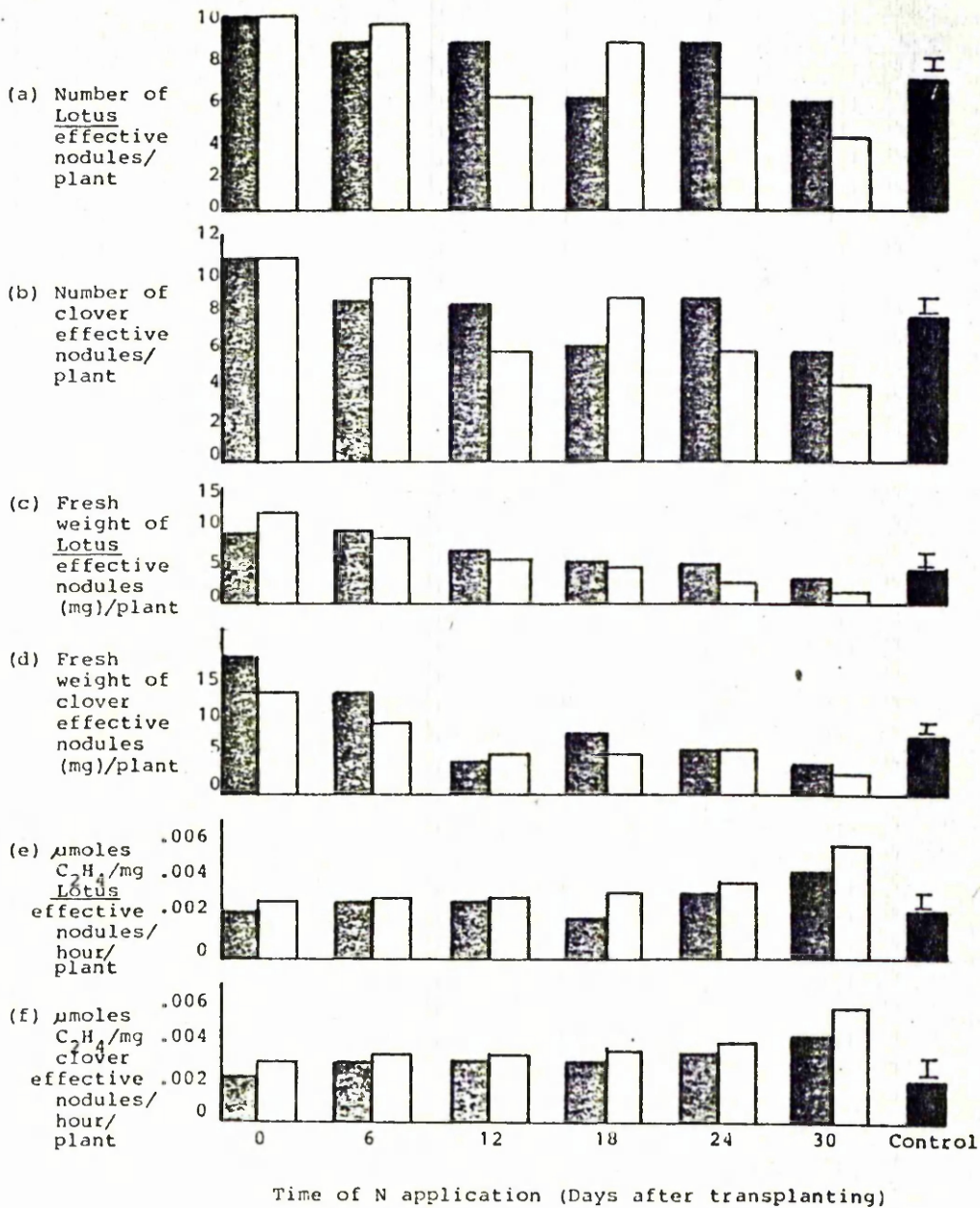
There was a significant increase ($P < 0.01$) in Lotus root dry weight on addition of 30 and 70 mg N/pot except on addition of 70 mg N/pot at twenty-four days after transplanting and addition of 30 and 70 mg N/pot on thirty days after transplanting. There was no change in clover root weight on addition of nitrogen throughout the experimental period.

Number of Effective Nodules (Fig. 5 a,b) (App. III : B : Table 5)

Addition of nitrogen at both levels on the day of, and 70 mg N/pot at six days after, transplanting significantly increased ($P < 0.001$) the number of Lotus and clover effective nodules above that of the control. However, for Lotus and clover there was a significant decrease ($P < 0.001$) in numbers compared with the control when 70 mg N/pot was added thirty days after transplanting.

Addition of 30 mg N/pot at any time after transplanting, up to a maximum of 30 days, had no effect on the effective nodule number of each species as also had 70 mg N/pot when applied at 12, 18 and 24 days after transplanting.

Fig. 5 The effect of KNO₃ at two rates (30 or 70 mg N/pot) applied at different days after transplanting (0, 6, 12, 18, 24 or 30) on number of effective nodules of (a) Lotus, (b) clover, fresh weight (mg) of effective nodules of (c) Lotus, (d) clover, μ moles C₂H₄/mg effective nodules/hour of (e) Lotus, (f) clover. Control plants received no nitrogen. Plants harvested at ten weeks old. Experiment 8. I = SED.



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Weight of Effective Nodules (Fig. 5 c,d) (App. III : B : Table 4)

Addition of nitrogen at both levels on the day of and six days after transplanting significantly increased ($P < 0.001$) the weight of Lotus effective nodules above the control as did addition of 30 mg N/pot 12 days after transplanting. However, as the number of days increased after transplanting before addition of nitrogen, the weight of effective nodules produced was similar to that of the control.

30 mg and 70 mg N/pot of KNO_3 applied on the day of transplanting and 30 mg N/pot added six days later significantly increased ($P < 0.001$) the weight of clover effective nodules compared with the control. The weight of effective clover nodules was reduced when 30 mg N/pot was applied at 12 and 30 days after transplanting and when 70 mg N/pot was added at the 30 day mark.

Acetylene Reduction (Fig. 5 e,f) (App. III : B : Table 6)

Addition of 70 mg N/pot at 30 days after transplanting resulted in a significant increase ($P < 0.001$) in activity of Lotus nodules. Other times of nitrogen addition throughout the study period had no effect on activity of Lotus nodules compared to that of the control.

Similar results were obtained for clover but addition of 30 mg N/pot on 30 days after transplanting also resulted in a significant increase ($P < 0.05$) in clover nodule activity.

8.4 Discussion

Stimulation of growth in both species was greatest when nitrogen was supplied at time of transplanting or six days later. The addition of nitrogen at these times presumably supplemented the nitrogen supplied by the embryo, boosted the growth of the seedling and carried it through the period of nitrogen hunger i.e. that period between exhaustion of embryo nitrogen and the full establishment of an effective symbiosis. The early stimulation of growth resulted in bigger plants thus aiding establishment, but the longer the delay in the addition of nitrogen to

the seedling, the less was the stimulation of growth in Lotus and the lack of increase in clover growth, suggesting that nitrogen was no longer the limiting factor in determining growth.

Addition of nitrogen appeared to benefit growth of clover only when supplied at time of transplanting or six days later, which corresponded with unfolding and expansion of the cotyledons. However, there was no effect on clover root growth which can be explained by suggesting that the shoots of clover compared with the roots were a very dominant sink for assimilate. Lotus responded on the other hand by increasing shoot production when nitrogen was added at all times. The greater sink activity of Lotus shoots compared with roots was only evident at twenty-four days after transplanting when 70 mg N/pot was added and at thirty days after transplanting when both levels of nitrogen were added.

In the present study increased shoot growth of both species and root growth of Lotus when nitrogen was added at the time of transplanting and six days later was due to vegetative growth from added nitrogen. However, increased growth of Lotus when nitrogen was added late at the 30 day mark was caused by an increase in the nitrogen fixing activity of the plants.

The increase in number and weight of effective nodules of both Lotus and clover when nitrogen was applied on the day of transplanting and six days later was probably due to the increased growth of the plants providing carbohydrate, and increase in root growth of Lotus may have resulted in a greater number of root hairs which act as infection sites. Reduction in numbers and weight of effective nodules of both species when 70 mg N/pot was added 30 days after transplanting and the rise in acetylene reduction indicated that the remaining reduced number of nodules had a higher specific activity therefore addition of nitrogen at this stage benefitted fixation. Pankhurst and Jones (1959) working with L. pedunculatus observed an increase in nitrogen fixation when NH_4NO_3 at 0.33 and 1.0 mg N/plant was added at the seedling stage and

when added as a low continual supply of 1 mg N/day/plant. When N was added as a single dose there was an increase of 66% in the amount of nitrogen fixed whereas there was an increase of 506.9% when nitrogen was continually supplied at a low level. The increase in specific activity, which was also shown in this experiment, was thought to be due to increased bacterial proliferation within the nodule as a result of the lower concentration of flavolan, which is toxic to rhizobia, in the plant roots.

The addition of 30 or 70 mg N/pot on the day of transplanting (i.e. 2 days after germination) or six days later gave the best total growth of both Lotus and clover plants without affecting fixation. The experiment has thus shown the importance of timing the application of nitrogen to boost certain aspects of the seedlings development without harming other aspects.

Since, in general, there was no difference between the effect of 30 mg N/pot and 70 mg N/pot, the lowest level would be used under field conditions for economic reasons. The results indicated the need to determine the optimal level of nitrogen which will benefit growth and fixation in the field so that the lowest amount required in commercial practice can be identified. The timing of addition of nitrogen supply under field conditions will be complicated by environmental conditions and leaching from the soil.

9. EXPERIMENT INVESTIGATING THE EFFECT OF A
COMMERCIAL COMPOUND FERTILISER "ENMAG"

9.1 Aim

The experiment was set up to investigate the effect of the slow release commercial fertiliser "Enmag". The composition of "Enmag" is as follows: 6% N, 9% Mg, 1% soluble P_2O_5 , 20% insoluble P_2O_5 and 10% K_2O although exact quantities vary within each batch commercially produced. It is used mainly in horticultural composts, as a slow release fertiliser for bedding-out plants and as a pre-seeding fertiliser for dune grass establishment.

9.2 Materials and Methods

The details of materials and methods used were similar to those in experiment 7 but in this instance the "Enmag" was applied at levels of 0, 30, 70 and 120 mg N/pot on the day of transplanting (i.e. two days after germination).

9.3 Results

Total Plant Dry Weight (Fig. 6 a) (App. III : A : Table 10; C : Table 1)

Addition of nitrogen as "Enmag" did not affect the total plant dry weight of either Lotus or clover.

Dry Weight of Shoot (Fig. 6 b) (App. III : A : Table 14; C : Table 2)

There was a significant increase ($P < 0.05$) in clover shoot weight as the nitrogen supply increased up to the level of 70 mg N/pot. The addition of 120 mg N/pot resulted in a significant decrease ($P < 0.05$) in clover shoot weight to below that of the control. There was no change in Lotus shoot weight at any level of N application.

Dry Weight of Root (Fig. 6 c) (App. III : A : Table 12; C : Table 3)

The root weight of Lotus was greater than that of clover at all levels of applied N. Addition of nitrogen had no significant effect on the root weight of Lotus or clover.

Fig. 6 The effect of "Enmag" slow-release fertiliser applied on the day of transplanting, at varying rates (0, 30, 70 or 120 mg N/pot) on (a) total dry weight (mg), (b) shoot dry weight (mg), (c) root dry weight (mg), (d) root length (cm); (e) shoot length (cm). Lotus \circ , clover \bullet . Plants harvested at ten weeks old. Experiment 9. \bar{x} = SED.

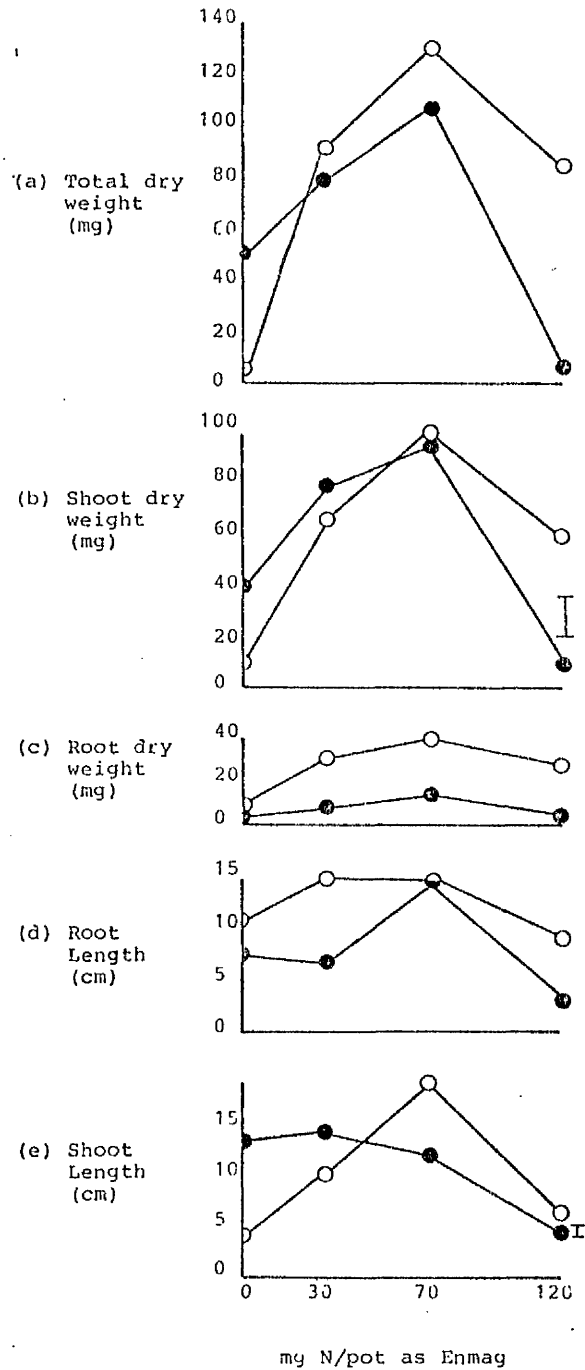
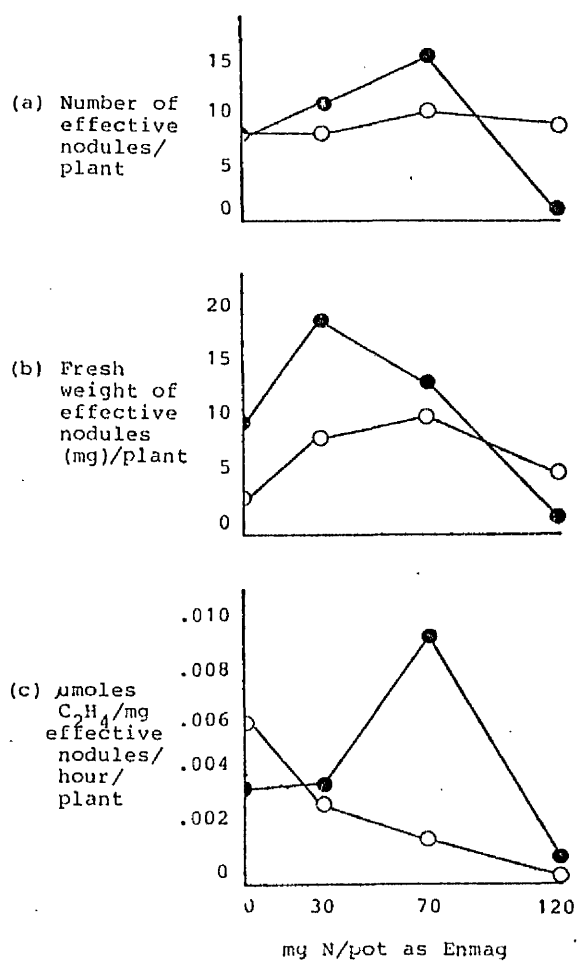


Fig. 7 The effect of "Enmag" slow-release fertiliser, applied on the day of transplanting, at varying rates (0, 30, 70 or 120 mg N/pot) on (a) number of effective nodules, (b) fresh weight of effective nodules (mg) and (c) $\mu\text{moles C}_2\text{H}_4/\text{mg}$ effective nodules/hour. Lotus \circ , clover \bullet . Plants harvested at ten weeks old. Experiment 9.



Shoot Length (Fig. 6 e) (App. III : A : Table 18)

There was a significant decrease ($P < 0.01$) in clover shoot length on addition of 120 mg N/pot but addition of nitrogen had no effect on Lotus shoot length.

Root Length (Fig. 6 d) (App. III : A : Table 16)

Lotus and clover root length was little affected by increasing nitrogen levels over the range 30-120 mg N/pot.

Number of Effective Nodules (Fig. 7 a) (App. III : A : Table 22; C : Table 5)

The addition of nitrogen over the range 30-120 mg N/pot had no effect on the number of Lotus effective nodules. The addition of 120 mg N/pot to clover plants, however, resulted in the complete inhibition of effective nodules.

Fresh Weight of Effective Nodules (Fig. 7 b) (App. III : A : Table 20;
C : Table 4)

Addition of nitrogen had no effect on effective nodule fresh weight of Lotus or clover although addition of 120 mg N/pot to clover plants resulted in almost complete inhibition.

Acetylene Reduction (Fig. 7 c) (App. III : A : Table 24)

The addition of "Enmag" had no significant affect on acetylene reduction by Lotus or clover although addition of 120 mg N/pot resulted in almost complete inhibition.

8.4 Discussion

The results from this experiment cannot be related solely to the effect of nitrogen because of the presence of Mg, K and P in the fertiliser. Due to the complex nature of the fertiliser "Enmag" comparisons between results from experiments 7 and 8 cannot be made.

The increase in clover shoot growth compared with root growth was similar to findings by Barta (1978) and in experiments 7 and 8 where NH_4NO_3 and KNO_3 were added to clover plants. This implies that the extra carbohydrate produced on the addition of "Enmag" equivalent to 30 and 70 mg N/pot was used for shoot growth and the shoot was therefore a

dominant sink for assimilate compared with the root. The increase in clover shoot growth was reflected in the increase in shoot length.

Lotus plant growth did not respond significantly to the addition of "Enmag" at quantities over the range of N used.

The inhibition of clover shoot growth, fixation, number of effective nodules and effective nodules fresh weight on addition of 120 mg N/pot "Enmag" cannot be wholly attributed to the addition of N but may have been due to a combination of effects by Mg, K, P and N although inhibition of fixation and associated measurements is known at this concentration of N. Infection of root hairs by rhizobium is correlated with nodule numbers and a reduction in nodule numbers may be due to a reduction in the number of infections. The addition of fixed N has been shown to prevent R. trifolii from accumulating in high numbers on clover root hair surfaces thus possibly reducing infection. It is not known whether fixed N prevents accumulation or formation of the carbohydrate - binding protein trifoliin to clover root hairs, or whether trifoliin that is present is somehow masked or modified so that it cannot specifically bind to unique carbohydrate structures found exclusively on the surface of R. trifolii (Dazzo & Brill, 1979). Reduction in acetylene reduction was probably due to loss of effective nodules and to a reduction in the amount or efficiency of nitrogenase in the remaining nodules.

The concentration range of "Enmag" used was of no value to Lotus plants and further work is required to determine if any levels will significantly increase growth without inhibiting fixation. Additions of low levels of "Enmag" equivalent to 30 and 70 mg N/pot would be of value to clover plants but care should be exercised when adding higher levels as both fixation and growth were reduced.

10. pH, Phosphate and Trace Element Experiment

10.1 Aim

The aim of the experiment was to examine and compare the growth and N_2 fixation of L. uliginosus cv. Maku and T. repens cv. S184 over a range of phosphate and pH levels in the presence and absence of trace elements.

10.2 Materials and Methods

One hundred and eighty, five inch diameter, pots were filled with 400 g of damp sphagnum peat. To each pot 0.18 g of muriate of potash (50% K_2O) equivalent to 72 kg K/ha was added. For both species thirty pots received no phosphate, thirty received superphosphate (20% P_2O_5) at a rate of 0.22 g/pot equivalent to 15 kg P/ha and the other thirty pots received 0.44 g/pot equivalent to 30 kg P/ha. Forty five pots of each species received 50 ml. of Dart and Pates (1959) trace element solution diluted 1 in 200 while the remaining forty five pots received no trace element solution. The trace elements added were as follows:
 H_3BO_3 , 2.86 g/pot; $MnCl_2 \cdot 4H_2O$, 1.81 g/pot; $ZnCl_2$, 0.11 g/pot;
 $CuCl_2 \cdot 2H_2O$, 0.05 g/pot; $Na_2MoO_4 \cdot 2H_2O$, 0.025 g/pot; all dissolved in 1 litre of distilled water.

For each species, the rooting medium was adjusted to give pH values: 3.5, 4.0, 4.5, 5.0 and 5.5. To obtain these levels of pH the following additions were made to the respective pots: 3 ml NH_2SO_4 and 0, 1.5 g, 3 g and 7 g of lime.

Seedlings were pregerminated on water agar and transplanted to pots, two days after germination. Twenty five pregerminated seedlings of L. uliginosus cv. Maku were added to each of ninety pots and into each of the remaining ninety pots twenty five pregerminated seedlings of T. repens cv. S184 were planted. One week after transplanting the seedlings were thinned to fifteen per pot. Inoculation of the seedlings was by adding 25 ml of a rhizobia plus tap water suspension to each pot 0, 3 and 10 days after sowing. Rhizobium strain cc 814S was added to L. uliginosus

cv. Maku seedlings and strain FA 6 to T. repens cv. S184 seedlings. The pots were watered daily labelled for identification and arranged in a randomised block design on a bench under mercury fluorescent lights in a glasshouse with a temperature of $20 \pm 5^{\circ}\text{C}$.

10.3 Results

Total Plant Dry Weight (Fig. 8) (App III: D. Table 1)

At pH 3.5 addition of 30 kg P/ha just failed to result in a significant increase in Lotus total plant weight. As the pH increased in the absence of phosphate, there was a significant increase ($P < 0.05$) in Lotus plant weight. As the pH increased from 3.5 to 4.5 on addition of 15 and 30 kg P/ha, there was a significant increase ($P < 0.05$) in Lotus plant weight but at pH 5.0 and 5.5 no further increase took place. At pH 4.0 and 4.5 as the phosphate level increased there was a significant increase ($P < 0.05$) in Lotus plant weight. At pH 5.0 the result was similar except there was no significant difference between the effects of the 15 and 30 kg P/ha levels. There was no significant difference in Lotus plant weight at pH 5.5 at the 0 kg P/ha level and that obtained at pH 4.5 with 30 kg P/ha, pH 5.0 with 15 kg P/ha and pH 5.5 on addition of both the 15 and 30 kg P/ha levels.

As the pH increased there was a significant increase ($P < 0.001$) in the total plant weight of clover and similar results were obtained with increasing phosphate levels. There was no significant acid times phosphate interaction.

The addition of trace elements had no significant effect on the total plant weight of Lotus but significantly increased ($P < 0.05$) clover total plant weight.

Fig. 8 Total dry weight (g) of (a) *L. uliginosus* cv. Faku and (b) *T. repens* cv. S184 grown in peat at varying pH levels (3.5, 4.0, 4.5, 5.0 or 5.5) and phosphate levels (0, 15 or 30 kg P/ha) with trace elements added. Plants grown under glasshouse conditions and harvested at ten weeks old. Experiment 10.
 I = SED.

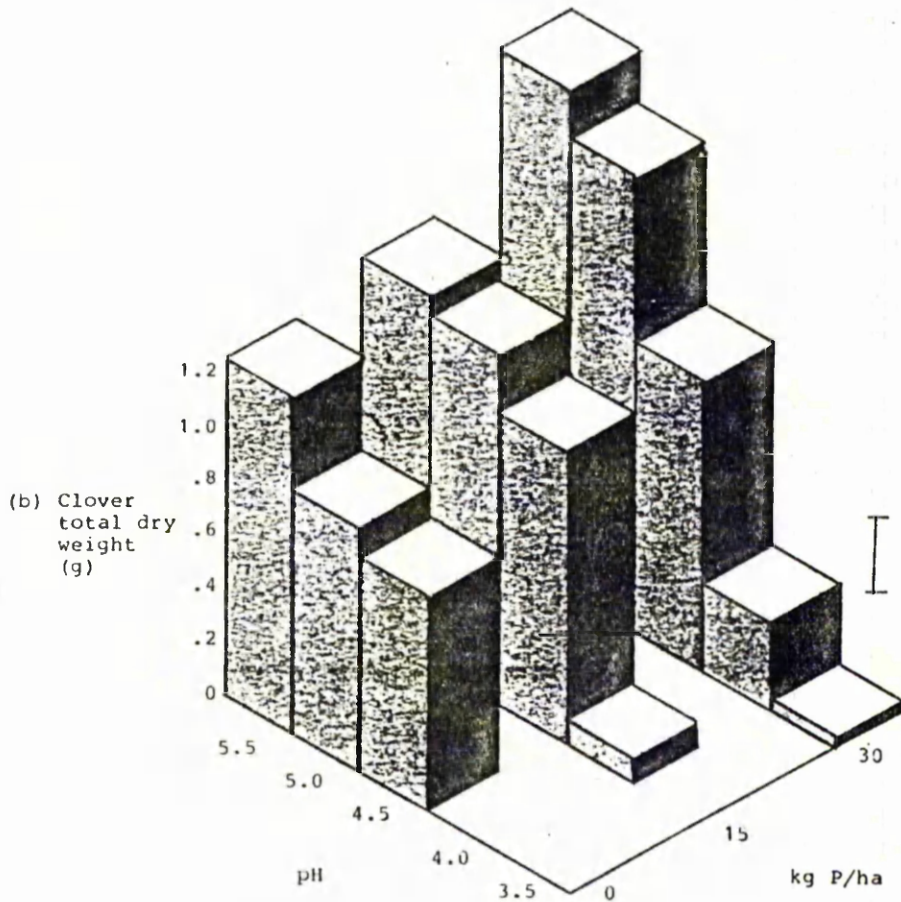
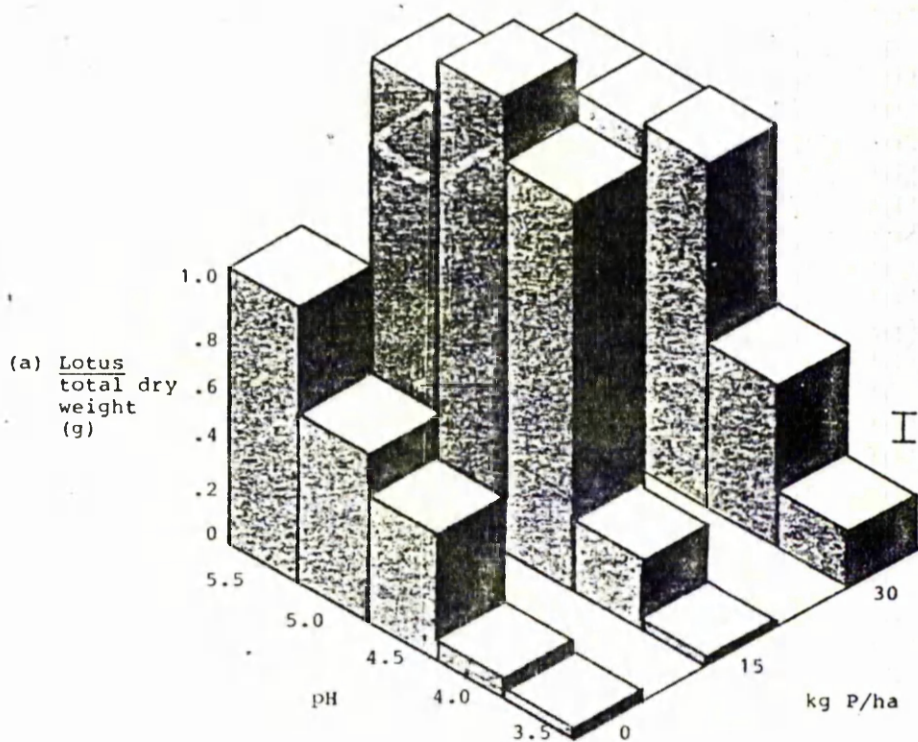


TABLE 4

Significance levels derived from measurements recorded from *L. uliginosus* cv. Maku and *T. repens* S184 plants at the termination, after ten weeks, of a glasshouse experiment with varying pH, phosphate and trace element solution (see appendix 7 for original measurements). Experiment 10

Parameters	Interactions	Sign	S.E.D.
Dry weight stem (g)	SPECIES	***	0.04
	ACID LEVEL	***	0.06
	PHOSPHATE	***	0.05
	ADD TE	*	0.04
	SPx ACID L	***	0.09
Dry weight root (g)	SPECIES	***	0.01
	ACID LEVEL	***	0.01
	PHOSPHATE	***	0.01
	ADD TE	*	0.01
	SPx PHOS	**	0.01
	SPx ACID L	***	0.02
Acetylene reduction	SPECIES	***	0.39
	ACID LEVEL	***	0.61
	ADD TE	***	0.39
	SPx ACID L	***	0.86
	SPx PHOS	*	0.67
Dry weight effective nodules (mg)	SPECIES	***	0.002
	ACID LEVEL	***	0.003
	ADD TE	***	0.002
	SPx ACID L	**	0.004
Number of effective nodules	SPECIES	***	2.81
	ACID LEVEL	***	4.45
	SPx ACID L	***	6.29
Number of non-effective nodules	SPECIES	***	4.49
	ACID LEVEL	***	7.11
	SPx ACID L	*	10.05

* P<0.05
 ** P<0.01
 *** P<0.001

Shoot Dry Weight (Fig. 10) (App II: A table 1a III : D Table 2)

As the pH increased in the absence of phosphate there was a significant increase ($P < 0.05$) in Lotus shoot weight. When the pH increased from 3.5 to 5.5 at the 15 kg P/ha level a significant increase ($P < 0.05$) in stem weight occurred but no further increase occurred at pH 5.5. At the 30 kg p/ha level there was a significant increase ($P < 0.05$) in shoot weight as the pH increased from 3.5 to 4.5 but as the pH increased from 4.5 to 5.5 there was no further increase. Only on addition of 30 kg P/ha at pH 4.0 was there a significant increase ($P < 0.05$) in Lotus shoot weight. There was no significant difference between the shoot weight obtained at pH 5.5 in the absence of phosphate and that obtained at pH 4.5 to 5.5 with the addition of 15 or 30 kg P/ha. At pH 4.5 as the level of phosphate increased there was a significant increase ($P < 0.05$) in Lotus shoot weight whereas at pH 5.0 there was a significant increase ($P < 0.05$) on addition of 15 kg P/ha but no difference between the effect of 15 and 30 kg P/ha.

With clover plants there was no significant acid times phosphate interaction. As the pH increased there was a significant increase ($P < 0.001$) in clover shoot weight and a similar result was obtained with increasing levels of phosphate.

The addition of trace elements significantly ($P < 0.05$) increased the shoot dry weight of clover but no effect was observed with Lotus.

There was a significant difference ($P < 0.001$) between the species with clover having a higher weight than Lotus.

Root Dry Weight (Fig. 9) (App II: A Table 1b III : D Table 3)

As the pH increased in the absence of phosphate there was a significant increase ($P < 0.05$) in Lotus root dry weight. When both 15 and 30 kg P/ha were added at pH's from 3.5 and 4.5, there was a significant increase ($P < 0.05$) in root weight. At pH 3.5 the addition of 30 kg P/ha resulted in

Fig. 10 Dry weight of shoot (g) of (a) *L. uliginosus* cv. Maku and (b) *T. repens* cv. S184 grown in peat at varying pH levels (3.5, 4.0, 4.5, 5.0 or 5.5) and phosphate levels (0, 15 or 30 kg P/ha) with trace elements added. Plants grown under glasshouse conditions and harvested at ten weeks old. Experiment 10. \pm = SED.

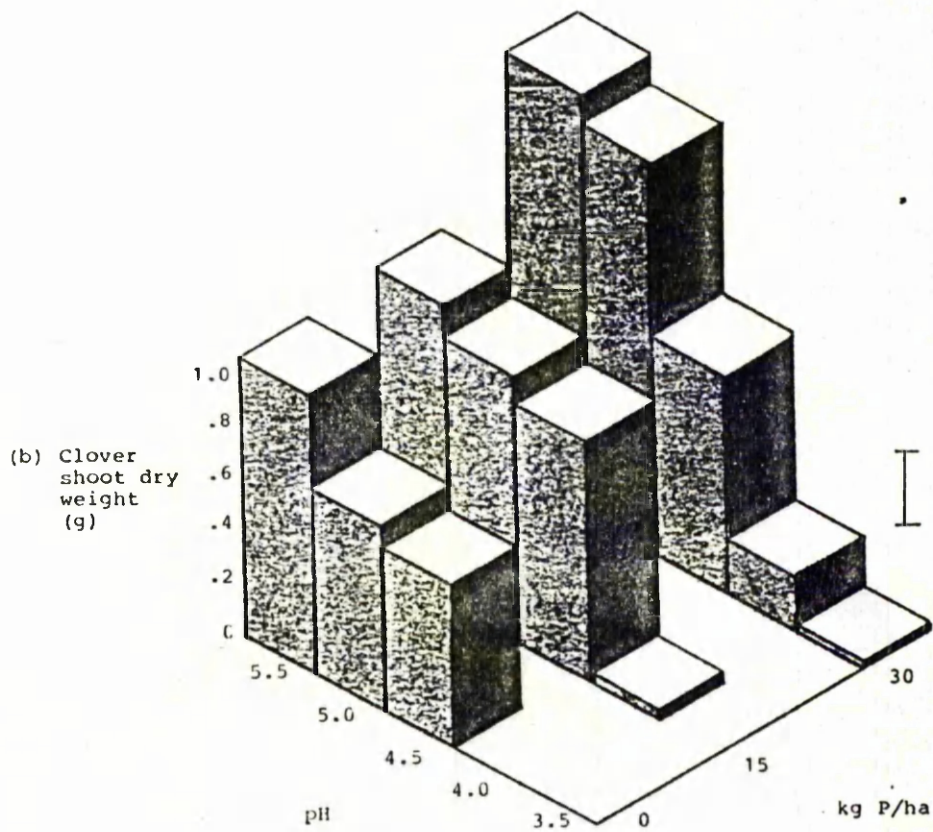
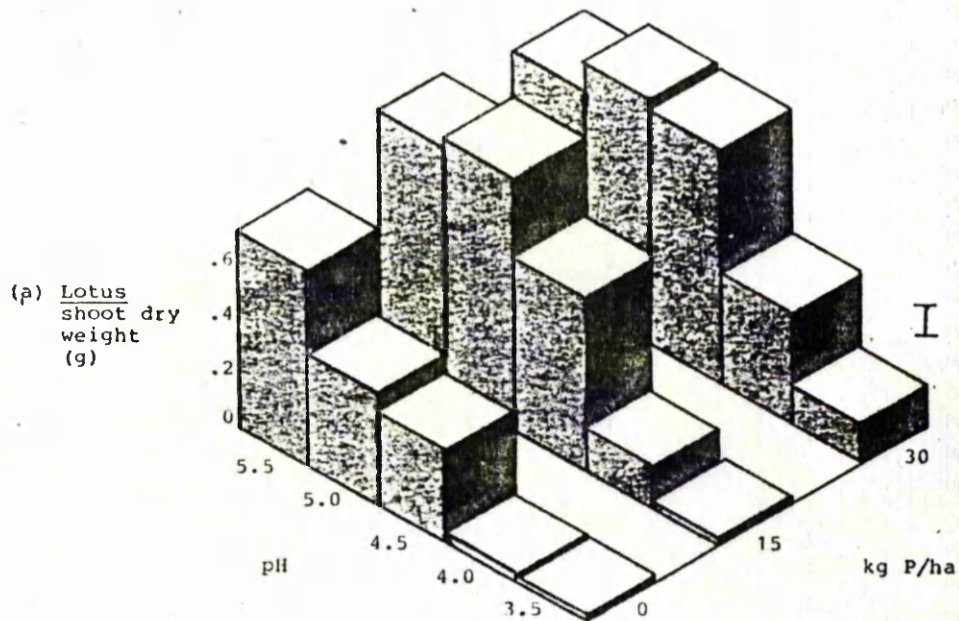


Fig. 9 Dry weight of root (g) of (a) *L. uliginosus* cv. Maku and (b) *T. repens* cv. S184 grown in peat at varying pH levels (3.5, 4.0, 4.5, 5.0 or 5.5) and phosphate levels (0, 15 or 30 kg P/ha) with trace elements added. Plants grown under glasshouse conditions and harvested at ten weeks old. Experiment 10. I = SED.

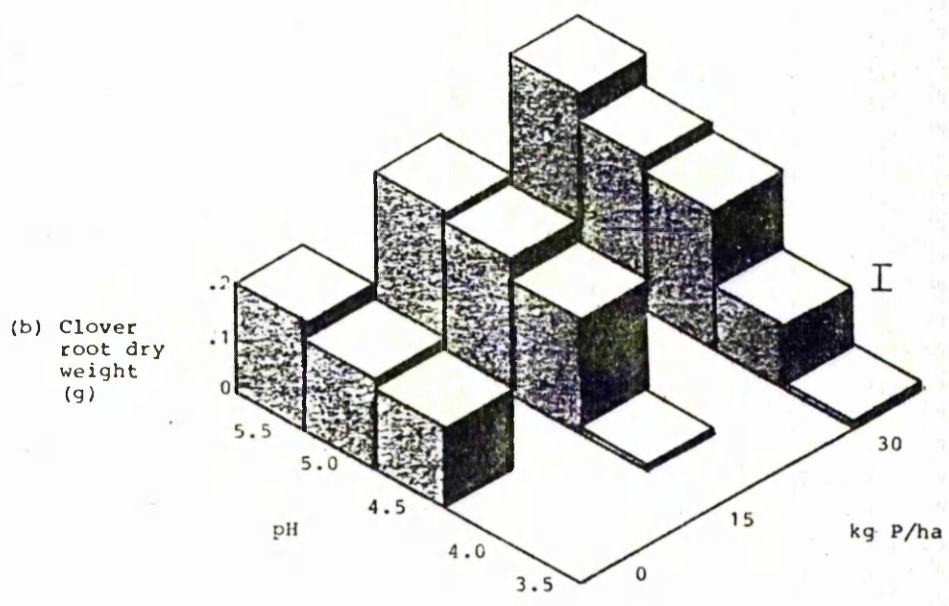
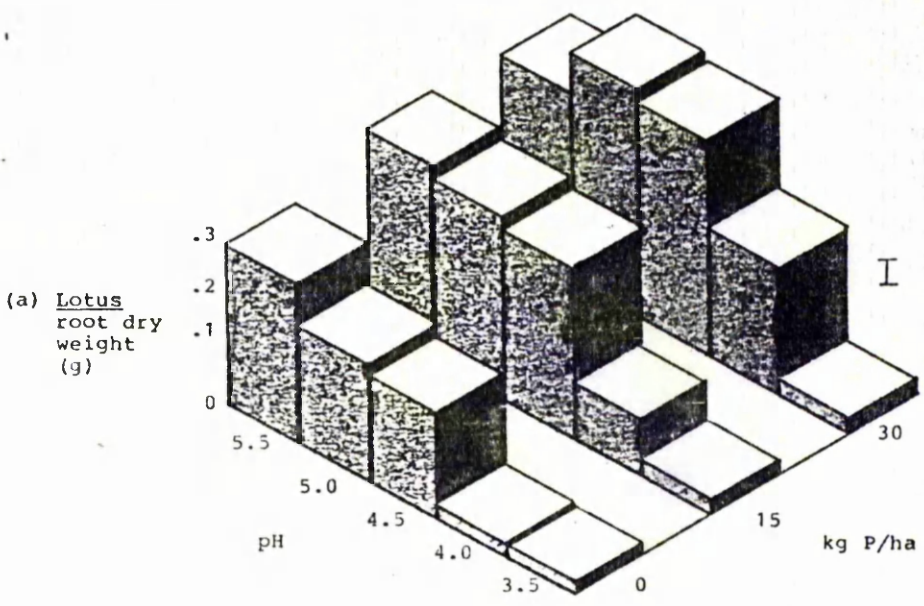
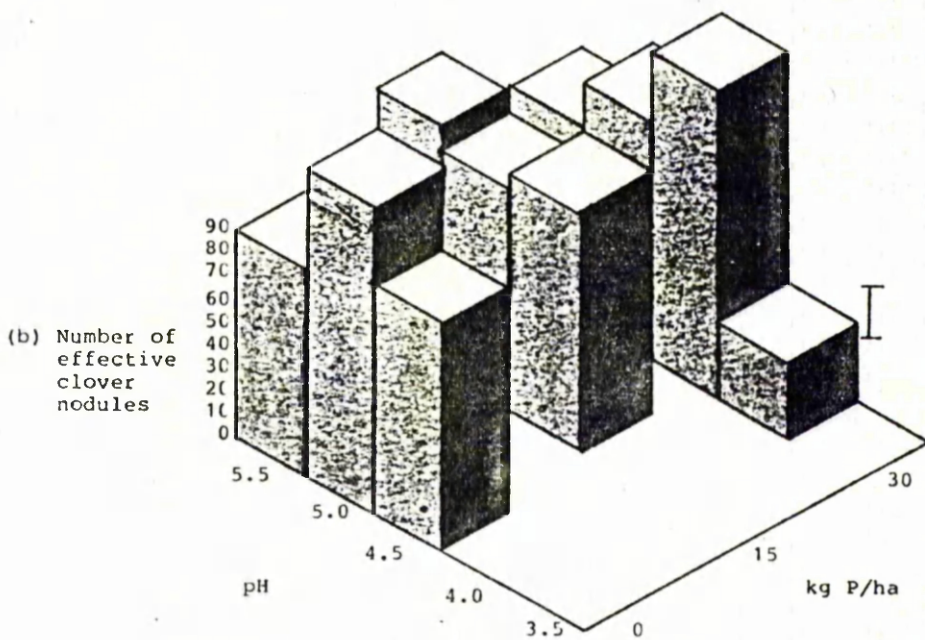
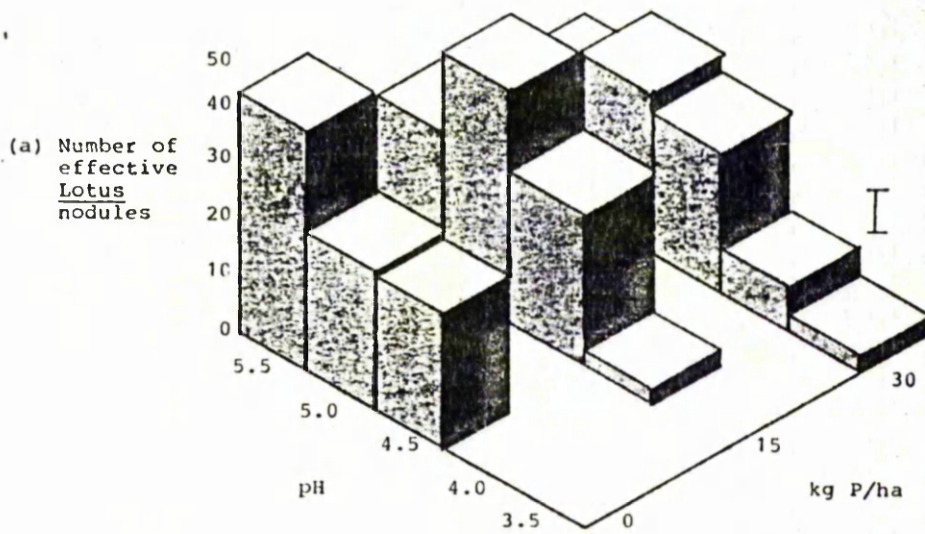


Fig. 12 Number of effective nodules on (a) *L. uliginosus* cv. Maku and (b) *T. repens* cv. S184 grown in peat at varying pH levels (3.5, 4.0, 4.5, 5.0 or 5.5) and phosphate levels (0, 15 or 30 kg P/ha) with trace elements added. Plants grown under glasshouse conditions and harvested at ten weeks old. Experiment 10. \square = SED.



a significant increase ($P < 0.05$) in root weights and at pH 4.0 and 4.5 as the level of phosphate increased there was also a significant increase ($P < 0.05$) in Lotus root weight. There was no significant difference between the root weight obtained at pH 5.5 over all the phosphate levels and pH 5.0 on the addition of 15 kg P/ha.

There was no significant acid times phosphate interaction but as the pH increase there was a significant increase ($P < 0.001$) in clover root weight. A similar result was obtained with increasing phosphate level.

The addition of trace elements significantly increased ($P < 0.05$) the root weight of clover but had no effect on Lotus.

There was a significant difference ($P < 0.001$) between the species with Lotus outyielding clover.

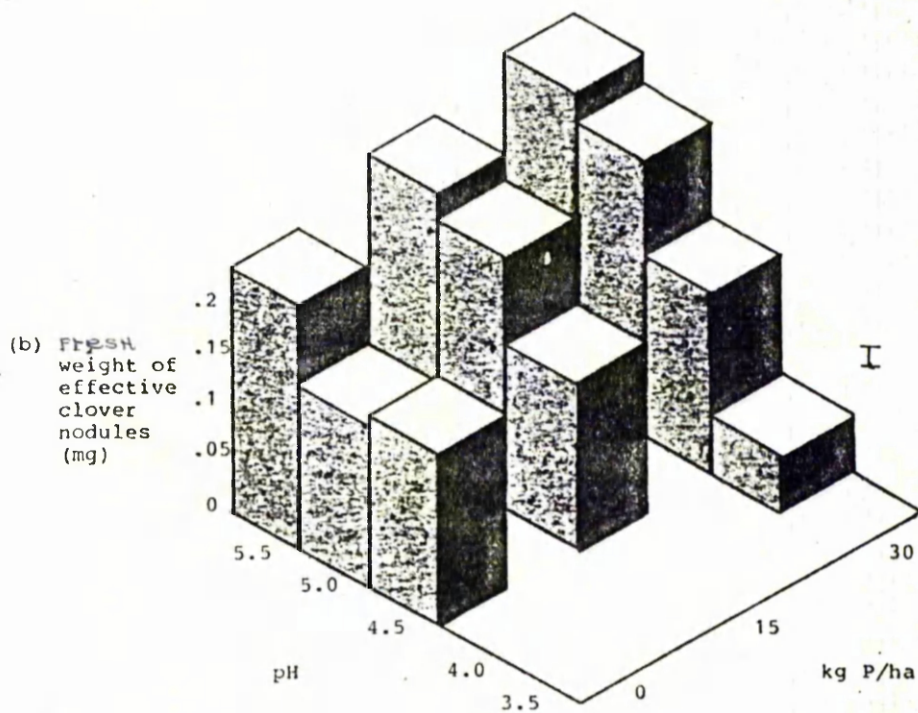
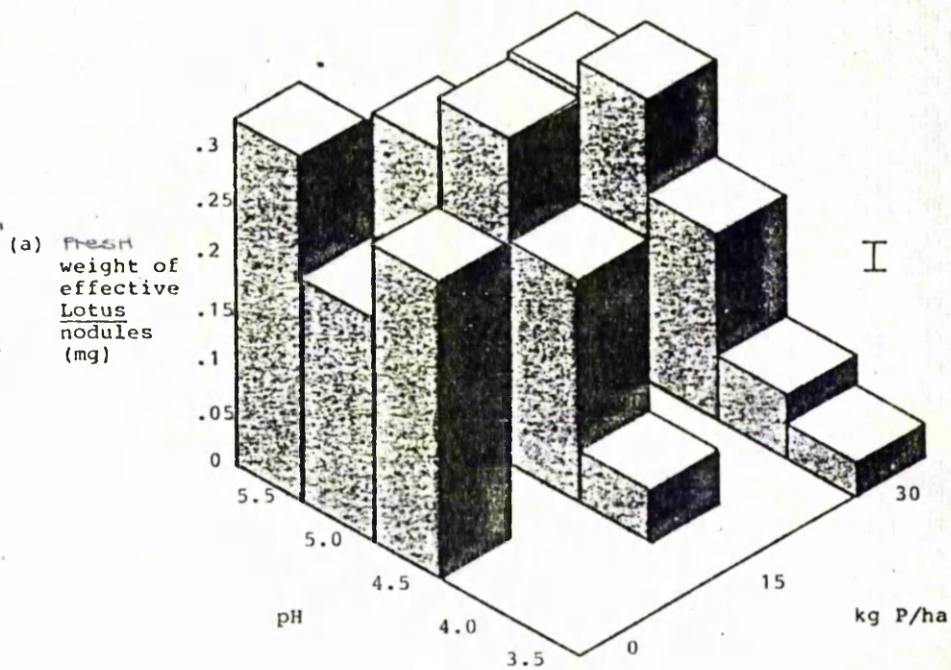
Number of Effective Nodules (Fig 12) (App II: A Table 1c

III: D Table 4.)

As the pH increased from 3.5 to 4.5 in the absence of phosphate, there was a significant increase in number of Lotus effective nodules however at pH 4.5 to 5.5 no further increase occurred. When phosphate was added at 15 or 30 kg P/ha at pH's from 3.5 to 5.0, there was a significant increase ($P < 0.05$) in number of Lotus effective nodules but at pH 5.5 no further increase took place. There was no significant difference between the number of nodules obtained at pH 4.5 and 5.5 when phosphate was absent with those obtained at pH 5.0 at the 30 kg P/ha level and pH 5.5 at the 15 kg P/ha level. At pH's 4.5 and 5.5 when phosphate was added, there was a significant decrease ($P < 0.05$) in the number of Lotus effective nodules but no significant difference between the effects of the 15 to 30 kg P/ha levels.

As the pH increased there was a significant increase ($P < 0.001$) in number of clover effective nodules but nodules were absent at pH 3.5. There was no significant differences between the results obtained at the

Fig. 11 Fresh weight of effective nodules (mg) on (a) *L. uliginosus* cv. Maku and (b) *T. repens* cv. S184 grown in peat at varying pH levels (3.5, 4.0, 4.5, 5.0 or 5.5) and phosphate levels (0, 15 or 30 kg P/ha) with trace elements added. Plants grown under glasshouse conditions and harvested at ten weeks old. Experiment 10. I = SED.



phosphate levels and no acid times phosphate interaction.

For Lotus addition of trace elements significantly increased ($P < 0.05$) the numbers of effective nodules but no significant effect was obtained for clover nodule numbers.

There was a significant difference ($P < 0.001$) between the species with clover having a higher number of effective nodules than Lotus.

Fresh Weight of Effective Nodules (Fig 11) (App. II: Table 1d, III :D

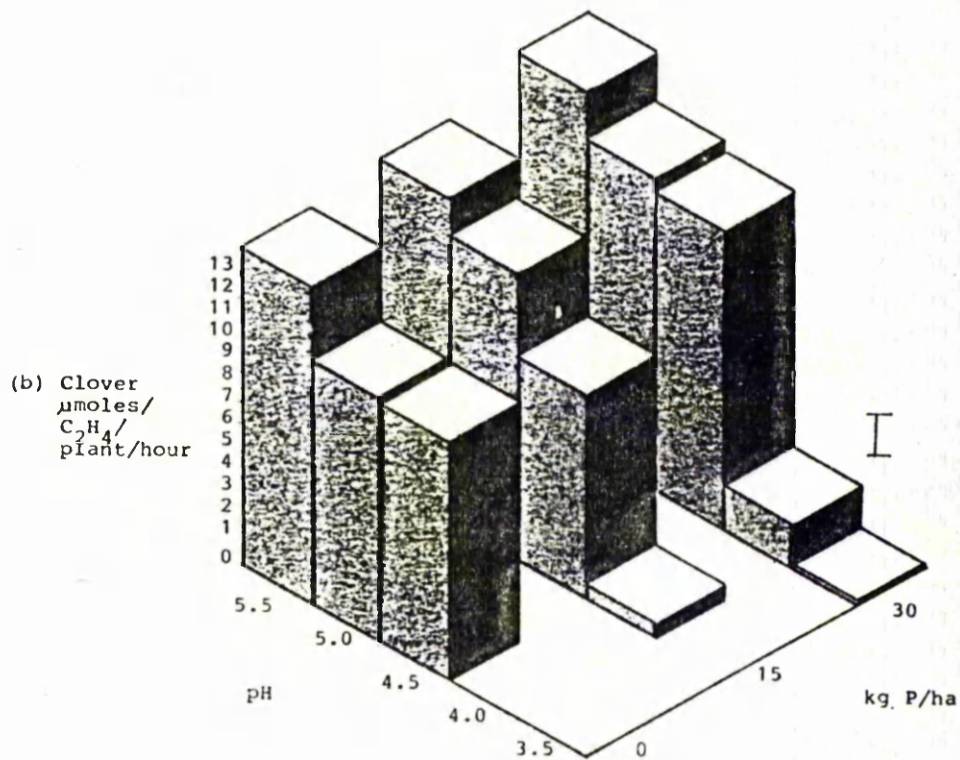
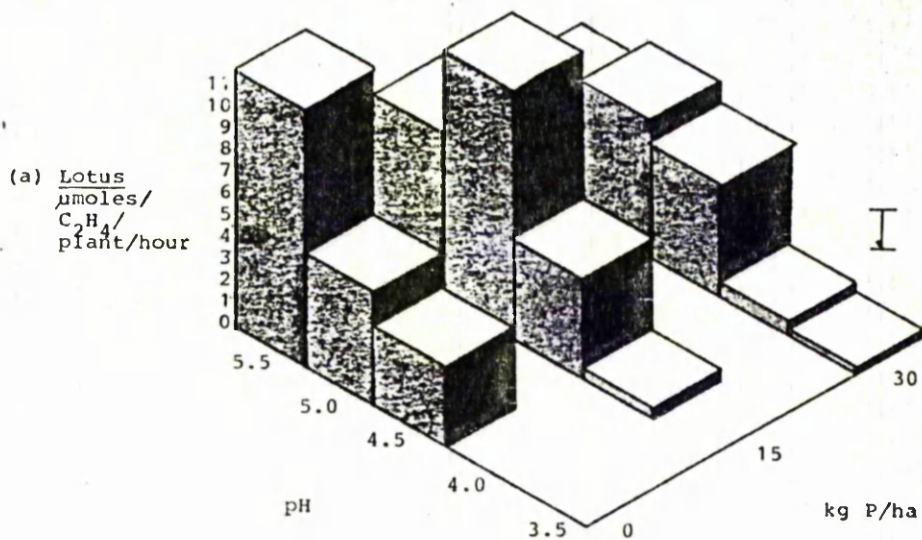
Table 6)

As the pH increased in the absence of phosphate there was a significant increase ($P < 0.05$) in the fresh weight of effective Lotus nodules. On addition of 15 kg P/ha there was a significant increase ($P < 0.05$) in fresh weight from pH 3.5 to 5.0 but there was no further increase at pH 5.5. Addition of 30 kg P/ha resulted in an increase as the pH increased from 3.5 to 4.5 but no further increase occurred at pHs 5.0 and 5.5. At pHs 3.5 to 4.5 over the three phosphate levels, there was no significant difference in fresh weight of Lotus effective nodules. At pH 5.0 addition of 15 kg P/ha resulted in a significant increase in fresh weight and this result did not differ significantly from that obtained at pH 5.5 in the absence of phosphate. At pH 5.5 addition of phosphate resulted in a significant decrease ($P < 0.05$) in Lotus effective nodule fresh weight but there was no significant difference between the effects of 15 and 30 kg P/ha.

There was no significant difference in the fresh weight of clover effective nodules when phosphate was added. There was however a significant increase ($P < 0.001$) as the pH increased with the maximum weight at pH 5.0. There was no acid times phosphate interaction.

Addition of trace elements significantly increased ($P < 0.001$) the fresh weight of both Lotus and clover effective nodules. There was a significant difference ($P < 0.001$) between the species with clover having a higher weight than Lotus.

Fig. 13 Acetylene reduction rate ($\mu\text{moles C}_2\text{H}_4/\text{hour/pot}$) of (a) *L. uliginosus* cv. Maku and (b) *T. repens* cv. S184 grown in peat at varying pH levels (3.5, 4.0, 4.5, 5.0 or 5.5) and phosphate levels (0, 15 or 30 kg P/ha) with trace elements added. Plants grown under glasshouse conditions and harvested at ten weeks old. Experiment 10. I = SED.



Acetylene Reduction (Fig 13) (App II: A Table 1e, III: D Table 8)

As the pH increased in the absence of phosphate there was a significant increase ($P < 0.05$) in acetylene reduction activity by Lotus. A significant increase ($P < 0.05$) in acetylene reduction occurred on addition of 15 kg P/ha as the pH increased, with the maximum activity taking place at pH 5.0. At the 30 kg P/ha level as the pH increased from 3.5 to 4.5 there was a significant increase ($P < 0.05$) in activity by Lotus but at pH 5.0 and 5.5 no further increase occurred. At pH 3.5 and 4.0 with all three phosphate levels no significant difference in Lotus activity occurred. The greatest level of activity was obtained at pH 5.5 without added phosphate and pH 5.0 when 15 kg P/ha was added. A significant decrease ($P < 0.05$) in activity took place at pH 5.5 on addition of phosphate.

As the pH increased there was a significant increase in acetylene reduction activity by clover. Addition of phosphate had no effect on acetylene reduction by clover neither was there an acid times phosphate interaction.

Addition of trace elements had no effect on acetylene reduction activity by Lotus but it significantly increased ($P < 0.05$) the acetylene reduction activity by clover plants.

There was a significant difference ($P < 0.001$) between the species with clover having a higher activity than Lotus. Acetylene reduction by both species was always highly correlated with number and weight of effective nodules (Table 5).

TABLE 5

Correlation coefficients to establish any relationships between measurements recorded for *L. uliginosus* cv. Maku and *T. repens* S184 at the termination, after ten weeks, of a glasshouse experiment with varying pH, phosphate and trace element solution. Experiment 10

df	SPECIES	1	2	3	4	5	6	7	8	9	
	SPECIES : LOTUS										
	Number of effective nodules	1	1.000								
	Number of non-effective nodules	2	0.695	1.000							
	Fresh weight of effective nodules	3	0.912	0.648	1.000						
	Fresh weight of non-effective nodules	4	0.381	0.790	0.429	1.000					
	Dry weight of effective nodules	5	0.886	0.530	0.942	0.277	1.000				
	Dry weight of non-effective nodules	6	0.466	0.834	0.503	0.974	0.381	1.000			
	Dry weight of stem	7	0.754	0.492	0.769	0.332	0.441	0.841	1.000		
	Dry weight of root	8	0.757	0.532	0.749	0.429	0.797	0.530	0.955	1.000	
	Acetylene reduction	9	0.816	0.560	0.892	0.305	0.372	0.778	0.687	0.955	1.000
	SPECIES : CLOVER										
	Number of effective nodules	1	1.000								
	Number of non-effective nodules	2	0.710	1.000							
	Fresh weight of effective nodules	3	0.732	0.308	1.000						
	Fresh weight of non-effective nodules	4	0.640	0.859	0.423	1.000					
	Dry weight of effective nodules	5	0.766	0.418	0.846	0.477	1.000				
	Dry weight of non-effective nodules	6	0.667	0.864	0.394	0.963	0.505	1.000			
	Dry weight of stem	7	0.882	0.690	0.709	0.699	0.810	0.742	1.000		
	Dry weight of root	8	0.836	0.672	0.731	0.731	0.760	0.906	0.906	1.000	
	Acetylene reduction	9	0.843	0.611	0.807	0.615	0.626	0.902	0.868	0.906	1.000

10.3 Discussion

At pH's 4.0 and 4.5 addition of 30 kg P/ha to Lotus resulted in the highest shoot and root weight, therefore at these low pH's high additions of phosphate benefited growth. At pH 5.0 both shoot and root weights of Lotus increased on addition of phosphate but both 15 and 30 kg P/ha had similar effects, indicating that addition of 30 kg P/ha had no benefit over addition of 15 kg P/ha. It also indicated that Lotus could utilise low levels of phosphate more efficiently than higher levels. This response by Lotus has been noted by various workers; Gibson et al (1975) showed that Lotus had a more marked response than clover, particularly at the low phosphate levels, and that only a slight effect was observed on further additions of phosphate. Brook (1973) also showed that Lotus at low phosphate yielded more and had a greater ability than T. repens to take up and accumulate phosphate. Clover on the other hand responded to increased phosphate by increasing shoot, root and total plant weight, thus highlighting the fact that clover had a greater response to a high level of phosphate compared with Lotus.

The main fact to emerge was that Lotus growing at pH 5.5 with zero phosphate had a similar yield to those plants growing at a lower pH, i.e. 4.5, 5.0 and the same pH (5.5) with added phosphate. Increasing the pH rather than adding phosphate therefore seems to be of benefit to the growth of Lotus. pH 5.5 is important, as field studies reported in this thesis (Expt. 15) showed that effective nodulation and N₂ fixation by L. uliginosus cv. Maku took place at a pH averaging 5.5. Effective nodulation at pH 5.5 in the absence of phosphate was similar to that obtained at pH 5.0 on addition of 15 and 30 kg P/ha, and pH 5.5 on addition of 15 kg P/ha. Increasing pH rather than increasing phosphate, more so with Lotus than clover, therefore benefited effective nodulation. This is similar to the findings of Greenwood (1961) who noted that even

in extremely phosphate deficient soil the addition of phosphate did not appear to be necessary for the presence of Lotus and clover rhizobia, provided lime had been added.

A decrease in the number of Lotus effective nodules occurred on addition of 15 and 30 kg P/ha at pH 4.5 but the fresh weight of effective nodules and acetylene reduction activity by Lotus were unaffected. This indicated that the nodules remaining had a higher specific activity. The addition of phosphate to Lotus at pH 5.5 resulted in a decrease in acetylene reduction and effective nodule fresh weight. This decrease may have been caused by a nutrient deficiency which increased as the phosphate level increased, under these particular growth conditions. The addition of trace element solution did nothing to alleviate the decrease, suggesting that it was caused by something other than a micronutrient deficiency. Brock (1973) found that Lotus was relatively less efficient in utilising additional phosphate in terms of fixing nitrogen, but offered no explanation for the cause. This phenomenon did not occur with clover but addition of phosphate at all pH's had no effect on nodule number, fresh weight of effective nodules and acetylene reduction activity by clover. A larger addition of phosphate was probably required to obtain a greater yield and acetylene reduction activity advantage, during this experiment.

A point of interest was the fact that when phosphate was added the increase in root and shoot growth, number and weight of effective nodules and acetylene reduction by Lotus, reached a level at pH 5.0 above which no further increase occurred. Increasing pH in the absence of phosphate on the other hand, resulted in an increase in all these measurements and highlighted the benefit of increasing pH rather than increasing the phosphate level. Clover benefited however from both an

increase in pH and phosphate level.

Lotus could grow but not nodulate at pH's 3.5 and 4.0 whereas clover could not grow at these low pH's without the presence of phosphate. This tolerance of Lotus for low pH's has been demonstrated by Greenwood (1961) who noted that although the addition of lime at pH 4.5 was beneficial to L. uliginosus and aided nodulation, it was not essential. However, it was essential for adequate nodulation of white and subterranean clover. Lowther (1976a) found that lime did not increase the growth of Lotus but did aid its establishment. The results cited here show an increase in growth on addition of lime but because this was a pot experiment the conditions of growth were probably akin to those of early establishment. These results are also similar to those obtained by Gibson et al (1975) who obtained an increase in yield for both Lotus and clover as the pH increased.

Clover would only survive at low pH sites on addition of high levels of phosphate, but as the pH and phosphate increased so also would clover yield. Lotus would therefore outyield clover at low fertility sites whereas competition from clover at high fertility sites would probably result in the elimination of Lotus.

The addition of trace elements benefited both growth and N_2 fixation by clover plants, but the main effect on Lotus plants was shown by improvement of effective nodulation. These results are similar to those obtained by Greenwood (1961) who found that addition of micronutrients resulted in a highly significant increase in the percentage of nodulated L. uliginosus plants and that the nodules were also much larger. Addition of this level of trace elements was adequate to increase growth and fixation by clover, thus aiding establishment.

The level of trace elements required to obtain an increase in growth and acetylene reduction by Lotus will therefore have to be further investigated.

Introduction

The effect of cutting on N_2 fixation and the root system of legumes has been monitored by several workers. Mitchell (1956) working with T. repens, T. subterraneum and L. uliginosus found that cutting stimulated root growth in white and subterraneum clover but made no difference at harvest in Lotus. The effect of defoliation and shading on the colour of the nodules and their number formed in T. repens, T. subterraneum and L. uliginosus was investigated by Butler et al (1959). Both treatments induced a rapid fading of the nodules and shading brought root growth almost or completely to a halt in all three species. The roots of all three species, under recurrent defoliation, underwent a cyclic pattern of decay and renewal although, compared to white clover, Lotus showed a slower capacity for recovery.

Moustafa et al (1969) found that the rate of acetylene reduction by white clover fell within twenty-four hours of defoliation and stayed at a low level for six days after which it increased, reaching levels similar to the undefoliated plants twenty-one days after treatment. Sinclair (1973) measured fixation in white clover, after defoliation, and found an immediate drop in acetylene reduction followed by a period of low activity lasting for approximately six days before acetylene reduction increased. Chu & Robertson (1974) showed that N_2 fixation decreased and remained at a low level for six days, but by the tenth day full recovery was obtained in white clover subjected to two shading and defoliation treatments. Changes occurred in nodule number and dry weight which were attributed to nodule decay, sloughing off and inhibition of nodule development. These factors were also closely related to losses in root dry weight.

11.1 Aim

The experiment was designed to measure the period of recovery in N_2 fixation after cutting and also to monitor any consequent changes in the root system of L. uliginosus cv. Maku.

11.2 Materials and Methods

148 pans 7" diameter were laid out on a bench in a heated glasshouse. The pans contained sphagnum peat, and fertiliser was added at the following rates: 46 kg GMP/ha, 12% P present, 20 kg superphosphate/ha, 8% P, 75 kg/ha muriate of potash, 60% K and lime at a rate of 1.6 tonnes/ha raising the pH from 4.0 to 4.8. The pans were watered when required.

Three seedlings were transplanted from water agar, two days after germination, to each pan and each plant was given a number. After ten weeks growth half the plants were chosen at random and cut back to their first node.

Acetylene reduction was measured on the following days after defoliation 1, 3, 5, 8, 11, 17 and 23. Ten undefoliated and ten defoliated plants were picked at random for each measurement.

After the soil was shaken from the root system each plant was placed in a universal bottle of 33 ml volume. A hole had been bored in the metal lid and a rubber septum fitted to facilitate the injection and withdrawal of gas. 1 ml of air was removed, 1 ml of C_2H_2 added and the system incubated for one hour in an incubation chamber set at 21°C with incadescent lighting. This was to ensure standard conditions for incubation of the different samples. Two 1 ml samples were removed, after incubation, using 1 ml plastic syringes. The syringes were stuck into rubber stoppers, for no longer than two hours, before injecting 0.4 ml of the sample into the GLC.

After incubation and gas analysis the roots of the plants were washed free of soil and examined. The results given are the mean of ten plants.

11.3 Results

Acetylene reduction was not immediately affected by cutting. A significant decrease ($P < 0.001$) was recorded eleven days after cutting followed by a significant increase ($P < 0.001$) in ethylene production recorded fifteen days after cutting. In the undefoliated plants ethylene production in general increased steadily throughout the study period except on day five when there was a reduction which coincided with a reduction in both shoot and root dry weight. A significant decrease ($P < 0.001$) in reduction rate was found in the defoliated plants compared with undefoliated plants (Table 6).

There was a significant decrease ($P < 0.05$) in the total number of nodules present on the defoliated plants throughout the study period (Table 8). No difference in the total number of ineffective nodules present was observed between treatments, however a highly significant decrease ($P < 0.001$) in the number of effective nodules present and nodule dry weight on the defoliated plants was noted (Table 8).

Acetylene reduction was positively correlated ($R = 0.66$) with the dry weight of nodules i.e. the decrease in acetylene reduction corresponded to a decrease in nodule weight (Table 7).

There was a significant decrease ($P < 0.01$) in root dry weight in defoliated plants. The results indicated that a drop in root dry weight occurred on the eighth day following cutting, at which time a decrease in nodule dry weight occurred (Table 8). A positive correlation ($R = 0.71$) between the root dry weight and the dry weight of nodules indicated that the greater the rooting system the greater the weight of nodules present. There was a significant decrease ($P < 0.001$) in shoot dry weight in defoliated plants (Table 8) and the shoot dry weight was positively correlated ($R = 0.75$) with the dry weight of nodules. Acetylene reduction was also correlated ($R = 0.61$) with shoot dry weight.

TABLE 6

Effect of cutting to the first node on acetylene reduction measured as $\mu\text{moles C}_2\text{H}_4$ produced/hour/plant, over the twenty three days after cutting, by L. uliginosus cv. Maku grown for ten weeks under glasshouse conditions. Experiment 11

<u>Days after harvest</u>	<u>Defoliated</u>	<u>Undeveloped</u>
1	0.022	0.041
3	0.030	0.086
5	0.015	0.018
8	0.014	0.083
11	0.008	0.148
17	0.157	0.256
23	0.142	0.414
Mean	0.055	0.149
S.E.D.	0.025	
Significance	***	

*** P<0.001

TABLE 7

Correlation coefficients to establish any relationships between measurements on L. uliginosus cv. Maku, recorded over a period of twenty three days after cutting to the first node. Experiment 11

Acetylene reduction	1	-			
Dry weight stem	2	0.61	-		
Dry weight root	3	0.40	0.60	-	
Dry weight nodules	4	0.66	0.75	0.71	-
		1	2	3	4

TABLE 8

Effect of cutting to the first node on various measurements from *L. uliginosus* cv. Maku plants grown under glasshouse conditions and recorded over a period of twenty three days after cutting. Experiment 11

Days after treatment	1	3	5	8	11	17	23	Mean	SE _D	Sig
a) <u>Total No. nodules</u>										
i) Defoliated	44.2	34.8	29.0	30.1	28.1	38.3	35.1	34.2		
ii) Unde-foliated	43.7	37.7	36.1	31.6	38.6	48.2	48.3	40.6	2.53	*
b) <u>Total No. effective nodules</u>										
i) Defoliated	9.3	6.9	6.3	5.6	3.8	4.9	4.8	5.94		
ii) Unde-foliated	15.4	9.4	9.3	12.5	10.6	11.8	14.0	11.86	0.919	***
c) <u>Total No. non-effective nodules</u>										
i) Defoliated	34.9	27.9	22.7	24.5	24.3	33.4	30.3	28.3		
ii) Unde-foliated	28.3	28.3	26.8	19.1	28.0	36.4	34.3	28.7	2.06	NS
d) <u>Fresh wt. of all nodules (mg)</u>										
i) Defoliated	18.51	15.18	15.82	14.24	11.52	18.86	16.93	15.87		
ii) Unde-foliated	20.02	17.44	17.18	18.51	24.84	31.12	34.93	23.44	1.681	***
e) <u>Dry wt. of all nodules (mg)</u>										
i) Defoliated	2.69	2.44	2.19	1.51	1.57	2.38	1.99	2.11		
ii) Unde-foliated	3.20	3.19	2.41	2.93	3.68	4.55	5.28	3.60	0.253	***
f) <u>Dry wt. root (g)</u>										
i) Defoliated	8.87	8.27	8.46	5.78	6.86	6.64	6.24	7.30		
ii) Unde-foliated	8.47	8.36	7.96	8.52	9.24	10.96	8.98	8.93	0.617	**
g) <u>Dry wt. stem (g)</u>										
i) Defoliated	7.07	6.44	10.08	4.70	6.34	10.04	11.67	8.05		
ii) Unde-foliated	25.50	26.66	17.60	23.22	23.13	25.50	34.78	25.77	1.568	***

* P<0.05

** P<0.01

*** P<0.001

11.4 Discussion

There were indications that after the fifth day following cutting, there was a continuing reduction in the number of effective nodules and in the dry weight of nodules. Under these conditions therefore cutting resulted in a gradual loss of effective nodules rather than an immediate loss, and this was further demonstrated by the gradual decrease in acetylene reduction over eleven days after cutting. Since there was no difference in numbers of ineffective nodules, the highly significant decrease in nodule dry weight was due to the loss of effective nodules, and the positive correlation of acetylene reduction activity and dry weight of nodules also indicated that the reduction in nodule weight was due to loss of effective nodules. Loss of effective nodules was probably due to sloughing off of individual nodules and parts of the root bearing nodules falling off after cutting.

The increase in acetylene reduction activity measured seventeen days following cutting corresponded with an increase, to the level of the control, in the dry weight and number of nodules. This increase in acetylene reduction may have been caused by the non-effective nodules, remaining after cutting, maturing into effective nodules. The low levels of activity prior to this would have been sustained by the effective nodules remaining after cutting.

12. EXPERIMENT INVESTIGATING EFFECT OF CUTTING, LIGHT AND TEMPERATURE REGIMES ON MAKU AND HUIA

12.1 Aim

The aim of the experiment was to investigate the effect of frequent cutting, under four regimes with varied light and temperature, on the N₂ fixing potential and dry matter production of both L. uliginosus cv. Maku and T. repens cv. Huia.

12.2 Materials and Methods

Fifteen pre-germinated seedlings of L. uliginosus cv. Maku and T. repens cv. Huia were transplanted into 7" pans. Each pan contained sphagnum peat to which fertiliser at the following rates had been added: 20 kg P/ha as superphosphate (20% P₂O₅), 46 kg P/ha as ground mineral phosphate (30% P₂O₅), 72 kg K/ha as muriate of potash (60% K) and lime at 16 tonnes/ha which brought the pH of the medium up to 4.7. To each pan 25 ml of a rhizobial suspension in tap water was added at 0, 4 and 10 days after planting. Rhizobium strain CC814s was used for inoculating Maku seedlings and strain FA6 for Huia seedlings.

The pans were randomly arranged in four environmental growth cabinets, each containing nine pans, five pans contained Maku seedlings and four containing Huia seedlings. The pans were replicated three times but pans containing control plants were not replicated. The pans were rotated within each cabinet daily. The plants were all grown under the same environmental conditions (i.e. 16 hour day; 21°C day/18°C night temperature regime and a relative humidity of approximately 70%) until they were ten weeks old. At this time all plants were cut back to 2.5 cm, with the exception of the controls, and the growth cabinet set to the following light/temperature conditions.

Cabinet	Light	Temperature
A	12 hours day 12 hours night	21°C day 18°C night
B	16 hours day 8 hours night	21°C day 18°C night
C	12 hours day 12 hours night	18°C day 15°C night
D	16 hours day 8 hours night	18°C day 15°C night

Acetylene reduction assays were carried out on plants two days prior to cutting, using the ten litre polypropylene buckets as incubation chambers, to check for variation within and between species. The incubation time was four hours, after which samples of gas were removed using either a syringe to store gas, in which case analysis of the sample took place immediately, or into 10 ml vacutainers. The vacutainers were used when immediate use of the GLC was prevented.

After incubation the plants and rooting medium were returned in their respective pans to the environmental growth cabinets.

After the first cut at ten weeks the plants were cut every twenty-one days. Acetylene reduction assays were conducted on the following days between cuts:

Assay Dates

After 1st cut (at 70 days): 1, 3, 6, 11, 17, 20

After 2nd cut (at 91 days): 1, 4, 6, 11, 14, 18, 20

After 3rd cut (at 112 days): 1, 4, 7, 11, 14, 18, 24, 32, 39

After 4th cut (at 133 days): 1, 4, 7, 11, 14, 18, 22

Only plants maintained under the 12 hour day, lighting regime (i.e. blocks A and C) were cut four times. After the fourth cut, their light regimes were returned to a 16 hour day but the temperatures were unaltered. Acetylene reduction activity was then followed for a further twenty-two days. This was done to check if a faster or higher level of recovery after cutting was obtained with an increase in light rather than a change of temperature. The plants subjected to a 16 hour day light regime (i.e. blocks B and D) were not cut a fourth time but less frequent acetylene reduction measurements were continued.

12.3 Results

Cut 1 (Fig. 14) (App. III : E : Tables 1, 2)

After cutting there was an immediate decrease in acetylene reduction for Lotus, with the exception of treatment C (12 hour day; 18/15°C). A drop in activity was obtained under all treatments with clover. Lotus acetylene reduction activity exhibited a lag phase (i.e. a period after cutting when there was no significant rise in activity), lasting seventeen days for plants grown under short day treatments (12 hour day), whereas under long day treatments (16 hour day) the lag phase lasted ten days. There was no significant increase thus indicating the presence of a lag phase, in clover activity as the number of days after cutting increased except under treatment B (16 hour day; 21/18°C) at twenty days after cutting.

Except on the day after cutting and three days after, there was a significant difference ($P < 0.001$) in the amount of acetylene reduced by Lotus plants between different light and temperature regimes. Thereafter, there was a significant difference ($P < 0.05$) between treatment B (16 hour day; 21/18°C) and A (12 hour day; 21/18°C) and between B and C (12 hours day; 18/15°C) and between C and D (16 hour day; 18/15°C). Activity was highest under long light and high temperature and lowest under short light and low temperature regimes. There was no significant difference between the acetylene reduction results obtained for clover under all four treatments. Full recovery of Lotus activity to that before cutting, occurred at between six and ten days under treatment B (16 hour day; 21/18°C), at ten days under treatment A (12 hour day; 21/18°C), and between ten and seventeen days under treatments C (12 hour day; 18/15°C) or D (16 hour day; 18/15°C). Full recovery of clover activity was only obtained at twenty days by plants grown under treatment B. Although Lotus plants recovered activity to the same level as prior to cutting, the uncut plants had a higher activity throughout the study period (Fig. 19). The

Fig. 14 1st cut: umoles C_2H_4 produced by ten week old (a) *L. uliginosus* cv. Maku and (b) *T. repens* cv. Huia. Measurements were made prior to cutting and at intervals throughout three weeks after cutting, when grown in peat in 7" pans under the following light; temperature conditions:

- A) 12/12; 21°/18°C ○
- B) 16/8; 21°/18°C ●
- C) 12/12; 18°/15°C ◐
- D) 16/8; 18°/15°C ◑

Fig. 14 (b): C = ◐
D = ◑

Experiment 12.

⊥ = SED.

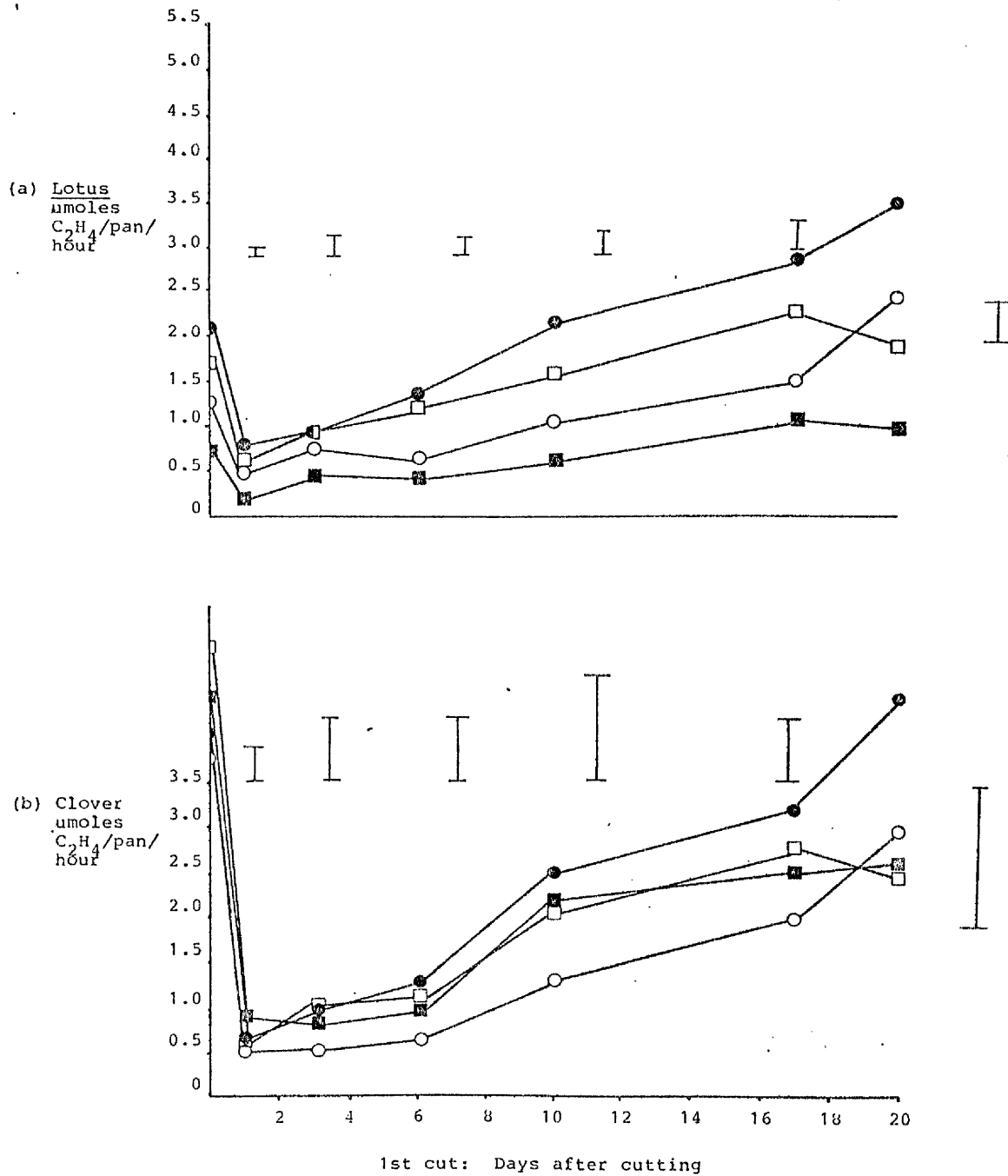
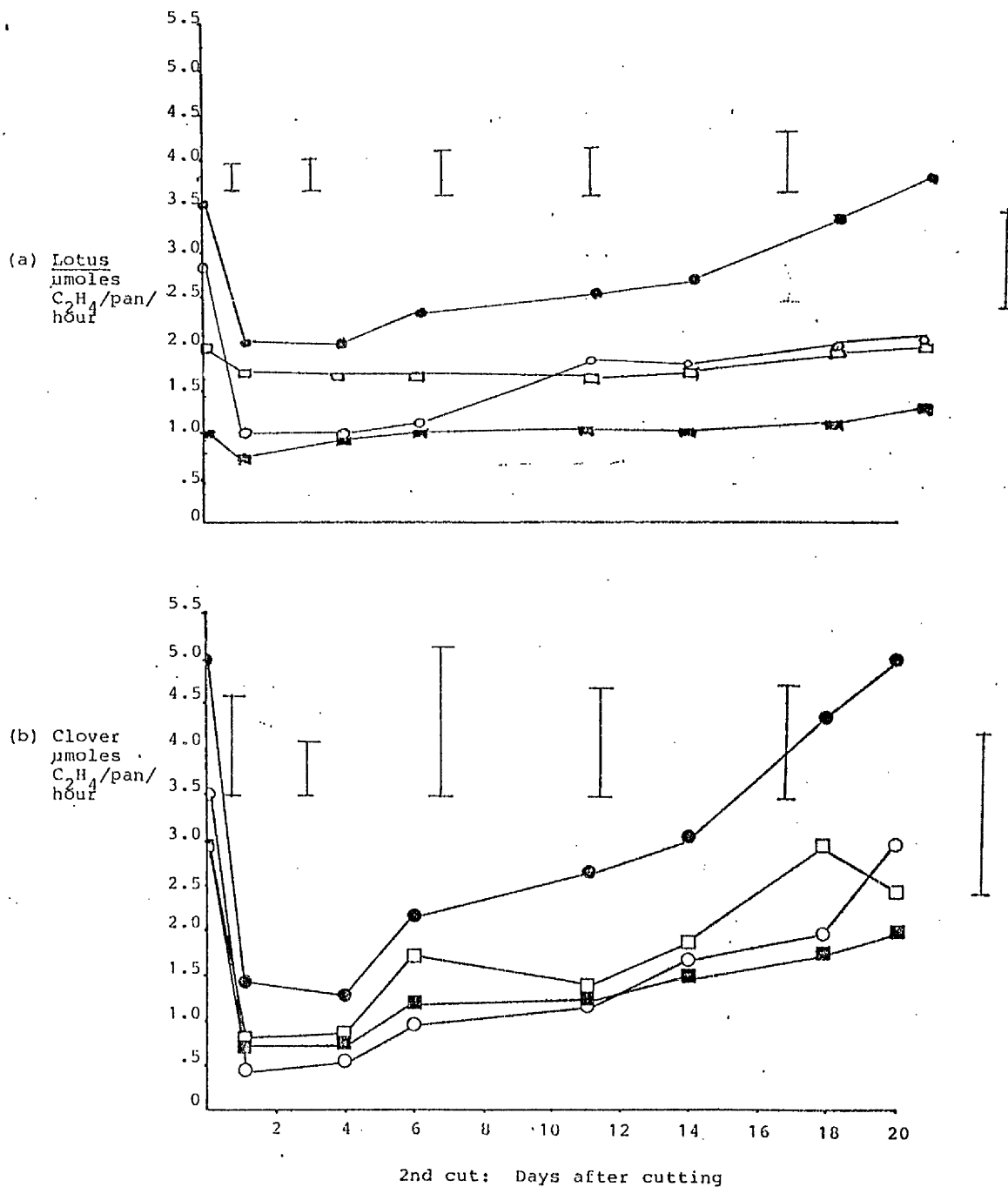


Fig. 15 2nd cut: $\mu\text{moles C}_2\text{H}_4$ produced by thirteen week old (a) *L. uliginosus* cv. Maku and (b) *T. repens* cv. Huia grown in peat in 7" pans under the following light; temperature conditions and cut three weeks after the first cut at ten weeks old. Measurements were made prior to cutting and at intervals throughout three weeks after cutting:

- A) 12/12; 21°/18°C ○
- B) 16/8; 21°/18°C ●
- C) 12/12; 18°/15°C ■
- D) 16/8; 18°/15°C □

Experiment 12.

± = SED.



activity by uncut clover plants was also always greater than that of cut.

Cut 2 (Fig. 15) (App. III : E : Tables 3, 4)

The apparent reduction in activity by Lotus plants after the second cut was not significant. There was no significant rise in activity by Lotus as the number of days after cutting increased. Activity under treatment B (16 hour day; 21/18°C) was significantly higher ($P < 0.05$) than that which was obtained under all the three other treatments.

A significant reduction in activity after the second cut occurred with clover plants grown under treatment B. The apparent drop in fixation by plants grown under treatments A, C and D was non significant. There was no significant increase in activity after cutting. Recovery back to the level of activity prior to the second cut was achieved by plants at fourteen to eighteen days, at eighteen to twenty days and at twenty days for treatments B, D and A respectively but was never attained under treatment C.

Activity by Lotus was highest under long day/high temperature conditions but there was no significant difference between the other three treatments. There was no significant difference between the rates of activity shown by clover plants under any of the four treatments.

Cut 3 (Fig. 16) (App. III : E : Tables 5, 6)

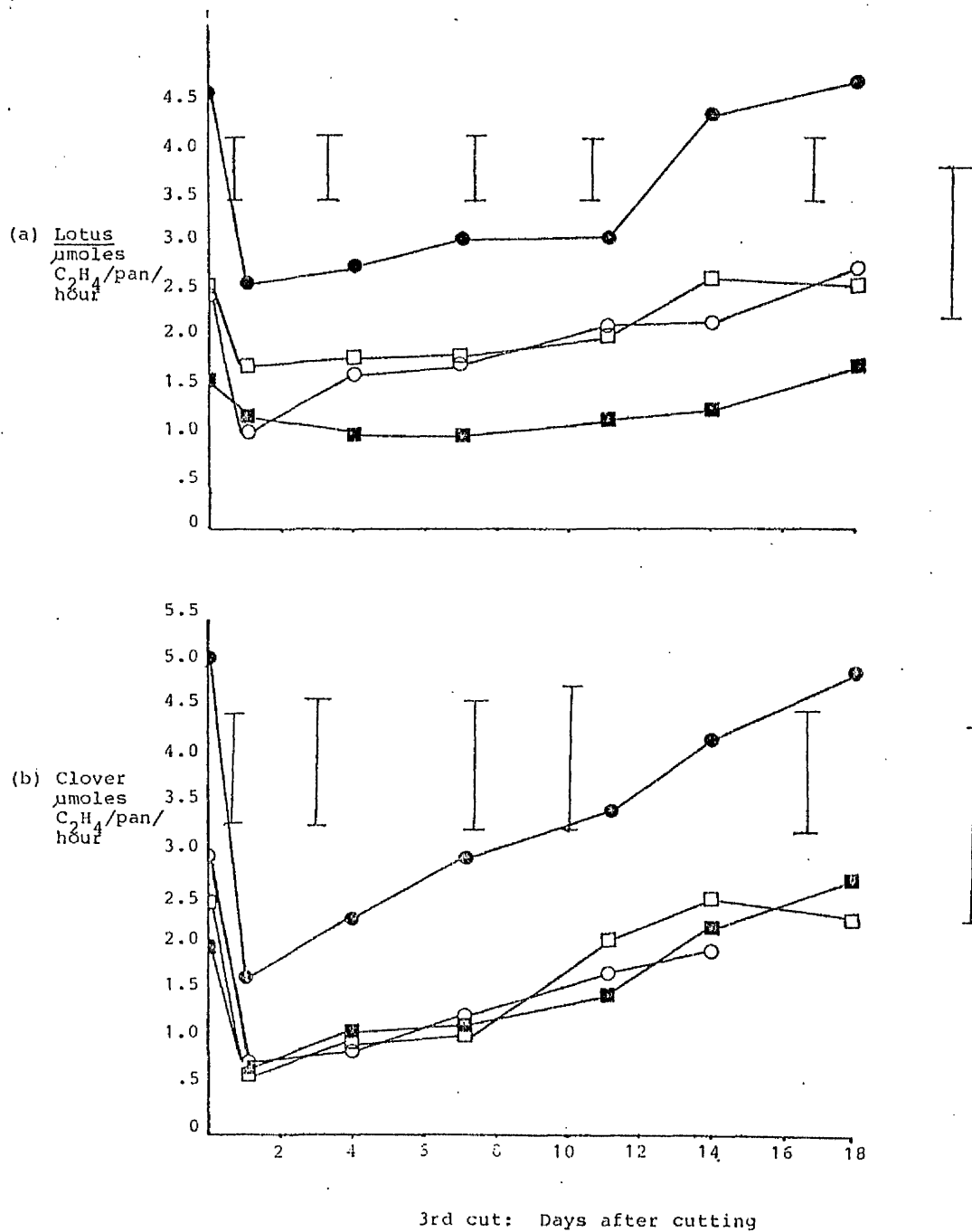
The apparent reduction in activity by Lotus and clover plants after the third cut was not significant. There was also no significant increase in activity by either species with an increase in days after cutting. Full recovery of Lotus activity to the level before the third cut was obtained fourteen days after cutting for treatments B or D and eighteen days after for treatments A or C. Full activity by clover plants equal to that obtained prior to the third cut, occurred fourteen days after cutting for plants maintained under treatments C or D and eighteen days after cutting for those kept under treatments A or B. There was a significantly higher ($P < 0.05$) activity by Lotus plants kept under treatment B (16 hour day; 21/18°C) compared with that obtained

Fig. 16 3rd cut: $\mu\text{moles C}_2\text{H}_4$ produced by sixteen week old (a) *L. uliginosus* cv. Maku and (b) *T. repens* cv. S184 grown in peat in 7" pans under the following light; temperature conditions and cut three weeks after the second cut at thirteen weeks old. Acetylene reduction measurements were made prior to cutting and at intervals throughout three weeks after cutting:

- A) 12/12; 21°/18°C ○
- B) 16/8; 21°/18°C ●
- C) 12/12; 18°/15°C ■
- D) 16/8; 18°/15°C □

Experiment 12.

⊥ = SED.



under the other three treatments. There was no significant difference between the activities by clover plants under any of the four treatments.

Cut 4 (Fig. 17)

Only plants under treatments A (12 hour day; 21/18°C) and C (12 hour day; 18/15°C) received a fourth cut and these were subsequently transferred to a longer light regime of 16 hour day i.e. A to B and C to D. Although a drop in activity by plants grown under the A and C treatment was apparent it was not significant. There was also no significant difference between the effects of the two treatments A-B or C-D. Full activity by Lotus plants equal to that obtained prior to cutting was obtained four days after the cut for those plants under treatment C-D and between six and ten days for those grown under treatment A-B.

There was no significant fall in clover activity after the fourth cut and no significant difference between the effects of the two treatments. Full recovery by clover plants under both treatments was attained between six and eleven days after the first cut.

Acetylene reduction activity continued to increase i.e. those plants which were not cut and grown under treatment B (16 hour day; 21/18°C) or D (16 hour day; 18/15°C). Plants kept under treatment B gave the highest activity for both species (Fig. 18).

Controls (Uncut Plants) (Fig. 19 and 20)

Both clover and Lotus plants showed a trend for increased activity with increased time. Due to the non replication of the controls no statistical analysis can be made. For both species, plants grown under treatment B (16 hour day; 21/18°C) appeared to give the greatest activity and plants maintained under treatment C (12 hour day; 18/15°C) the lowest. In Lotus plants as the time increased the difference between the effects of treatment B compared to the effects of the other treatments increased.

Fig. 17 4th cut: $\mu\text{moles C}_2\text{H}_4$ produced by nineteen week old (a) *L. uliginosus* cv. Maku and (b) *T. repens* cv. Huia plants kept under 12/12 light and 21°/18°C temperature and kept under 12/12 light and 18°/15°C temperature, transferred to 16/8 light and same temps. The plants were cut three weeks after the third cut (Fig. 16) and then transferred. Acetylene reduction measurements were made prior to the growth cut at nineteen weeks old and at intervals of 1, 4, 6, 11, 14, 18 and 20 days after cutting.

(a) A = ●
C = □

(b) A = ○
C = ■

Experiment 12.

I = SED.

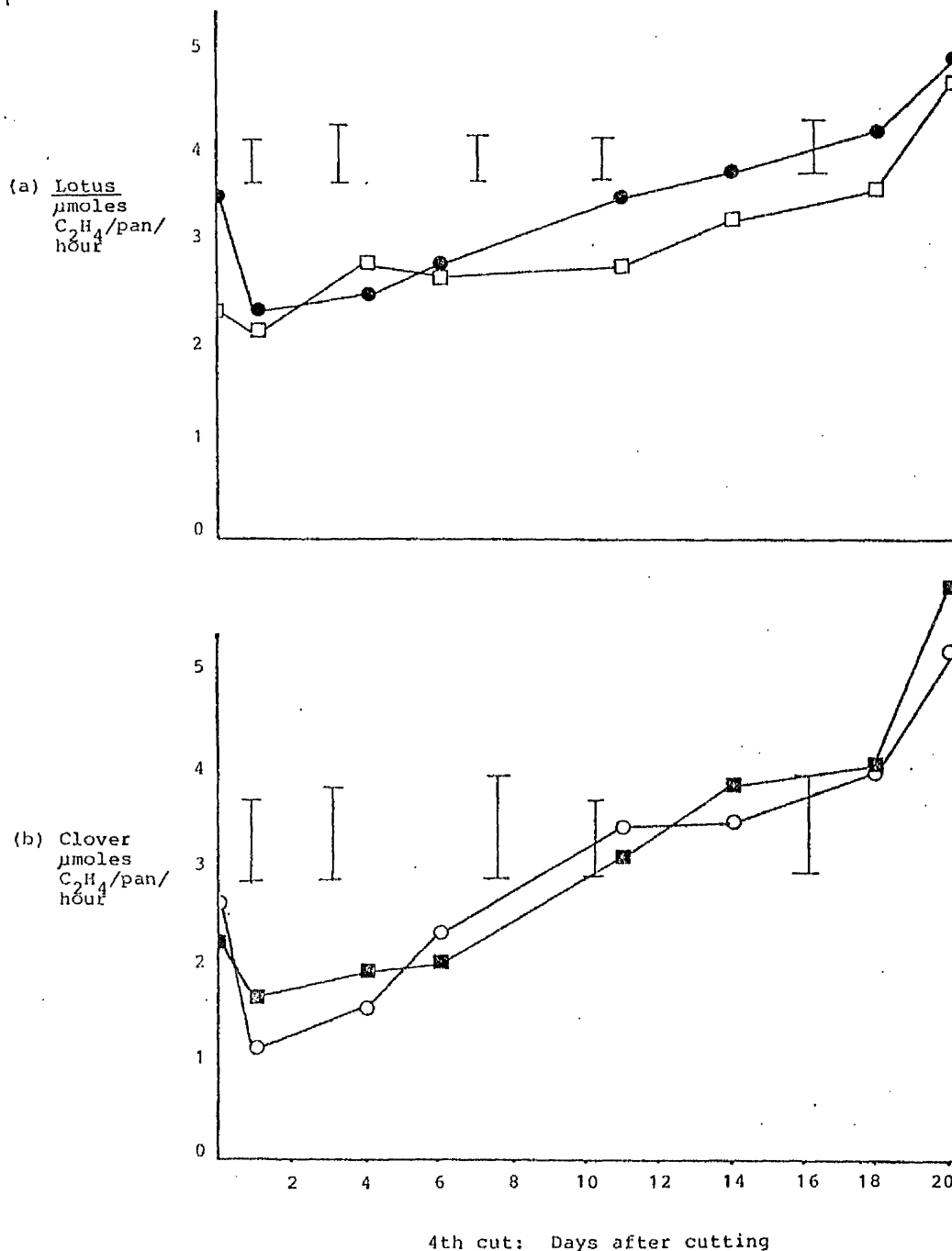


Fig. 18 $\mu\text{moles C}_2\text{H}_4$ produced by (a) *L. uliginosus* cv. Maku and (b) *T. repens* cv. Huia plants kept under 16 hour day and 21°/18°C day/night temperature or 16 hour day 18°/15°C temperature. Acetylene reduction measurements were made at 24, 32 and 40 days after the third cut at sixteen weeks old. (See Fig. 16) \bar{x} = SED.

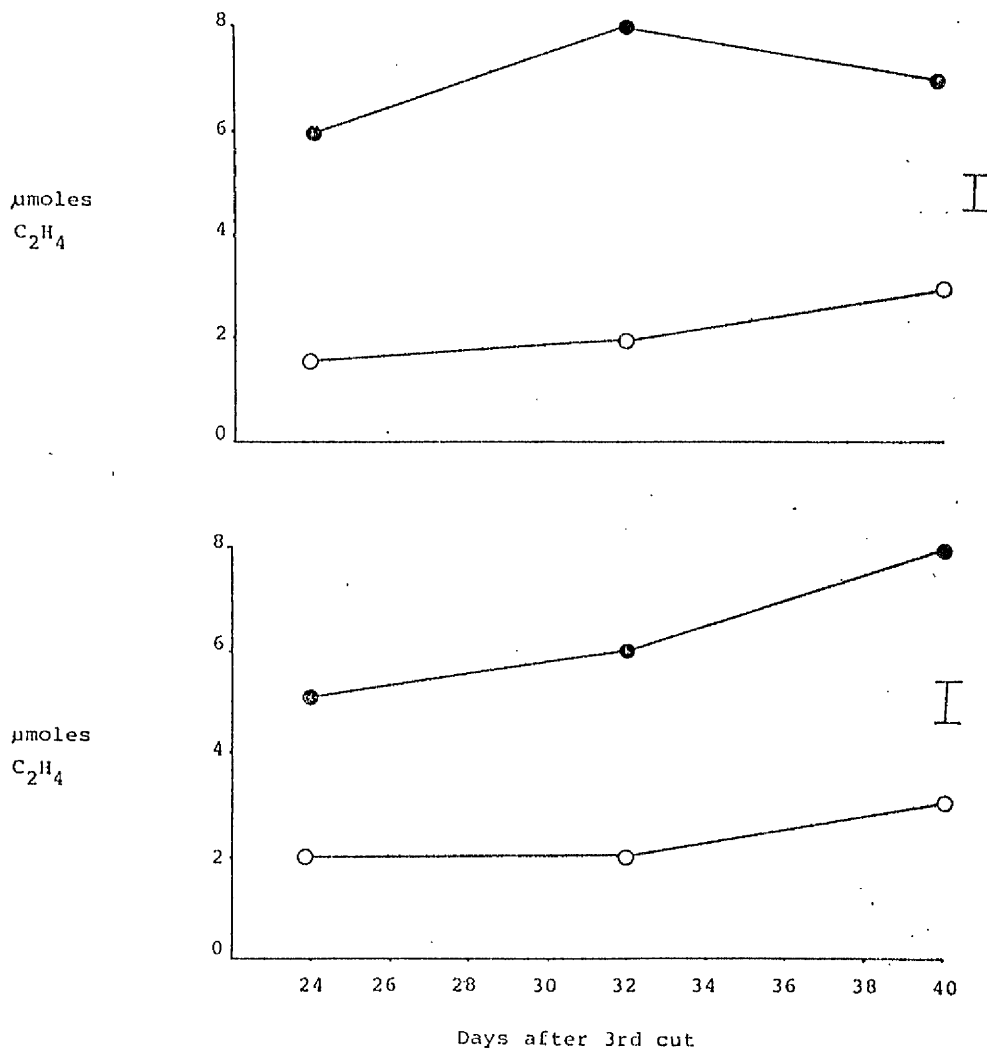


Fig. 19 $\mu\text{moles C}_2\text{H}_4$ produced by *L. uliginosus* cv. Maku plants grown in peat in 7" pans under the following treatments:

- A) 12/12; 21°/18°C ○
- B) 16/8; 21°/18°C ■
- C) 12/12; 18°/15°C ●
- D) 16/8; 18°/15°C □

These plants acted as controls and were not cut throughout the experimental period. Experiment 12. I = SED.

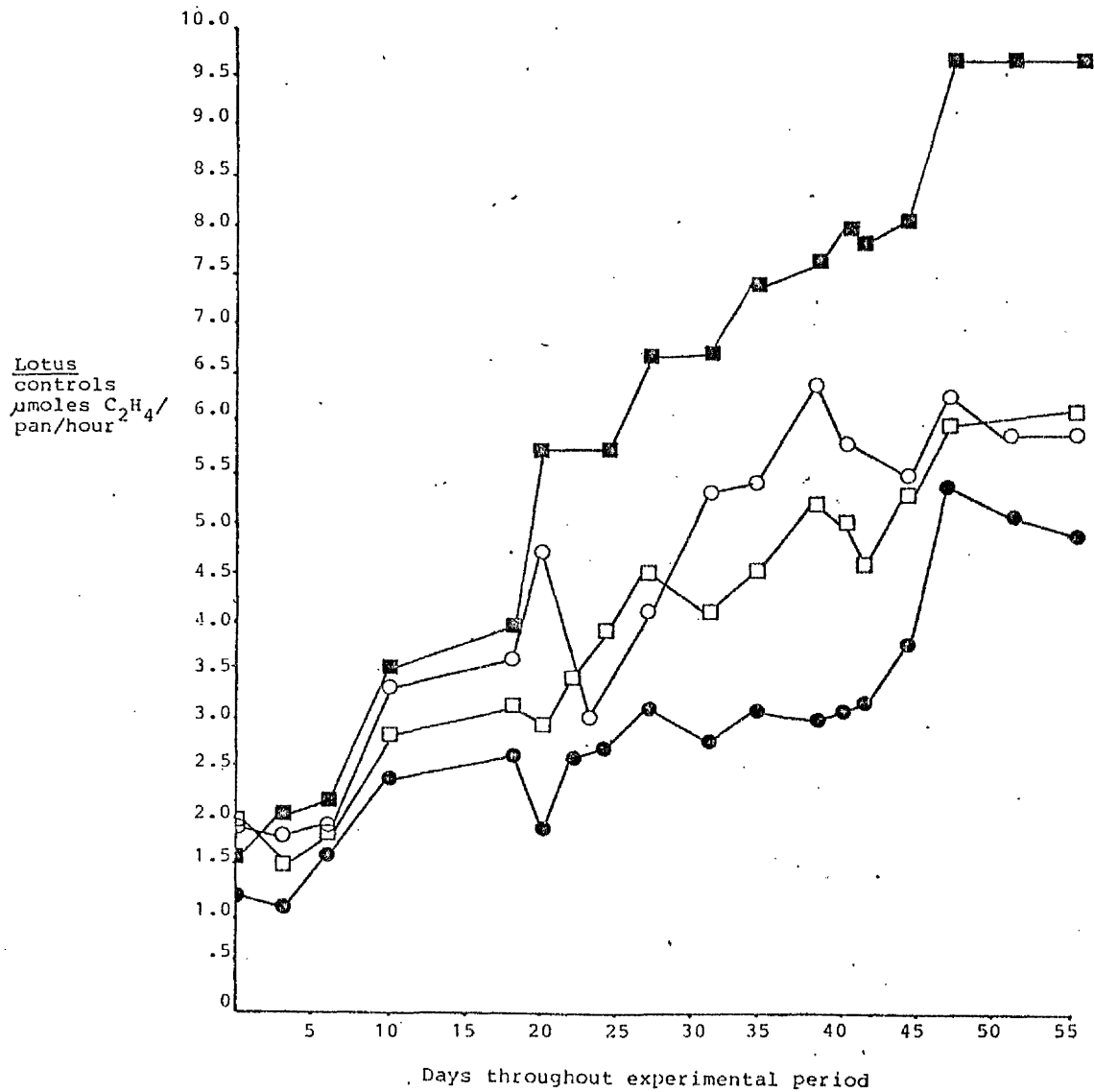


Fig. 20 $\mu\text{moles C}_2\text{H}_4$ produced by *T. repens* cv. Huia plants grown in peat in 7" pans under the following treatments:

- A) 12/12; 21°/18°C ○
- B) 16/8; 21°/18°C ●
- C) 12/12; 18°/15°C ■
- D) 16/8; 18°/15°C □

These plants acted as controls and were not cut throughout the experimental period. Experiment 12. \bar{x} = SED.

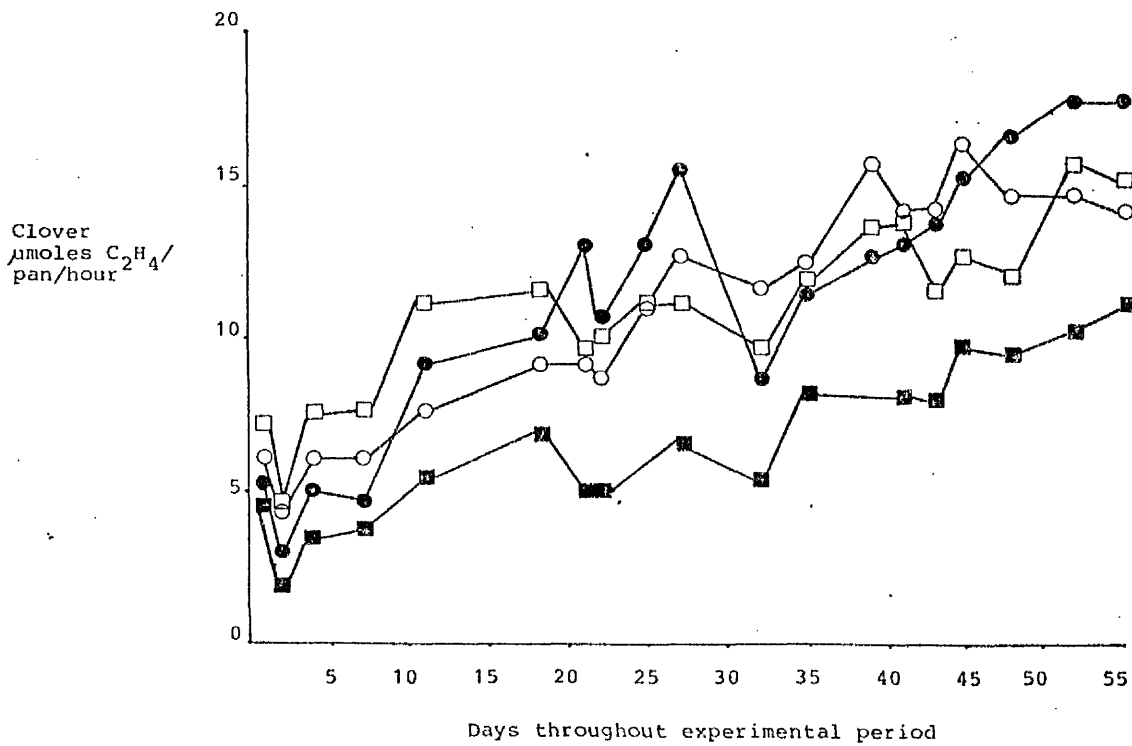
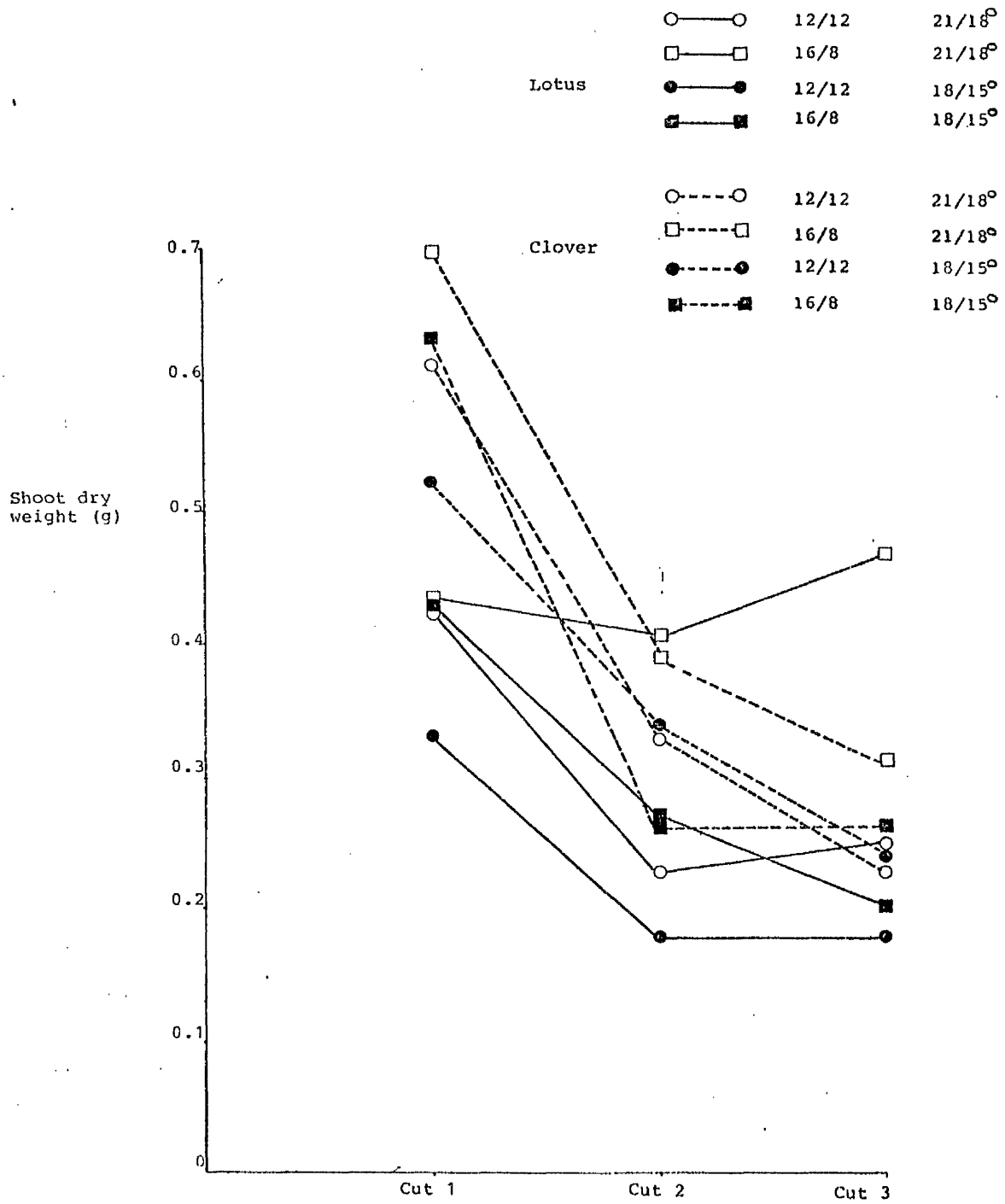


Fig. 21 Shoot dry weight (mg) of *L. uliginosus* cv. Maku and *T. repens* cv. Huia recorded after each cut.



Throughout the study period uncut plants of both species had a greater acetylene reduction activity under all treatments.

Shoot Dry Weight (Fig. 21) (App. III : E : Table 7)

Lotus shoot dry matter at cuts two and three was similar to that at cut one under conditions of long light and high temperature. There was a reduction in shoot dry matter at cuts two and three under conditions of shorter light and high temperature. For clover plants as the number of cuts increased the shoot dry matter yield decreased in all treatments.

12.4 Discussion

The main effect of cutting was the immediate drop in acetylene reduction activity. This phenomenon shown by both species has been monitored by several workers e.g. Butler et al. (1959); Moustafa et al. (1961); Chu & Robertson (1974); Halliday & Pate (1976); Haystead & Lowe (1977) and Barta (1978). In all cases cutting caused a significant loss of nodules and in plants which were repeatedly cut there was a rapid turnover of both root and nodule tissue by clover, but not so rapid in the case of Lotus. The reduction in activity at cut one was probably a combination of the effect of cutting and the transfer of the plants into different lower light/temperature regimes.

Where there were significant differences obtained between the treatments i.e. after cuts 1, 2 and 3, for Lotus species, long day/high temperature conditions resulted in the greatest activity and the least activity was obtained under the remaining treatments. The low activity under these conditions supports the theory of Hardy et al. (1971), who postulated that the decrease in nitrogenase activity per plant and nodule efficiency was due to the reduced photosynthetic period and a reduced rate of photosynthesis caused by the lengthening of the preceding dark period. Nitrogen fixation in plants is A.T.P. dependent and A.T.P. is supplied to the nodules via photosynthesis; any effect therefore which slows down or inhibits photosynthesis will result in a lowering of

nitrogenase activity. Work cited in the Rothamsted annual report (1971) showed that L. corniculatus and L. hispidus were tolerant of cold temperatures (3°C) but that nitrogenase activity declined with low temperatures. It was assumed that, as the leaves expanded on the defoliated plants, the supply of carbohydrate to their nodules was increased and thus was responsible for the partial recovery of nitrogen fixation activity.

The fact that no fall in acetylene reduction activity by Lotus plants and clover plants with the exception of treatment B occurred after the second and third cuts was due to the persistent low level of growth and activity associated with the treatments. The lack of a significant difference for clover plants, between the effect of the treatments indicated that the variation in treatment was not sufficiently great.

When plants under treatments A or C were cut for a fourth time and then transferred to a sixteen hour day, there was a reduction in the time taken for the plants to achieve the rate of activity similar to that prior to the fourth cut, compared with the time taken for recovery under the 12 hour day regimes. This was presumably due to the increased total photosynthate available due to the longer day length. Day (1972) noted that the effect of day length on nodulation was more dramatic than that of higher intensity. Short days completely inhibited or severely reduced the number of nodules formed; thus, conditions favouring the transport of assimilates to the root, e.g. long days, were found to favour nodule production.

Work carried out by Mitchell (1956) showed that L. uliginosus grown under full natural day length and low temperature had a large root system which carried numerous larger pink nodules. McKee (1962) found with L. corniculatus that nodulation under photoperiods of 9 and 11 hours was greatly inferior to nodulation under natural (undefined) and 15 hour photoperiods. Nodulation and thus fixation was therefore reduced by

decreasing photoperiod.

The amount of acetylene reduced by white clover was always higher than that by Lotus. The level of activity by Lotus tended to increase after each cut, more so under long day treatments, whereas the activity of clover after cutting was reduced, especially under the low temperature treatments, with the exception of those plants maintained under treatment B. This cutting regime under the different treatments therefore appeared in general to have a greater deleterious effect on clover activity than on Lotus. This may be due to the growth form of Lotus where the shoot emerging from the crown formed a close mass. When cut back to 2.5 cm a greater amount of leaf may have been left compared with that present when clover, with its upright leafy petioles, was cut to the same level.

Cutting appeared to have on both species, the initial effects of an immediate lowering of acetylene reduction activity which remained at a low level between cuts. Acetylene reduction activity by both species never returned to the level obtained with the uncut plants.

Lotus plants grown under the long day/high temperature conditions gave dry matter levels, at cuts two and three, similar to those obtained by all plants at cut one. Since the levels from cut one reflected ten weeks growth whereas the levels from cuts two and three reflected three weeks re-growth, these conditions must stimulate Lotus shoot growth after cutting. The large drop in Lotus shoot dry matter under conditions of long days and low temperatures was similar to the results obtained by Mitchell (1956), also working with L. uliginosus.

It was noted in this study that low temperatures reduced Lotus shoot growth more subsequent to cutting than did higher temperatures. Mitchell (1956) found that Lotus shoot weight/unit length was reduced at low temperature (11.6°C) but was not reduced at high temperature (22.2°C). A combination of long light and high temperature was therefore required to obtain substantial growth and this would limit establishment until both light and temperature had reached certain levels. There was a marked

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contrast between the high shoot dry weight of plants given long days and higher temperatures and their low weight on transfer to cooler conditions. This partially explains the poor establishment of L. uliginosus during early spring conditions of short days and low temperatures in hill lands. Under the longer photoperiods and higher temperatures of mid summer, the ability of Lotus seedlings to compete with associated plant species might be enhanced and therefore aid establishment.

Clover yielded more dry matter than Lotus except at cut three under long day and high temperature conditions. However, there was a reduction in dry matter production after each cut under all treatments for clover, but not for Lotus, indicating that this cutting regime had a deleterious effect on clover shoot growth. Mitchell (1956) showed that while stem initiation in clover was slower than in Lotus, its first leaf production was faster and this may explain why, under most treatments, clover outyielded Lotus. The results indicated that the cutting regime had little affect on shoot production of Lotus and may have in fact stimulated it during long day/high temperature conditions, whereas with clover shoot production decreased as the number of cuts increased, under all treatments.

Both species grew and fixed nitrogen under short day and low temperature conditions. The fact that clover fixed nitrogen to a greater extent than Lotus under these conditions indicated that it had a potential for quicker establishment; Lotus, however, under long day/high temperature conditions appeared to be more persistent under this cutting regime than did clover.

13. MEASUREMENT OF RHIZOME PRODUCTION

13.1 Aim

The importance of rhizome production by L. uliginosus cv. Maku first became apparent during studies into establishment, when it was found, for example, that new shoots arising in spring at a distance from parent crowns had their origin on an extensive rhizome system. It is thought that winter survival of L. uliginosus is dependent on the production, at the end of the growing season, of rhizomes which act as storage and extension organs. It has been widely claimed (Grueb & Wedin, 1971) that Lotus maintains a low carbohydrate level in its rhizomes during the growing season, only building up this level at the end of the season. The time during which rhizomes are produced is important from the point of view of formulating cutting or grazing managements. If plants are grazed late into the growing season there may not be adequate top growth left to provide the energy required for rhizome production, which may result in winter kill of the plant. This feature will be particularly important in the establishment year when there has been only limited build up of carbohydrate reserves by the plants.

The aim was to monitor rhizome development by L. uliginosus cv. Maku and to confirm reports (Sheath, 1977) that maximum rhizome development occurs in late summer/early autumn.

13.2 Materials & Methods

The site was on the upland plateau east of Sorn, Ayrshire, (Map ref. NS 607273) at an altitude of approximately 206 m. The soil was a moderately wet peat, carrying Calluna/Molinia wet heath vegetation. In 1977 the area was fenced and ten 5 m x 4 m plots were treated with 7.5 t/ha ground limestone, 45 kg/ha ground mineral phosphate (30% P_2O_5), 20 kg/ha superphosphate (20% P_2O_5) and 72 kg/ha muriate of potash (60% K_2O). The pH of this soil in August 1979 recorded from stratified soil cores was

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TABLE 9

Mean monthly weather readings for 1978 and 1979 recorded at the Meteorological station at Auchincruive 44.7 metres above sea level (m.a.s.l.)

	Temperature °C Air		Temperature °C Soil at 10 cm		Total Rainfall (mm)		Total Hours of Sunshine	
	1978	1979	1978	1979	1978	1979	1978	1979
Jan	4.6	0.6	2.0	0.7	79.8	74.7	59.0	51.5
Feb	2.4	1.6	0.7	0.6	41.3	14.8	85.0	89.3
March	6.2	4.0	4.5	2.8	125.9	96.8	82.8	96.2
April	6.4	6.7	5.7	5.8	20.3	58.0	128.1	136.7
May	11.5	8.6	11.5	8.9	7.2	41.8	211.5	197.4
June	12.7	13.1	14.3	13.4	40.9	45.9	182.4	162.4
July	13.5	13.8	14.4	13.9	47.3	83.2	180.0	102.4
Aug	13.8	13.4	14.1	13.3	93.6	146.6	97.3	162.7
Sept	12.8	12.1	12.1	11.5	149.3	65.1	84.8	125.9
Oct	11.7	10.9	10.6	9.4	66.6	115.1	88.3	78.1
Nov	8.1	7.0	7.5	5.8	127.1	182.5	51.8	45.3
Dec	3.4	4.9	2.8	4.1	67.4	121.6	41.2	53.2

5.66 at 0-2.5 cm depth, 4.72 at 2.5-5.0 cm and 3.83 at 10.0-12.5 cm.

In May 1977 and June 1979 L. uliginosus cv. Maku seed inoculated with Rhizobium strain CC 814 s was sown by hand at a rate of 3 kg/ha onto previously rotavated ground and raked in.

Preliminary studies were carried out in 1978 on Maku plants sown in May 1977, to investigate rhizome production in second year plants. Twenty plants were removed monthly from August until November 1978. This work was continued in 1979 when twenty plants from the same sowing (May 1977) were again removed monthly from April to August and weekly during September and October with a final collection in November. Plants sown in 1979 were sampled in their first year between August and November 1979. All plants were washed and separated into shoot, crown and rhizome components. A division was made between short shoots i.e. those produced from new rhizome at the end of the year and tall aerial shoots i.e. those produced by the crown and rhizome throughout the growing season. All fractions were dried at 100°C for twenty hours and the resulting dry weight of each expressed in grams.

Environmental data (Table 9) were obtained from the nearest Meteorological Station at the West of Scotland Agricultural College, Auchincruive, Ayr.

13.3 Results

Growth

The following sequence of morphological developments was observed.

First Year

After germination plants produced aerial shoots and, later, small crowns, with not more than two shoots per crown. There was a rapid increase in shoot dry weight during August and the first part of September, with a peak in early October (Table 10). Crown dry weight continued to increase throughout the autumn. Flowering did not occur in first year plants.

Rhizomes were produced by 36% of the crowns. These arose from the base of the crowns and had no nodes or adventitious buds. Production of rhizomes began in the third week of September and on average two rhizomes were produced per crown. A tap root occasionally developed, but more commonly first year plants had a forked root system, with lateral roots emerging from just below the crown. Dry weights are shown in Table 10.

Second Year

Growth initially was comprised of short aerial shoots formed from the tips of rhizomes laid down in the previous autumn, but later tall aerial shoots arising from the crown made up the bulk of the vegetation. Shoot dry weight increased significantly ($P < 0.01$) from the end of September, reaching a maximum during October. This was followed by a significant decrease ($P < 0.01$) in November (Table 10). Flowering occurred from the end of July until the beginning of September.

Rhizome development in second year plants began in early August. Most production occurred during the first three weeks in October after which it levelled off. The amount of old rhizome and crown also increased during this period (Table 10). New rhizomes emerged from crowns, from nodes on existing rhizomes (up to a maximum of four new rhizomes per node), and latterly from nodes on newly formed rhizomes (up to a maximum of two rhizomes per node). From these nodes adventitious roots also developed, the earliest of which were well nodulated and the later ones less so. The terminal growing points of all new rhizomes eventually formed short aerial shoots, which also arose from nodes elsewhere on the new rhizomes.

Third Year

Short aerial shoots were initiated from nodes on existing rhizomes, including nodes which had not previously borne either new rhizomes or aerial shoots. Later the terminal growing points of existing rhizomes or, if these had been killed, the nodes immediately behind, formed tall aerial shoots; these also developed from the crowns.

Shoot dry weight increased rapidly during June and July, reaching a peak in early August, followed by a significant decline ($P < 0.05$). A further non-significant increase in late September preceded senescence (Table 10). Short aerial shoots were again formed from nodes on newly formed rhizomes. Flowering occurred between late July and the beginning of September.

Rhizome development was similar to that described in the second year. The total amount of rhizome did not alter significantly during the early part of the year and new rhizomes began to emerge in early August. Production reached a peak in September and continued at a reduced rate during October and early November. Increases in the amount of old rhizome and of woody rhizome also occurred in September. Crown dry weight increased slowly during the year (Table 10).

In October of the third year three types of rhizome could be identified viz. 'new rhizome', Plate 1, 'old rhizome', Plate 2, and 'woody rhizome', Plate 3. New rhizome was recognized by its white colour and pink pigmentation at the point of emergence from the node. Old rhizome was darker in colour and more pliable than woody rhizome, which was thick and brown. Most of the new rhizomes arose from old rhizomes (i.e. of the previous year) and any which emerged from nodes on woody rhizomes tended to be short and thin. Few rhizomes emerged from the crown in the third year, and in some cases the crown appeared to be dead (Plate 4).

By the end of the third year, therefore, an extensive rhizome system had developed around each of the original crowns. Rhizomes were generally confined to the top 5.0 cm of the soil, occasionally penetrating to between 10.0 and 12.5 cm. Rhizomes occasionally emerged above the soil surface and grew horizontally along the surface for a time, becoming green on the upper side, before turning down into the soil again. Frost damage to rhizomes growing near to the soil surface frequently led to the formation of isolated rhizome sections (Plate 5).

TABLE 10

Dry weight measurements (g) of rhizome, crown and shoot production in L. uliginosus cv. Maku plants taken from hill land (Sorn) plots Experiment 13

(a) 1st year plants (Sown June 1979, analysed during 1979)			
	New rhizome (g)	Crown (g)	Shoot (g)
9th Aug	0	0.014	0.049
6th Sept	0	0.016	0.217
19th Sept	0.003	0.016	0.240
5th Oct	0.012	0.020	0.314
23rd Oct	0.010	0.022	0.216
2nd Nov	0.008	0.030	0.270

(b) 2nd year plants (Sown June 1977, analysed during 1978)				
	New rhizome (g)	Old rhizome (g)	Crown (g)	Shoot (g)
11th Aug	0.09 ± 0.12	0.20 ± 0.12	1.21 ± 0.18	4.27 ± 0.77
27th Sept	0.64 ± 0.13	0.26 ± 0.61	4.10 ± 0.49	7.20 ± 1.16
23rd Oct	5.89 ± 0.84	1.84 ± 0.49	2.57 ± 0.37	18.55 ± 3.32
22nd Nov	4.85 ± 0.66	1.18 ± 0.26		13.50 ± 2.39

(c) 3rd year plants (Sown June 1977, analysed during 1979)						
	New rhizome (g)	Old rhizome (g)	Woody tissue (g)	Crown (g)	Tall aerial shoots (g)	Short aerial shoots (g)
4th April	5.72 ± 0.82	2.23 ± 0.62		1.20 ± 0.20	0.53 ± 0.08	
8th May	6.35 ± 0.88	1.05 ± 0.32		1.20 ± 0.14	0.50 ± 0.08	
5th June	5.55 ± 1.02	1.30 ± 0.45		0.94 ± 0.16	2.81 ± 0.50	
10th July	4.18 ± 0.70	0.97 ± 0.19		1.19 ± 0.22	15.71 ± 2.37	
7th Aug	0.16 ± 0.04	6.48 ± 0.68	0.70 ± 0.19	1.63 ± 0.23	34.02 ± 3.19	
6th Sept	1.40 ± 0.35	5.28 ± 1.58	1.38 ± 0.30	1.17 ± 0.19	22.37 ± 3.72	
21st Sept	3.61 ± 0.51	10.60 ± 1.40	2.21 ± 0.43	1.30 ± 0.20	31.57 ± 3.14	
28th Sept	4.71 ± 0.81	9.37 ± 1.19	3.34 ± 0.69	1.46 ± 0.25	30.37 ± 3.88	0.58
8th Oct	4.83 ± 0.58	11.62 ± 1.27	2.40 ± 0.63	2.05 ± 0.38	26.19 ± 3.19	1.18
15th Oct	5.60 ± 0.79	8.94 ± 1.23	2.29 ± 0.49	1.55 ± 0.32	19.24 ± 2.73	1.77
8th Nov	5.98 ± 0.67	9.40 ± 0.97	2.21 ± 0.28	1.41 ± 0.22	14.86 ± 2.21	

Plate 1

New rhizome showing adventitious roots with attached nodules. Also shows development of new shoots from rhizome tip and nodes.

N = New shoot formed from tip of rhizome

E = Effective nodules

S = New shoot formed from node on rhizome

A = Adventitious roots with effective nodules

Plate 2

Old rhizome growth

C = Crown

O = Old rhizome

Plate 3

Woody tissue showing tap root and crown

C = Crown

T = Tap root

W = Woody tissue

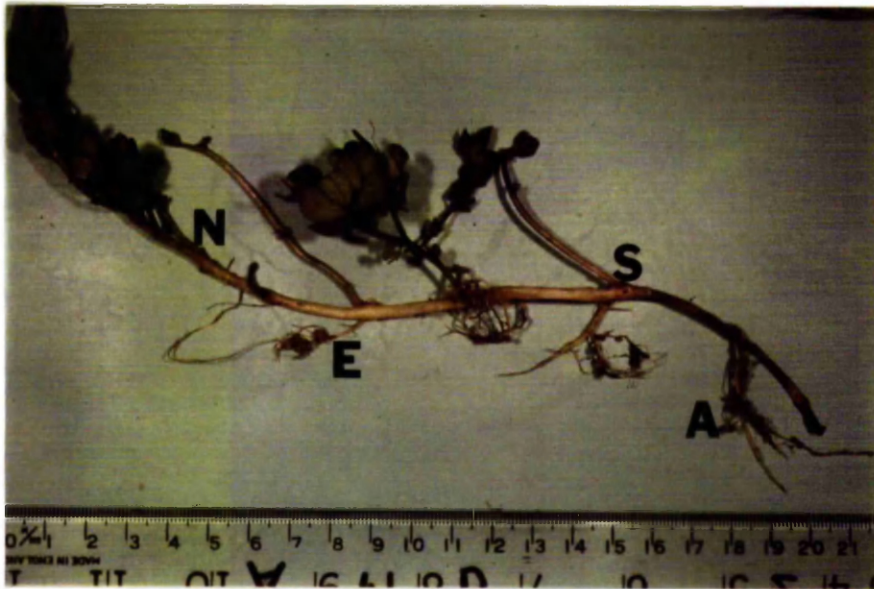


PLATE 1



PLATE 2

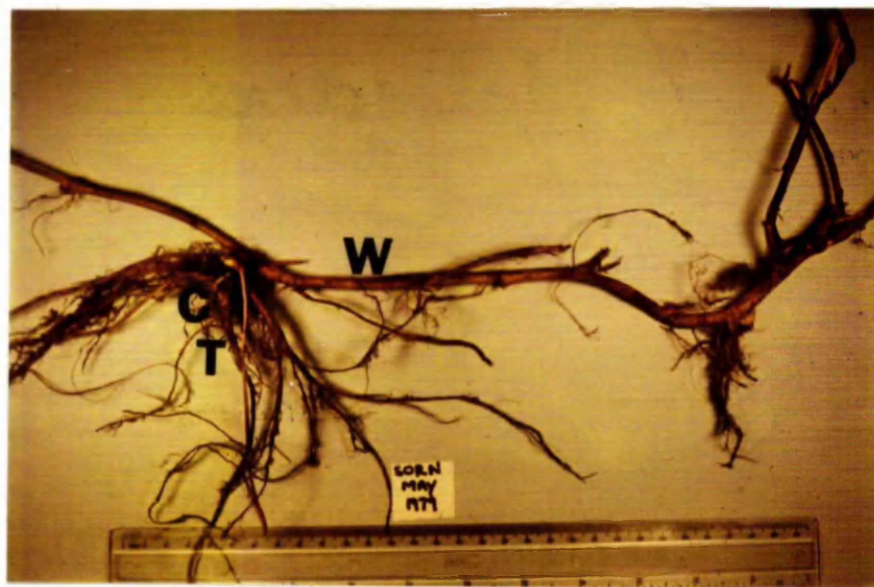


PLATE 3

135(8)

Plate 4

Crown with tap root and associated laterals

C = Crown

T = Tap root

L = Lateral roots

N = New rhizome

Plate 5

New rhizome showing signs of degeneration and new spring shoot productio

D = Degenerated root

S = Newly formed short shoots

N = New rhizome

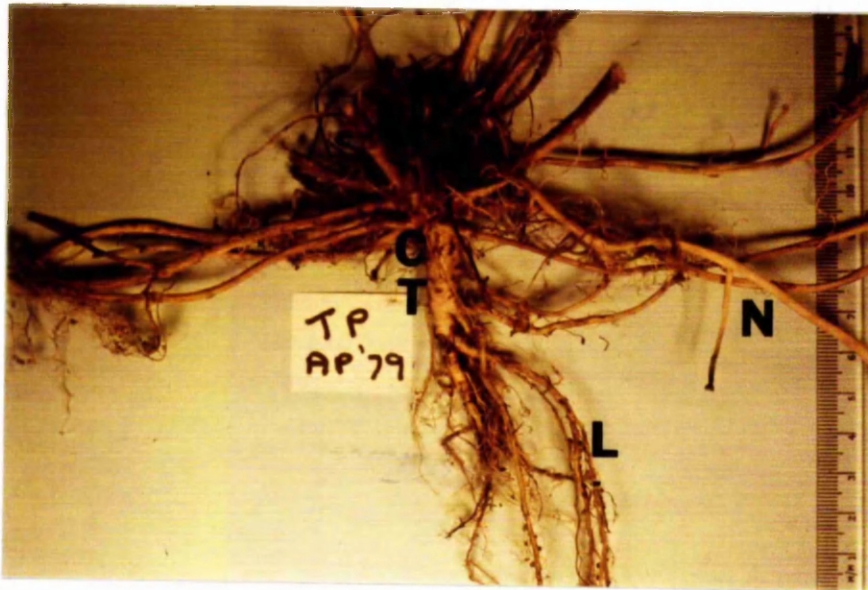


PLATE 4

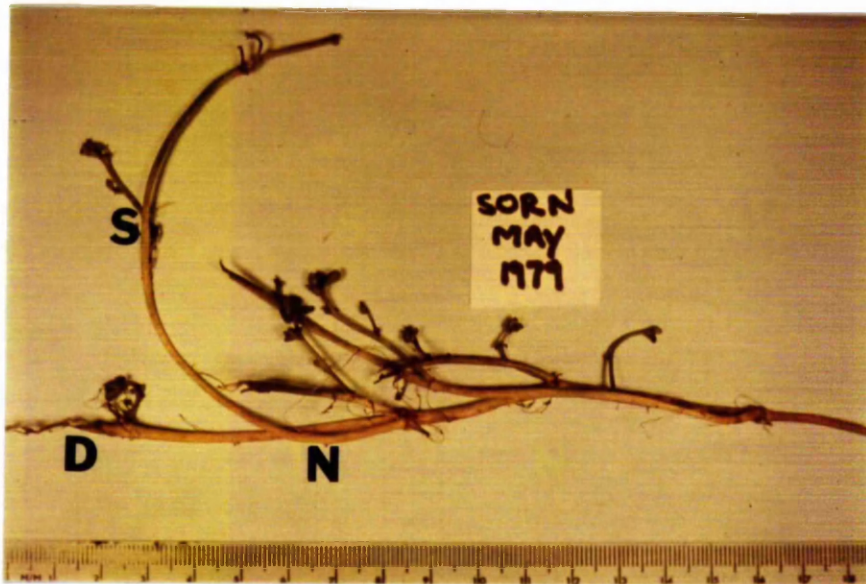


PLATE 5

13.4 Discussion

Growth

These results indicate a marked seasonality in both rhizome and shoot production in L. uliginosus under upland conditions in south-west Scotland.

Rhizomes

In the first autumn after sowing relatively few rhizomes were formed and most plants overwintered in the form of a small crown. A number of plants died during the winter, but this did not appear to be associated with the presence or absence of rhizomes.

The subsequent pattern of rhizome development was similar in both the second and third years, although the timing varied. In both years rhizome emergence commenced in early August, when soil temperature and solar radiation levels were relatively high for the year (Table 9). However, maximum rhizome production coincided in both years with decreases in temperature and radiation and occurred some three weeks earlier in 1979, which was in general cooler and duller than 1978.

Mitchell (1956) found that initial rhizome emergence in L. uliginosus seedlings took place at low temperature or under high light intensity, and a similar result was obtained for temperature alone by Sheath (1977). Precise inferences cannot be drawn from the limited data available, but it appears therefore that annual variations in soil temperature especially may produce corresponding shifts in the timing of rhizome production.

Total production of 'new rhizome' dry matter was no greater in the third year than in the second, while that of existing rhizome components increased by approximately twofold. This implies that a limited amount of energy is available for rhizome production, which must be shared among the different rhizome types. Rhizome extension is therefore likely to be greatest in establishing plants, whereas in established plants, three years old or more, the available energy will be increasingly utilised in the form of storage products.

These well-developed rhizomes are an important feature of established plants of L. uliginosus in relation to lateral spread, shoot production and overwintering. Their role is less clear, however, in first year plants, many of which survived without having formed rhizomes, and in which initial crown development appears to be particularly relevant.

Shoots

In both first and second year plants maximum shoot dry weight was recorded in October, coinciding in the second year particularly with maximum rhizome production. Also in the second year shoot production was low during August and September, when flowering and seeding were taking place.

In the third year the situation was more complex. Shoot production was more extensive and less dependent on the crowns. An initial peak in shoot dry matter in early August was followed by a significant decline during flowering. A further non-significant increase in shoot production in September again coincided with maximum production of all three rhizome components.

Final shoot senescence in the third year (1979) was also about two weeks earlier than in the second year and coincided with the development of short aerial shoots from the newly formed rhizomes. The fate of these short shoots was not definitely established, but at Sorn few survived unless protected by a mat of decaying, tall, aerial shoots or other vegetation. In more favourable locations they may provide a basis for renewed aerial growth in the spring.

This work was carried out in ungrazed conditions and further work should attempt to elucidate the effect of cutting and grazing management on the production of new rhizome. Sheath (1977) has indicated that severe or frequent defoliations result in later and lower production of new rhizome.

14. MEASUREMENT OF SEASONAL FIXATION

14.1 Aim

The aim of the studies was to determine the seasonal pattern for N₂ fixation by L. uliginosus cv. Maku growing under field conditions.

14.2 Materials and Methods

Two sites were used to record seasonal fixation. One was at plots situated at Sorn described on page 162. The second site was situated at Eaglesham (O.S. Ref. NS 55 49), which is a wet peat site, with the layout of the plots as below. These plots were 5 m x 3 m and were sown in May 1971 with four L. uliginosus cultivars: Beaver, Border, Grasslands 4703, Grasslands 4704 and T. repens cv. S100. The site, unlike that at Sorn, is continually grazed by sheep. The acetylene reduction assays were conducted on seven 5 cm diameter by 7.5 cm depth cores per 4.6 l glass incubation jars with an incubation time of one hour.

SOUTH

WHITE CLOVER	BORDER	4703	4704
4704	BEAVER	WHITE CLOVER	WHITE CLOVER
BEAVER	4704	BEAVER	4703
4703	WHITE CLOVER	4704	BORDER
BORDER	4703	BORDER	BEAVER

NORTH

14.3 Results Grazed Site: Eaglesham

For all the cultivars of L. uliginosus studied, acetylene reduction activity oscillated throughout the study period (App. II : C : Table 3).

White clover started off at a higher level of activity than, but with an overall activity similar to, that of the L. uliginosus cultivars. There was an immediate increase in activity during June followed by a rapid decline during October, until a low level of activity occurred in November, for all L. uliginosus cultivars.

14.4 Discussion Grazed Site

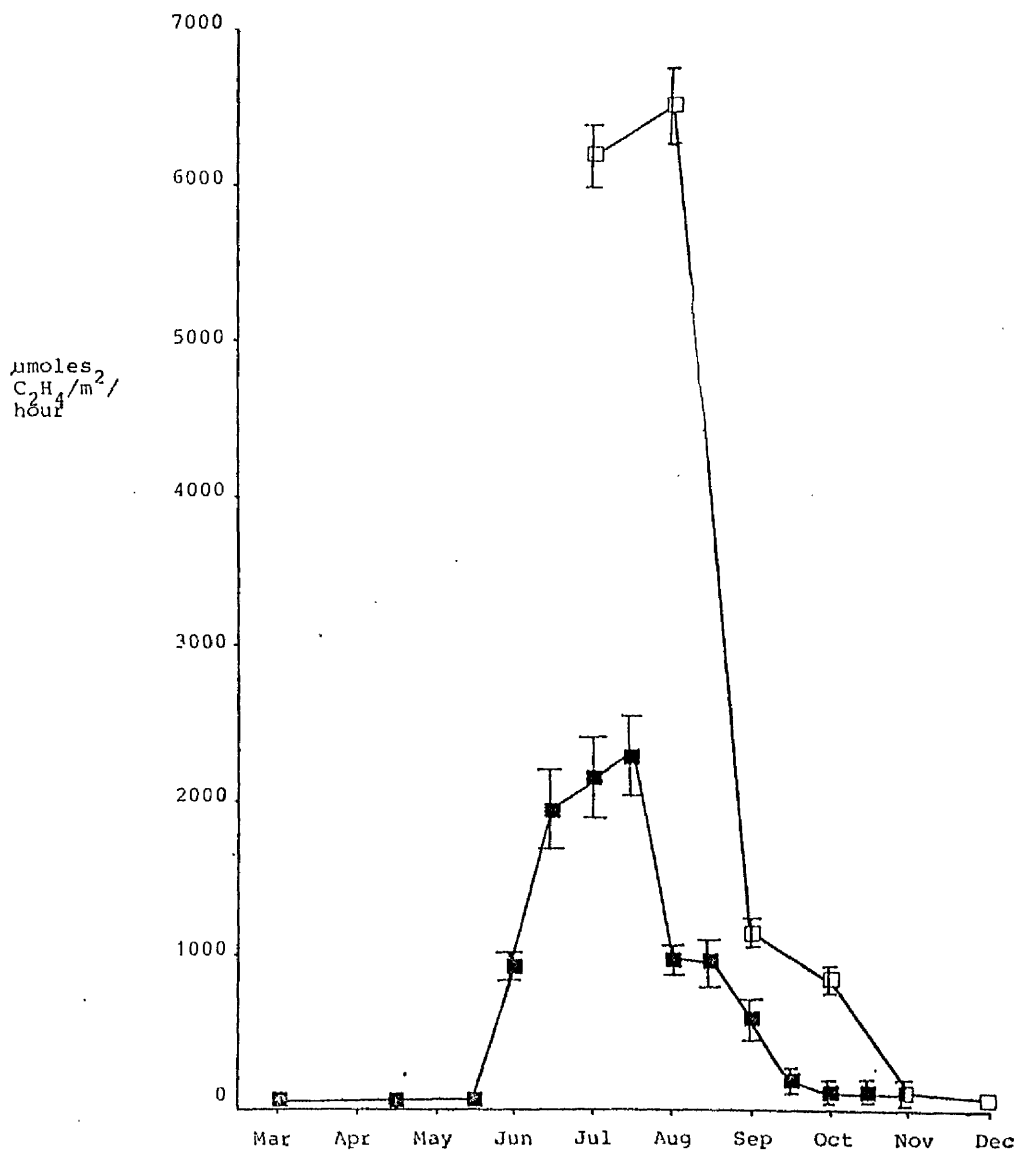
No worthwhile conclusions could be drawn from the data because there was no control over grazing by sheep and no environmental parameters such as soil temperature or rainfall were measured. The falls in activity may have coincided with periods of grazing while the increases may have been due to periods of regrowth. The effect of flowering was not applicable to this study as the plants never reached the flowering stage, due to grazing. Oscillations in activity throughout the study may also have been due to the wetness of the site. The site became waterlogged quickly after periods of rain and fixation would therefore have been at a lower level at these times compared to when the site was drier.

14.5 Results Ungrazed Site: Sorn

In 1978 fixation was not measured using cores until late July and the highest level of recorded activity occurred at the beginning of August which was followed immediately by a marked decline. There was a more gradual decline from September onwards, with virtually no activity taking place in November and December (Fig. 22) (App. II : C : Table 4).

In 1979 fixation was measured from March to November and remained at a very low level until the beginning of June. There was a rapid increase in activity throughout June and early July followed by a gradual decrease until the middle of August when it levelled out. A further decline occurred in September and virtually no activity took place in October and November (Fig. 22).

Fig. 22 Seasonal fixation in 1978 \square and 1979 \blacksquare as $\mu\text{moles C}_2\text{H}_4$ produced by the mean measurements from six replicate plot sets, each set consisting of seven 5 cm diameter x 7.5 cm depth cores, taken from an ungrazed field site (Sorn) sown in June 1977 with *L. uliginosus* cv. Maku $\text{I} = \text{SE}$



14.6 Discussion Ungrazed Site

Fixation rates during 1978 were higher and fixation continued for longer than in 1979, although the pattern was similar in both years. The lower overall level of fixation in 1979 is attributed to a combination of higher rainfall, lower soil temperatures and lower solar radiation levels, than in 1978. The initial rise in fixation in 1979 coincided with an increase in soil temperature (Table 9), suggesting that fixation was previously inhibited by low temperature; similar results were obtained for T. repens by Masterson & Murphy (1973). However, the same authors also showed that inhibition of fixation at the start of activity was associated less with low soil temperatures than with increased soil moisture. Rainfall at Sorn was high during April and May 1979, the period immediately preceding the commencement of fixation. Similarly, the initial decline coincided in both years with an increase in rainfall to the maximum for the year up to that point, which occurred a month earlier in 1979 than in 1978. After this initial decline there was a reduction in rainfall in both years and the fall in fixation was arrested. Inhibition of fixation due to high rainfall is probably caused by the effect of waterlogging on the activity of the nodules (Sprent, 1979).

Flowering, which in both years occurred from the end of July until the beginning of September, probably also contributed to the initial decline in fixation. Flowering has been shown to be associated with degeneration of existing nodules and to inhibit new nodule and root formation (Pate, 1958). Shoot production itself, however, had a good positive correlation ($R = 0.67$) with N_2 fixation.

The final decline in fixation in both years was again associated with a combination of high rainfall and low soil temperatures. The channelling of energy into production of new rhizomes may also have contributed to this decline as rhizome production and N_2 fixation during 1979 were highly negatively correlated ($R = -0.94$). Lotus, unlike white clover, does not

show a second peak in activity prior to senescence (Haystead & Lowe, 1977;
Masterton & Murphy, 1973).

15. Measurement of Fixation in Stratified Cores

15.1 Aim

L. uliginosus has been widely claimed to grow and nodulate at low pH levels usually specified as being around pH 4.5. These pH's have been determined in complete soil cores of up to 15 cm in depth so that any alterations in pH with increasing depth are likely to have been masked.

The aim was to investigate pH and phosphate levels at different soil depths and to obtain correlations between these and nitrogen fixation (measured by acetylene reduction) at corresponding depths.

A visual estimation of Lotus growth was also carried out on plots which had been laid out to investigate the effect of varying fertiliser rates and the presence and absence of added lime on establishment of Lotus.

15.2 Materials and Methods

Table 11 shows the layout of the plots and their treatments which were sown with L. uliginosus cv. Maku at Sorn in June 1977 at a seeding rate of 3 kg/ha.

Two limed and two unlimed plots were investigated in August 1978 and four limed plots in August 1979. All had phosphate and potassium applied in June 1977 at the following rates: 10 kg P/ha as GMP, 10 kg P/ha as supers, 20 kg K/ha as 60% muriate of potash; lime was applied at 7.5 tonnes/ha. Fifteen 5 cm diameter x 15 cm depth cores were removed from each plot, care being taken to include a Lotus plant or part of one in each core. In the unlimed plots where no plants were visible cores were removed at random. Each core was divided into five segments each 2.5 cm long and the bottom 2.5 cm discarded. The corresponding segments of each core were bulked in incubation jars, subjected to an acetylene reduction assay and incubated for one hour in the field. The contents of each jar were then analysed for pH, P, K and moisture content.

Plot size = 5 m x 2 m

Plot number = 84

Replication = x 3

<u>Fertiliser Treatment per Plot</u>	<u>Code</u>
10 kg P/ha as GMP + 10 kg P/ha as supers (i.e. 23 kg P ₂ O ₅ = 10 kg P/ha)	20P
15 kg P/ha as GMP + 15 kg P/ha as supers (i.e. 34.5 kg P ₂ O ₅ = 15 kg P/ha)	30P
40 kg P/ha as GMP	40P
20 kg K/ha as 60% muriate of potash (i.e. 24 kg K ₂ O/ha = 20 kg K/ha)	20K
40 kg K/ha as 60% muriate of potash (i.e. 48 kg K ₂ O/ha = 40 kg K/ha)	40K
20 kg P/ha as GMP + 20 kg P/ha as supers + 60 kg K/ha as muriates	P+K
No fertiliser added	NF
7.5 tonnes/ha lime added	+
No lime added	-

Cover score

Scored out of ten (complete cover) for L. uliginosus growth
visually assessed August 1979.

TABLE 11

Layout, fertiliser treatment and cover score of Sorn plots sown
June 1977 with *L. uliginosus* cv. Maku

1,1 30P + 8	1,2 20P + 8	1,3 P+K + 9	1,4 40P + 10		1,5 20P - 2	1,6 40K - 0	1,7 NF - 0	1,8 20K - 0
2,1 30P + 9	2,2 20K + 8	2,3 40K + 9	2,4 NF + 9		2,5 40P - 0	2,6 P+K - 0	2,7 30P - 0	2,8 30P - 0
3,1 NF + 6	3,2 40P + 5	3,3 40K + 4	3,4 20K + 5		3,5 20P - 0	3,6 40K - 0	3,7 NF - 0	3,8 40P - 1
4,1 NF + 10	4,2 30P + 6	4,3 P+K + 9	4,4 20P + 9		4,5 P+K - 1	4,6 20K - 0	4,7 40P - 1	4,8 20P - 0
5,1 40K + 7	5,2 20K + 7	5,3 40P + 5	5,4 P+K + 8		5,5 40K - 1	5,6 20K - 0	5,7 30K - 0	5,8 P+K - 0
6,1 20K - 0	6,2 40K - 0	6,3 P+K - 0	6,4 NF - 0		6,5 20P + 9	6,6 P+K + 9	6,7 40P + +	6,8 40K + +
7,1 20P - 1	7,2 40P - 1	7,3 NF - 0	7,4 30P - 0		7,5 20P + 10	7,6 30P + 8	7,7 NF + +	7,8 20K + +
8,1 P+K - 0	8,2 20K - 0	8,3 40P - 1	8,4 30P - 1		8,5 NF + 10	8,6 40P + 9	8,7 P+K + +	8,8 40P + +
9,1 NF - 0	9,2 40K - 0	9,3 20P - 0	9,4 NF - 0		9,5 40P + 7	9,6 30P + 7	9,7 20K + +	9,8 20P + +
10,1 40p - 2	10,2 P+K - 0	10,3 20K - 1	10,4 30P - 0		10,5 20K + 7	10,6 40K + +	10,7 20P + +	10,8 30P + +
		11,1 20P - 0	11,2 40K - 0		11,3 NF + 6	11,4 P+K + +	+ +	+ +

A visual cover score out of ten, for growth of Lotus was awarded to all plots sown in August 1979 (Table 11).

15.3 Results

Data for August 1978 (Table 12) showed marked gradients in all parameters measured with the exception of K content. Acetylene reduction occurred down to, but not below, 10 cm depth indicating this as being the lower limit of N_2 fixation. N_2 fixation was greatest in the top 2.5 cm with a marked decrease between the second and third core segments. Over 95% of the activity took place in the first 7.5 cm.

The pH level was highest in the upper core segment and low (pH 3.7) below 10 cm depth with maximum decreases occurring between segments two and three (pH 5.01-4.32). It is therefore clear that in this soil the affect of added lime was concentrated in the upper layers. It also appears that the pH level shown in segment three may be limiting for N_2 fixation which here required the addition of sufficient lime to raise the pH at least to the level of segment two (pH 4.9).

A high level of available phosphate was detected only when both lime and phosphate had been applied to the peat.

Regressions were calculated for N_2 fixed (measured as $\mu\text{moles } C_2H_4$ produced/hour) against pH, phosphate, potassium and moisture levels (Table 13). These showed that the higher the pH and phosphate level, the higher the rate of fixation which occurred under these conditions i.e. pH 5.6 and available phosphate of 16 parts per hundred thousand. It also indicated that a low rate of fixation was linked with low pH and low available phosphate.

Since N_2 fixation did not take place below 10.0 cm, cores of 7.5 cm depth were chosen for subsequent studies on field N_2 fixation.

Table 14 for August 1979, shows a marked gradation in all measured parameters as depth of core increased, with the exception of K content which increased between depths 5 to 10 cm.

In all plots the highest levels of N_2 fixation took place in the first 2.5 cm with decreases in activity occurring as the depth of core increased. Fixation was detected down to 12.5 cm. In most plots over 95% of the N_2 fixation took place in the first 7.5 cm.

The greatest drop in fixation occurred between 2.5 and 5.0 cm and correspondingly the highest drop in phosphate content also occurred between these two segments. N_2 fixation gradually decreased until a low level was obtained at 10 and 12.5 cm. Phosphate content followed a similar pattern to that of fixation and these two parameters were highly correlated (Table 15).

The highest drop in pH occurred for all plots, with the exception of plot 1, between 2.5 and 5.0 cm. The level of pH also decreased as the depth of core increased until it reached a steady level at 10 and 12.5 cm. Table 15 shows that pH and N_2 fixation were also highly correlated.

N_2 fixation was also highly correlated to the moisture content of each segment (Table 15). As the depth of core increased the moisture content decreased. N_2 fixation was not correlated to the K content of the soil.

The obvious result from the visual scores of the plots (Table 11), was the absence of Lotus in plots with no lime added. The unlimed plots which had some Lotus plants present were those to which phosphate had been added. Addition of K to unlimed plots did not increase growth of Lotus (Plate 6).

TABLE 12

Various measurements from cores removed from hill land (Sorn) plots in August 1978. The plots had been treated with varying quantities of phosphate, potassium and lime (as shown in Tab 11) and sown with *L. uliginosus* cv. Maku in June 1977. Experiment 15

	Core	Segment depths	AR	pH	P	K	Total moisture content of cores	% activity of total acetylene reduction in cores
<u>Plot</u>	1	(0-2.5 cm)	5.10	5.49	16	26	7.2	47.31
1, 3	2	(2.5-5 cm)	5.30	4.87	9	23	7.1	49.17
	3	(5.0-7.5 cm)	0.30	4.18	3	25	7.1	2.78
	4	(7.5-10.0 cm)	0.08	3.87	3	27	6.2	0.74
	5	(10.0-12.5 cm)	0.00	3.69	2	21	6.4	0.00
<u>Plot</u>	1		7.80	5.64	16	13	8.0	69.15
4, 3	2		2.90	5.18	8	13	7.2	25.71
	3		0.50	4.46	5	15	6.0	4.43
	4		0.08	4.02	3	18	6.0	0.71
	5		0.00	3.57	2	17	6.0	0.00
<u>Plot</u>	1		0.00	4.07	7	19	7.0	0.00
2, 6	2		0.00	3.85	6	16	6.8	0.00
	3		0.00	3.75	7	24	6.8	0.00
	4		0.00	3.68	5	24	7.2	0.00
	5		0.00	3.54	1	20	6.0	0.00
<u>Plot</u>	1		0.00	3.80	4	16	5.6	0.00
4, 5	2		0.00	3.71	4	19	6.0	0.00
	3		0.00	3.63	2	19	6.2	0.00
	4		0.00	3.61	1	17	6.2	0.00
	5		0.00	3.54	1	17	6.2	0.00

TABLE 13

Correlation coefficients to establish any relationships among acetylene reduction ($\mu\text{moles C}_2\text{H}_4/\text{hour}$) and pH, phosphate, potassium and moisture levels recorded from cores removed in August 1978. Experiment 15

Plot	AR:pH	AR:P	AR:K	AR:Moisture
1, 3	0.93	0.90	-0.04	0.71
4, 3	0.89	0.99	-0.77	0.97

TABLE 14

Various measurements from cores removed from hill land (Sorn) plots in August 1979. The plots had been treated with varying quantities of phosphate, potassium and lime (as shown in Tab 11) and sown with *L. uliginosus* cv. Maku in June 1977. Experiment 15

Core	Segment depths	AR	pH	P	K	Total moisture content of cores	% activity of total acetylene reduction in core
<u>Plot</u>	1 (0-2.5 cm)	5.69	5.66	16	11	8.0	83.82
1, 3	2 (2.5-5 cm)	0.69	4.80	6	16	6.6	8.69
	3 (5.0-7.5 cm)	0.30	4.16	5	26	6.4	4.42
	4 (7.5-10.0 cm)	0.10	3.87	2	18	5.8	1.47
	5 (10.0-12.5 cm)	0.12	3.82	1	11	6.0	1.77
<u>Plot</u>	1	4.70	5.18	16	12	8.8	70.39
4, 3	2	0.99	4.50	8	29	6.2	14.83
	3	0.59	4.08	3	28	5.8	8.84
	4	0.10	3.83	2	22	4.8	1.50
	5	0.10	3.65	1	17	5.6	1.50
<u>Plot</u>	1	3.96	6.26	25	23	8.0	72.79
6, 6	2	1.09	4.89	14	25	6.0	20.04
	3	0.15	4.09	7	26	6.0	2.76
	4	0.15	3.79	4	22	5.2	2.76
	5	0.10	3.64	2	20	5.0	1.84
<u>Plot</u>	1	4.70	5.55	17	16	7.0	77.48
8, 7	2	0.87	4.68	9	25	7.2	14.34
	3	0.25	4.14	5	24	6.4	4.12
	4	0.25	3.84	1	15	6.0	4.12
	5	0.25	3.76	1	15	5.2	4.12

TABLE 15

Correlation coefficients to establish any relationships among acetylene reduction ($\mu\text{moles C}_2\text{H}_4/\text{hour}$) and pH, phosphate, potassium and moisture levels recorded from cores removed in August 1979. Experiment 15

<u>Plot</u>	AR:pH	AR:P	AR:K	AR:Moisture
1, 3	0.90	0.96	-0.48	0.96
4, 3	0.93	0.96	-0.50	0.82
6, 6	0.97	0.96	0.07	0.95
8, 7	0.93	0.93	-0.44	0.50

Plate 6

Field site at Sorn showing L. uliginosus cv. Maku growth in limed plots
(1979)

A = Limed plots

B = Unlimed plots

PLATE 6



15.4 Discussion

The results from the two core depth investigations showed that N₂ fixation was highly correlated to both pH and available phosphate content. The greatest activity was concentrated in the segments near to the soil surface. These results were similar to those obtained by Sinclair et al. (1976) from work carried out on grass/clover pasture. N₂ fixation was concentrated close to the soil surface and over 80% of the activity was found in the surface 7.5 cm. Hoglund & Brock (1978), working on ryegrass white clover pasture, similarly found that 70% of activity occurred in the top 7.5 cm.

N₂ fixation was recorded at a greater depth in August 1979 compared to August 1978. This was probably due to the growth of root and nodules which had occurred between these two times of measurement. Cores of 7.5 cm in depth continued to be used for subsequent field work as activity was still greatest within this depth.

The absence of, or low, fixation at pH's of 3.54-3.69 indicated that the surrounding soil environment was not suitable for effective nodulation. This may have been due to the restricted diffusion of air down into the soil and to the absence of suitable rhizobia. Greenwood (1961) considered that Lotus rhizobia would be absent in soils of low pH i.e. 4.5 which had a low fertility but addition of lime alone resulted in the soil becoming a relatively favourable medium for both clover and Lotus rhizobia. Greenwood (1961) also found that even in an extremely phosphate deficient soil the addition of phosphate did not appear to be necessary for the presence of rhizobia provided lime had been added.

The quantity of available phosphate measured in plots to which phosphate but no lime was added was similar to that obtained in plots with no phosphate or lime added. The addition of phosphate was therefore of no value when the pH of the soil it was added to was low i.e. the phosphate was immediately absorbed by the soil and there was no appreciable higher amount made available to the plants (Russell 1973).

Some of the results obtained from these two investigations confirm findings obtained from a laboratory experiment carried out varying pH and phosphate levels (Experiment 10). Under laboratory conditions no fixation by Lotus occurred at low pH i.e. 3.5 and 4.0 unless high levels of phosphate i.e. 30 kgP/ha were added. Similarly, as shown in Table 112 no fixation took place in plots 2,6 and 4,5 under low pH and low available phosphate. The results from the laboratory experiment also showed that fixation tended to be inhibited slightly at high pH's with high additions of phosphate. Table 14 shows that in plot 6,6 at high pH and high available phosphate the fixation level is lower than that obtained on plots with slightly lower pH and available phosphate levels.

The addition of lime and phosphate resulted in the greatest fixation, thus the importance of raising the pH is emphasised before phosphate additions can be beneficial to fixation.

The visual estimations of the plots in August 1979 emphasised the need for addition of lime to raise the pH to allow establishment and growth of Lotus to occur. The only plots where slight growth resulted, in the absence of lime, were those with additions of phosphate.

N_2 fixation was highly correlated to moisture content, the results obtained being comparable to those obtained by Hoglund & Brock (1978). They found in long term studies that, as the surface layers of a ryegrass white clover pasture dried, N_2 fixation only occurred at greater depths, reducing the specific moisture effects on N_2 fixation per unit area.

N_2 fixation was not correlated to K content indicating that addition of potassium is not so critical to the maintenance and increase of N_2 fixation of Lotus as is the addition of phosphate.

16. MEASUREMENT OF NODULE DEVELOPMENT AND DISTRIBUTION

16.1 Aim

The aim was to follow the development and distribution of nodules on the root system of L. uliginosus cv. Maku plants over a growing season and to correlate these measurements with N_2 fixation.

16.2 Materials and Methods

Twenty eight pipes of 380 mm diameter and 620 mm height were filled with sphagnum peat, and fertiliser was added at the following rates: 20 kg P/ha as superphosphate (20% P_2O_5); 60 kg K/ha as muriate of potash (60% K) and lime at 1.6 tonnes/ha which brought the pH to 4.5. Seeds were sown into the pipes in May 1978 and Rhizobium strain CC 814 s was added in a water suspension after germination had occurred. One plant was removed from each pipe monthly and because the peat was loosely packed there was little disturbance to the surrounding plants. The following measurements were noted: root length, shoot length, shoot and root dry weight, number and weight of effective and non-effective nodules and their position on the root, and acetylene reduction. Later, the presence of rhizomes was monitored. Acetylene reduction for the first month was measured using 33 ml universal bottles fitted with rubber septa in the lids and, as the plants were larger in the following months, 4.6 litre glass jars were used as incubation chambers.

The failure of seed to germinate and of the plants to grow well due to lack of rain, and later to survive the winter caused a second sowing to be carried out in June 1979 and the study repeated using the same methods.

16.3 Results and Discussion

Throughout the 1978 study period the total weight of the plant increased, with both shoot and root dry weight increasing. The total weight of the plant also increased during the 1979 study period but a

TABLE 16

Measurements of growth recorded for *L. uliginosus* cv. Maku plants grown on pipes outdoors at Auchincruive. Experiment 16

	Column	Description	1	2	3	4	5	6	7	8	9	10	11	12
	1	Root length (cm)												
	2	Stem length (cm)												
	3	Main root division below crown (cm)												
	4	Number of stems												
	5	Root dry weight (mg)												
	6	Shoot dry weight (mg)												
	7	Total plant weight (mg)												
	8	% Stem												
	9	% Root												
	10	Number of Rhizomes												
	11	Rhizome length (cm)												
	12	Rhizome dry weight (mg)												
(a) Plants sown May 1978 and removed monthly during 1978														
10 July	4.29	1.78	1.03	*	2.46		5.65	8.84	69.67	30.33	0	0	0	0
16 Aug	8.54	4.86	0.79	*	13.36		32.01	45.74	70.55	29.45	0	0	0	0
11 Sept	9.06	7.30	*	2	29.98		83.82	114.17	73.66	26.34	0	0	0	0
16 Oct	12.32	8.47	0.76	4	107.46		219.91	352.19	67.17	32.83	1.25	2.86	13.23	34.36
2 Nov	16.76	13.76	1.00	6	171.89		361.84	588.14	67.79	32.21	1.39	4.54		
(b) Plants sown June 1979 and removed monthly during 1979														
25 July	6.90	5.76	0.73	3	6.14		26.39	32.53	81.13	18.87	0	0	0	0
20 Aug	11.60	10.90	0.50	3	48.00		184.00	232.00	79.31	20.69	0	0	0	0
17 Sept	14.25	12.91	0.57	4	83.13		274.00	369.39	76.72	23.28	0	0	0	0
15 Oct	16.84	20.67	0.58	6	252.38		1005.19	1379.75	72.85	18.29	2.30	5.10	80.03	61.11
5 Nov	16.75	15.38	0.45	4	134.53		327.50	536.27	61.07	25.09	2.00	5.16		

reduction occurred in November and this pattern was followed by both shoot and root. The number and length of stems present increased during the 1978 period and the pattern obtained during the 1979 period was similar to that for total plant weight. During both study periods stems arose from the crown. In a few instances a single stem only was present and it tended to be tall and erect. The first stem to emerge from a crown was usually the tallest. These results were similar to those obtained by Sheath (1977) who found that the developing crown in an establishing Maku plant was weak, only one to six shoots developing from it depending on the weather conditions. The reduction in percentage shoot in October and November was due to an increase in rhizome production increasing the total plant weight and also the dying back of stem tissues. No flowering occurred during either of the two study periods (Table 16).

The main root division, where the branching of the roots first began, remained similar throughout the study periods (Table 16). The root system could be divided into the tap root and laterals. Although the root length was measured, and showed an increase throughout the study periods, the roots did not tend to grow down into the peat but spread out through the peat and formed a net work of lateral roots near to the surface (Table 16). Unlike L. corniculatus a thick tap root, which grows down deeply thus allowing the plant some drought resistance (Macdonald, 1946), was not formed. In those plants which were removed at the start of the study periods, July-August, the root system usually consisted of a single thin tap root. At this stage the length of root measured usually corresponded with the depth to which the root had grown. As the study periods progressed, the root system became more compacted and consisted of a bunch of lateral roots emerging from the crown and a more extensive branching system emerging from the tap root. The tap root did not appear to become thicker and thus became difficult to distinguish from the laterals, especially if branching had started near to the crown. Later in the study periods there was extensive growth of the laterals

TABLE 17

Nodule measurements and acetylene reduction ($\mu\text{moles C}_2\text{H}_4/\text{hours/plant}$) by *L. uliginosus* cv. Maku plants grown in pipes outdoors at Auchincruive. Experiment 16

Column	Description	1	2	3	4	5	6	7	8	9	10	11
1	Depth of root below the crown on which nodules are first formed											
2	Depth of root below the crown where nodule formation ceases											
3	Number of effective nodules formed on the tap root											
4	Number of effective nodules formed on lateral roots											
5	Number of non-effective nodules formed on the tap root											
6	Number of non-effective nodules formed on lateral roots											
7	Total number of effective nodules											
8	Total number of non-effective nodules											
9	Dry weight (mg) of effective nodules											
10	Dry weight (mg) of non-effective nodules											
11	$\mu\text{moles C}_2\text{H}_4$ Produced/hour/plant											
		16th Aug	2.81									
		17th Sept	1.90	*	2	0	2	0	3	0.39	0.34	0.003
		16th Oct	2.36	4.48	2	3	6	4	8	0.37	*	0.102
		21st Nov	1.60	2.38	1	5	1	25	26	7.04	4.55	0.547
				1.63	1	17	2	49	51	13.63	7.02	0.848
(a) Plants sown May 1978 and removed monthly during 1978												
		20th Aug	2.00									
		17th Sept	2.35	4.40	2	0	2	0	3	0.39	0.34	0.003
		15th Oct	1.92	6.95	3	3	6	4	8	0.37	*	0.102
		5th Nov	1.49	8.26	1	5	1	25	26	7.04	4.55	0.547
					1	17	2	49	51	13.63	7.02	0.848
(b) Plants sown June 1979 and removed monthly during 1979												
		20th Aug	2.00	4.40	2	0	2	0	3	0.39	0.34	0.003
		17th Sept	2.35	6.95	3	3	6	4	8	0.37	*	0.102
		15th Oct	1.92	10.01	1	5	1	25	26	7.04	4.55	0.547
		5th Nov	1.49	8.26	1	17	2	49	51	13.63	7.02	0.848

horizontally through the peat. At the end of both study periods there was no sign of root degeneration and root and shoot dry weight were highly correlated throughout the study periods but the correlation between shoot and root length was not so good (Table 18).

In both study periods the production of rhizomes was seen first in September (Table 16). At this time the rhizomes were small projections measuring less than a centimetre and were formed on only a few plants. Greater production was detected in October and in the 1978 study there was an increase in their number in November whereas in 1979 there was a slight decrease. The rhizomes were white with a slight pinkish pigmentation, at the point of emergence from the crown. They grew horizontally just under the peat surface and at the end of the study periods the tips of most rhizomes had emerged above ground and formed a small green shoot. There was no branching of rhizomes during either study period. The dry weight of rhizomes was highly correlated with both shoot and root dry weight during the two study periods.

Under both study periods the first nodules (Table 17), effective and non-effective, were detected in August. The nodules were small and the effective ones varied in colour from dark red to pink and displayed only a small amount of acetylene reduction. The nodules were not clustered around the base of the crown but were scattered over the root surface. This was due to the application of rhizobia via a water suspension, once the seeds had germinated, rather than on the seed coat. Initial infection by the rhizobia was therefore not limited to the crown area of the plant. The first nodules were found within the 2 cm region of the tap root below the crown during both study periods and the position of the first nodule did not vary throughout the study periods. The correlation between where the nodules start and when root branching occurred was poor.

Throughout the study periods both number and weight of effective and non-effective nodules increased as did the ability of effective nodules

TABLE 18

Correlation coefficient to establish any relationships among recorded measurements of *L. uliginosus* cv. Maku plants grown in pipes outdoor at Auchincruive. Experiment 16

Column	Description	1	2	3	4	5	6	7	8	9	10	11
1	Stem length correlated with root length											
2	Number of stems correlated with stem length		*									
3	Number of stems correlated with root length		0.41		*							
4	Root length correlated with dry weight of root	0.57	0.75	0.51	*		0.78	0.32	0.17	0.36	0.24	0.36
5	Root length correlated with dry weight of stem	0.81	0.60	0.71	*		0.96	0.46	0.42	0.41	0.52	0.93
6	Dry weight of root correlated with dry weight of stem			0.54	0.89	0.87	0.70	0.96	0.94	0.95	0.91	0.90
7	Total number of nodules correlated with dry weight of stem	0.70		0.54			0.57	0.30	0.72	0.88	0.94	0.72
8	Total number of nodules correlated with dry weight of root											
9	Total nodule weight correlated with dry weight of stem											
10	Total nodule weight correlated with dry weight of root											
11	Acetylene reduction correlated with dry weight of effective nodules											

Column	Description	1	2	3	4	5	6	7	8	9	10	11
1	Stem length correlated with root length											
2	Number of stems correlated with stem length		*									
3	Number of stems correlated with root length		0.41		*							
4	Root length correlated with dry weight of root	0.65	0.20	0.15	*		0.79	* 0.40	* 0.59	* 0.40	* 0.38	* 0.37
5	Root length correlated with dry weight of stem	0.35	0.20	0.25	*		0.88	0.66	0.29	0.63	0.55	0.89
6	Dry weight of root correlated with dry weight of stem	0.11	0.38	-0.14	*		0.82	0.79	0.85	0.84	0.55	0.95
7	Total number of nodules correlated with dry weight of stem	0.78	0.48	0.73	0.95	0.76	0.84	0.79	0.78	0.84	0.55	0.95
8	Total number of nodules correlated with dry weight of root	0.22	0.13	0.34	0.38	0.29	0.81	0.78	0.66	0.73	0.60	0.72
9	Total nodule weight correlated with dry weight of stem											
10	Total nodule weight correlated with dry weight of root											
11	Acetylene reduction correlated with dry weight of effective nodules											

* missing figures

to reduce acetylene (Table 17). During the 1979 study period a drop in all these parameters occurred in November. The number of non-effective nodules outyielded the effective ones, during the 1978 study period, but the weight of effective nodules was greater than that of non-effective. This indicated that the non-effective nodules were of a smaller mass than the effective. During the 1979 study period both the number and weight of effective nodules outyielded the non-effective. This plus the overall better growth of the 1979 plants indicated that a more efficient symbiosis was established than by the plants grown during 1978. The largest increase in numbers of nodules occurred between October and November during 1978 and September and October in the 1979 study period. This indicates that infection of the 1978 plants was slower than those in 1979 and partially explains why the 1978 plants were weaker than the 1979 plants. The 1978 study showed that both non-effective and effective nodules were found on the lateral roots rather than the tap root indicating the presence of more infection sites on the lateral roots. The total number of nodules was well correlated to both root and shoot dry weight especially at the end of the study periods when the symbiosis between the rhizobia and the plants was well established. The same can also be said for total nodule weight and root and shoot dry weight. Acetylene reduction was always highly correlated with the dry weight of effective nodules (Table 18).

A comparison between the two study periods revealed that smaller plants were obtained in 1978 compared to those which grew during 1979. The 1979 plants appeared to reach their peak growth in October and by November had started to die back, whereas the 1978 plants continued to increase their growth in November. The growth of the 1978 plants in November, however, did not exceed the growth of the 1979 plants at their peak in October.

The 1978 seeds were sown one month earlier than in 1979, and, a considerable dry period after sowing inhibited germination (Table 9).

The growth of plants from seeds which did germinate was initially stunted and therefore it took longer for an efficient symbiotic relationship, with the added rhizobia, to establish. This poor early establishment was reflected in the further poor growth of the plants. The plants sown in June 1979 were not subjected to this particular stress factor. There was adequate rainfall to allow more or less immediate germination and growth, affording an early establishment of the rhizobia-plant symbiosis which was reflected in the larger plants produced throughout the growth period.

See plates 7-9 for visual description of 1978 plants.

Plate 7

Plants removed from pipes in August 1978

Plate 8

Plants removed from pipes in October 1978

L = Lateral roots

N = Effective nodules

T = Tap root

Plate 9

Plants removed from pipes in November 1978

R = New rhizome



PLATE 7

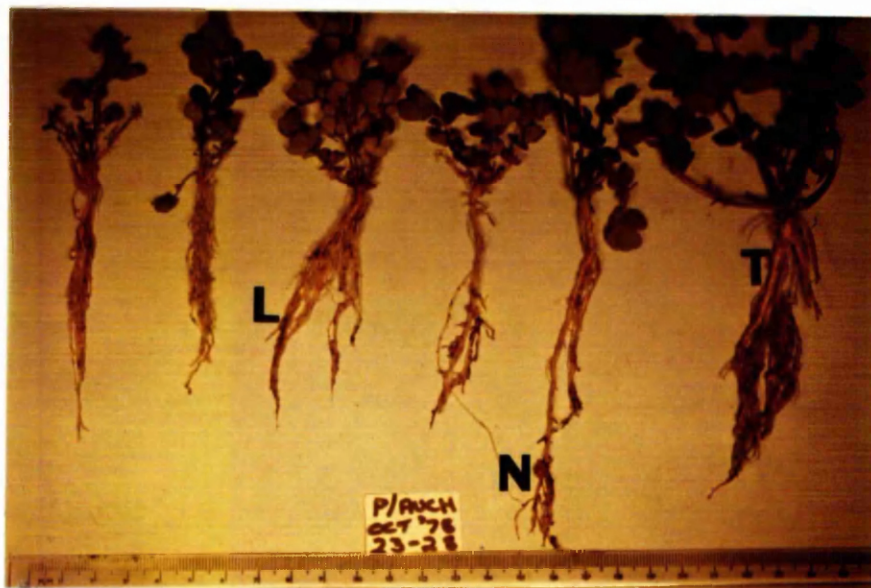


PLATE 8



PLATE 9

17. EXAMINATION OF VARIABILITY IN MORPHOLOGY WITHIN
A POPULATION OF L. ULIGINOSUS CV. MAKU PLANTS

17.1 Aim

The aim was to investigate the variability present within L. uliginosus cv. MaKu under lowland conditions at Auchincruive.

17.2 Materials and Methods

One hundred and ninety plants were grown over winter in pots containing sphagnum peat adjusted to pH 4.5 with fertiliser added at the following rates: 20 kg P/ha as super phosphate (20% P₂O₅), 46 kg P/ha as ground mineral phosphate (30% P), 7.5 kg K/ha (60% K) and lime at 7.5 tonnes/ha. The plants were transferred to a plot at Auchincruive on the 13th June 1978 and were planted twelve inches apart. All plants were cut back to a height of 2.5 cm on 10th October 1978 and their top growth dry weight noted. (Table 19). The survival of plants during the winter of 1978 was noted and throughout 1979 plants were scored for initiation of flowering and pod formation. In October 1979 the plants were again cut to a height of 2.5 cm and the dry weight noted.

17.3 Results and Discussion

Only twenty four plants out of one hundred and ninety planted out survived the winter. These twenty four plants must therefore carry genes for winter survival. The plants which survived the winter had high dry matter yields when harvested the previous October.

New growth started to emerge in the middle of May 1979. Flowering was initiated in the middle of July and the last initiation of flowering occurred in the middle of August. Flowering continued in all plants until the middle of October when the plants were harvested (Table 20).

The state of pod development was studied at harvest. Pods were found on the majority of plants and were at various stages of development

TABLE 19

Shoot dry weight (g) measurements of *L. uliginosus* cv. Maku plants transferred from glasshouse conditions to a plot at Auchincruive in May 1978 and harvested in October 1978. Experiment 17

Plant	Weight	Plant	Weight	Plant	Weight	Plant	Weight	Plant	Weight
1	34.44	39	8.22	77	20.00	115	18.41	153	42.23
2	18.94	40	13.35	78	32.27	116	24.46	154	16.30
3	36.42	41	32.33	79	42.37	117	4.74	155	13.62
4	15.26	42	23.38	80	21.36	118	5.45	156	12.83
5	18.00	43	31.26	81	10.29	119	50.00	157	18.42
6	23.00	44	24.85	82	9.22	120	16.45	158	8.22
7	19.15	45	22.00	83	26.96	121	13.37	159	14.86
8	13.37	46	14.00	84	18.83	122	51.18	160	17.35
9	23.00	47	37.58	85	13.88	123	13.41	161	5.09
10	10.00	48	44.07	86	26.51	124	29.67	162	7.88
11	45.00	49	23.27	87	21.00	125	16.78	163	18.07
12	38.00	50	27.66	88	4.70	126	19.97	164	9.20
13	31.53	51	26.37	89	7.73	127	10.32	165	14.75
14	14.30	52	8.53	90	18.58	128	21.05	166	4.18
15	28.67	53	17.37	91	27.27	129	22.62	167	3.79
16	30.58	54	10.74	92	9.00	130	20.61	168	4.62
17	24.64	55	6.00	93	11.89	131	23.56	169	7.25
18	22.00	56	3.51	94	3.51	132	33.08	170	6.40
19	17.32	57	6.72	95	21.66	133	36.97	171	4.54
20	26.00	58	8.89	96	27.63	134	33.89	172	5.57
21	27.63	59	21.90	97	15.57	135	45.35	173	15.29
22	2.82	60	8.25	98	62.28	136	5.23	174	15.47
23	12.16	61	8.88	99	26.86	137	15.38	175	13.82
24	20.38	62	4.20	100	44.47	138	19.37	176	7.20
25	4.52	63	4.56	101	47.15	139	50.77	177	2.58
26	29.34	64	29.00	102	41.60	140	33.43	178	30.89
27	13.74	65	22.35	103	8.33	141	37.16	179	8.09
28	61.56	66	36.59	104	36.59	142	27.45	180	3.96
29	18.00	67	40.73	105	18.07	143	0.16	181	5.62
30	45.28	68	21.39	106	7.20	144	20.00	182	12.33
31	18.42	69	15.09	107	32.46	145	28.48	183	5.22
32	8.52	70	5.47	108	6.63	146	12.93	184	3.49
33	6.40	71	13.99	109	0.86	147	15.58	185	11.27
34	0.86	72	12.61	110	40.75	148	19.97	186	10.00
35	26.00	73	9.53	111	21.36	149	19.41	187	5.30
36	3.00	74	39.40	112	10.74	150	13.00	188	3.51
37	11.75	75	19.91	113	49.37	151	22.33	189	1.29
38	8.10	76	20.56	114	8.27	152	41.41	190	16.52

TABLE 20

Shoot dry weight, date of flowering and formation of pods by *L. uliginosus* cv. Maku plants planted outdoors at Auchincruive in May 1978 and harvested in October 1979. Experiment 17

Plants	Dry wt Oct 78	Dry wt Oct 78	Date of flowering 1979	Pods (16.10.79)
1	34.44	14.65	18th July	No pods
3	36.42	76.82	23rd July	Green, non split, FHP
20	26.00	44.90	13th August	Green, non split, FHP
21	27.63	281.65	18th July	Green, greenish brown, non split, FHP
26	29.34	76.99	1st August	No pods, still flowering
28	61.56	208.32	18th July	Yellow, reddish brown, FHP
51	26.37	38.49	13th August	Yellow-green, non split, FHP
47	37.58	142.39	1st August	No pods, still flowering
64	29.00	104.09	18th July	Yellow-brown, reddish brown, some split
67	40.73	121.14	23rd July	Green, non split, FHP
69	15.09	28.02	5th August	Brown, split
74	39.40	57.68	5th August	No pods, still flowering
76	20.56	99.76	23rd July	No pods, still flowering
116	24.46	26.65	20th August	No pods
119	50.00	213.21	23rd July	Green, non split, FHP
120	16.45	39.47	20th August	Green, non split
122	51.18	106.54	18th July	Various stages, yellow reddish brown, non split
145	28.48	13.50	25th August	Reddish-green, non split
151	22.33	57.41	13th August	No pods, still flowering
152	41.41	65.44	13th August	Brown split, FHP
153	42.23	98.47	18th July	Green brown, non split, FHP
173	15.29	22.67	25th August	Green brown, non split, FHP
174	15.47	43.04	20th July	No pods, still flowering
190	16.52	48.93	1st August	No pods, still flowering

FHP = Flowering head present

within a plant. The pods on plants which had flowered first had almost reached maturity at the time of harvest. Signs of pod splitting were detected on only two of the plants. The high level of rainfall which occurred during the pod development probably reduced the level of pod splitting, as the pods require to dry out before splitting occurs. It was therefore not possible accurately to distinguish plants which may have carried a gene repressing pod splitting.

The variation in dry matter production for the 1979 growth season was high, as would be expected from the heterozygous nature of the cultivar. The plants with the greatest dry matter were also found to be those which flowered first. The results indicate that dry weight of plant material at the end of a growth season may be a character which could be used as a winter hardy selection criterion, and may be linked to earlier flowering.

18. GENERAL DISCUSSION

The slow rate of establishment associated with L. uliginosus cv. Maku was shown to be linked with the slow initiation of N_2 fixation. Data from plants sown in pipes under lowland conditions (Expt. 16) showed that fixation first occurred approximately eight weeks after seedling emergence and addition of rhizobia. On the other hand, in pot experiments in glasshouse and growth chambers effective nodulation occurred and fixation was initiated about three weeks after the rhizobia were added to the pregerminated seedlings. This was probably due to the fact that pot experiments were always carried out under the higher temperature conditions, of a glasshouse or growth chamber, than those experienced in the pipes kept outdoors. Poor establishment of Maku seedlings was found to be correlated with climatic conditions; studies illustrated that a dry period after sowing inhibited germination and when germination eventually occurred the establishing plants made poor growth (Expt. 16). The period between the ending of the source of N in the seed and the initiation of N_2 fixation will be one of nitrogen deficiency and will limit plant growth and thus establishment. Results from experiment 7 showed that establishment could be improved if a low level, i.e. 30 kg N/ha, of KNO_3 was added at seedling emergence or up to six days later, causing an increase in growth without inhibiting fixation rate. The added N at this time presumably helped alleviate the nitrogen deficiency occurring between the end of the seed N and the commencement of fixation. If a balance between growth and fixation is required, care should be taken not to add high levels (180 kg N/ha) at transplanting as this was found to inhibit growth.

Under field conditions N_2 fixation by Maku was practically non-existent until the beginning of June, coinciding with an increase in soil temperature and thus suggesting that fixation was previously inhibited by low temperatures. N_2 fixation by first year Maku plants grown in pipes

(Expt. 16) was found to increase throughout the growing season and, since no flowering took place, at the end of the growing season there was no rapid decrease in fixation. The level of fixation during the first year remained fairly low as the symbiotic system had not fully developed. Brock (1973) has stated that an effective N_2 fixing system in Lotus may take a year to develop.

Conditions for the growth of Maku plants were studied in pots where pH, phosphate level and the presence or absence of trace elements were varied (Expt. 10). The findings were in line with those shown by workers in New Zealand, when growing Lotus under field conditions (Lowther, 1976a; Brock, 1973; Greenwood, 1961). The results of the pot experiment were supplemented with field results obtained from a study of fixation by Maku plants in soil cores (Expt. 15). Maku plants can therefore grow and fix N_2 under conditions of lower pH and utilise low levels of phosphate more efficiently than white clover. Lowther (1976a) showed that total legume growth could be increased by sowing a Lotus and clover mixture in areas in which white clover growth was limited by acid soils. The greatest potential for Lotus, however, is under conditions of low pH and low fertility where white clover cannot readily colonize. Effective nodulation of Maku plants was shown to be aided by the addition of trace elements (Mn, Mo, B, Zn, Cu) immediately prior to sowing. Significantly better nodulation along with increased plant growth was obtained for both Lotus and clover when trace elements were added.

Once the plants have become established under field conditions, management plans have to be formulated to ensure persistence of the species.

The effect of cutting Maku plants in pots (Expt. 11) gave results similar to those obtained by other workers for legumes both in pots and the field (Butler et al, 1959; Chu and Robertson, 1974; Haystead and Lowe, 1977). The rate of recovery of fixation after cutting was found to be longer for Lotus than that reported for clover. The rate of

recovery, however, was found to be higher under conditions of long day (16 hours) and high temperature (21°C day/18°C night). The effect of grazing or cutting in mid-summer will therefore not be as deleterious as grazing or cutting in the spring. Lotus was found to be more persistent than clover under a three-cut regime (i.e. three cuts consisting of one at ten weeks, one at thirteen weeks and one at sixteen weeks) although the rate of fixation by Lotus was lower. The quantity of nitrogen supplied to the surrounding vegetation by Lotus would therefore presumably be less than that by clover, after cutting, although the growth of foliage after cutting would be quicker.

The development of rhizomes by Maku plants has been shown to play a main part in the persistence of the species. A close relationship between rhizome production and nitrogen fixation has been demonstrated (Expt. 13). The production of new rhizome material contributed to the end of season decline in fixation during both years of study. The channelling of energy into rhizome production may account for the different patterns of seasonal fixation by white clover and Lotus. The second peak which is present in the seasonal pattern of clover does not occur with Lotus. In established plants of L. uliginosus a defined sequence of events emerges. A peak in nitrogen fixation and shoot dry matter production in mid summer is followed by a rapid decline in both, coinciding with flowering and early rhizome development. After flowering and seed production nitrogen fixation continues to decline while production of both shoot and rhizome dry matter increases. These various events show a degree of interdependence and their timing also appears to be related to environmental factors including temperature and rainfall.

Knowledge of these seasonal patterns allows tentative plans for management to be prepared. Grazing regimes allowing maximum crown

development in first year plants, prior to overwintering, will differ from regimes applied to established plants where a balance between utilising shoot production and encouraging maximum development of rhizomes, in autumn, is desired. Sheath (1977) has already shown, for example, that defoliation of this species before and during the period of rhizome development both delays rhizome emergence and reduces total rhizome production.

Earlier in the year adequate grazing pressure may have to be maintained in order to prevent flowering with reduction in flowering having important side-effects on the overall pattern of shoot production and nitrogen fixation. Although a lower rate of fixation would be expected throughout the year under a grazing situation, fixation may be extended for a longer period since the decrease due to flowering would be avoided.

This particular cultivar Maku will not grow to any significant level in early spring when it would be required after lambing. Maku will require a lax system of grazing to allow a suitable time for recovery of fixation and a rest period from grazing will have to occur, for established plants, some time during September or October to allow maximum growth of rhizome material. Light and infrequent grazing has already been advocated by Brock & Charlton (1977) for successful Lotus establishment in mixed pastures. Growth of Maku is therefore not realistic for use in intensive grazing on young stands i.e. first and second year plants. Its main value lies in use as a colonizer of land of low pH and low fertility. Further, in this way it can be used to improve land so that, in the future, ingression of white clover might take place. Maku would be suitable for a system of lax grazing and may therefore be useful in a two pasture system where sheep could be placed on it for a short period of time to allow other areas of land to recover from grazing. Maku would also be suitable for areas where white clover would not grow at all, even with some pH improvement, e.g. under wet conditions.

Results from the present study have given some useful guidelines for use in future field work concerning L. uliginosus cv. Maku.

Seed Bed Preparation

No comparative work was done concerning seed bed preparation but successful establishment was shown under Calluna/Molinia wet heath vegetation which was rotavated, fertilised and sown within a week.

Fertiliser Requirement

With soil at pH 3.5 lime should be added at 6-7 tonnes/ha which will raise the pH to between 5 and 5.5. Phosphate should be added at 30 kg P/ha and potassium at 72 kg K/ha. Establishment of plants can be aided if N at 30 kg N/ha is applied at sowing. Both growth and effective nodulation have been shown to increase on the addition at sowing of trace elements. A suitable composition of these is: H_3BO_3 0.29 kg/ha, $MgCl_2 \cdot 4H_2O$ 0.18 kg/ha, $ZnSO_4 \cdot H_2O$ 0.02 kg/ha, MoO_3 0.008 kg/ha and $CuSO_4 \cdot 5H_2O$ 0.008 kg/ha. Seed should be inoculated with the appropriate rhizobia, prior to sowing at a rate of 3-5 kg/ha. If a peat inoculum is used, sowing should take place within twenty-four hours of applying the inoculum to the seed.

Management

Current information on rhizome production by Maku would suggest that careful management is required for the first two to three years. Little or no grazing should take place in the first year because of slow Maku growth and damage which might occur to the crowns. Management practices in the second year should take account of the seasonal production of shoot and rhizome material, therefore no grazing from late September until the end of October when rhizome material is laid down is advised. However, grazing could take place for a short period after this time to allow use of the shoot material produced during October. Until more work is carried out on the effect on the rhizome system of cutting or grazing, management plans for plants over two years old cannot yet be stated.

19. FUTURE WORK

Having further identified the potential of L. uliginosus as a species for use in areas of low fertility and low pH, future work should concentrate on developing a management system to ensure good establishment, particularly in the first year, and persistence of the species together with high herbage dry matter.

Good establishment can be achieved if germination occurs quickly and is followed closely by infection by rhizobia, nodule production and initiation of nitrogen fixation. The earlier in the season this occurs then the longer the growing season that will result and thus allow the legume to become better established. Under Scottish conditions germination will therefore take place at temperatures of 6°C which will necessitate the selection of Lotus cultivars which will germinate under these low temperatures. Development of the cultivar Maku, which has considerable variability present due to its heterogeneity through outbreeding, may be a suitable starting point. Selection of Rhizobium strains which will infect, form nodules and fix nitrogen under low temperatures will also have to take place. These two separate studies should proceed together with alternate testing to obtain an efficient symbiosis i.e. a suitable strain of Rhizobium which will give optimum effectiveness with a particular cultivar of Lotus. It has been shown that varieties of white clover differ considerably in their selection or preference for strains of Rhizobium and that the preference is genetically controlled by the host (Jones & Hardarson, 1979). Similar work should be carried out on cultivars of L. uliginosus and once an effective symbiosis has been developed, cuttings from that plant can be taken and the same Rhizobium strain, associated with the parent plant used for further inoculation. To obtain seed set by the cultivar, however, some variability within the cultivar would be required.

The strain of Rhizobium utilised with Maku, in this thesis, was isolated in Australia and therefore it could be expected to have a different efficiency under Scottish conditions to which it was unadapted. Recent studies (Yong, 1980) have shown that the strain of Rhizobium used (cc814s) for inoculating Maku plants may not have been the most efficient i.e. fixation by Maku was greater when associated with a different Rhizobium strain (cc829). Work on the isolation of indigenous strains of Rhizobium and determination of their level of effectiveness should be carried out in an attempt to obtain a strain which is an efficient N₂ fixer under Scottish conditions and which can also compete successfully against other less effective indigenous strains of Lotus rhizobia.

The slow initiation of fixation (page) partially explains why the rate of plant establishment is slow. The results from pot experiments indicate that work requires to be extended to the field to enable the identification of levels of nitrogen application which will allow maximum growth and fixation from a minimum nitrogen input. The value of placement of nitrogen close to the legume seeds and timing of N application in the field both merit further study. The long term effects of added nitrogen on final establishment and persistence of the species should be studied. Haystead (cited in HFRO, 1979) has shown that white clover given 90-120 kg N/ha after nodule initiation, suffered a depression in nodulation and fixation but not growth, whereas grass growth showed improvement in the second year but by this time the depressant effect on clover nodulation and N₂ fixation had disappeared. In cases where Lotus is sown in a seeds mixture with companion grasses the addition of nitrogen will stimulate grass growth apart from stimulating legume growth, resulting in greater competition effects for the legume. A study is therefore required to determine the level of N which will give a balance between boosting legume growth and reducing as much as is feasible the competition from grasses.

When Lotus is being used in a seeds mixture to improve pasture, a careful choice of companion grasses is required. The grasses would have to be adapted for areas of low pH and low fertility, have fairly high nutritional value and have minimal competition with the Lotus during its seedling stage. Charlton (1971) noted that the following grasses would not offer formidable competitive stress under areas of bog conditions due to their slow germination: perennial ryegrass (S23), cocksfoot (S134), meadow fescue (S215 or S53). New Zealand workers have grown L. uliginosus in mixtures with bred varieties of perennial ryegrass and cocksfoot without any problems being encountered from the competitive aspect (Barclay & Lambert, 1970).

Studies determining the effect of date of sowing on establishment should be carried out under field conditions. Results cited by Davis (1969) showed good initial growth of L. uliginosus after sowing in July but there was severe dieback in winter after which the material never regained any degree of prominence. Sowings carried out in early August by Charlton (1971) failed to survive the winter but seedlings emerged in the spring presumably from hard seed. Sowing in early to mid spring may provide the species with the greatest opportunity of a rapid and successful establishment.

Once the plant has become established under field conditions management plans have to be formulated to ensure persistence of the species. A pattern for seasonal fixation in an ungrazed situation has been established (page) and this can be used as a basis for further investigations into the effect of management practices on fixation in the field. In this way practices may be developed which can enhance fixation.

The grazing pressure to which first year plants can be subjected, if any, to allow their survival during the first winter and emergence the following spring and the means by which first year plants survive the winter has still to be investigated. Cutting can be used to

simulate grazing pressures and the effect of different cutting regimes should be investigated to find the best system for production of dry matter, N₂ fixation and survival of the plants.

The function of rhizomes as sites for regrowth, colonization of surrounding areas and winter survival of the plant should be exploited. To understand the physiology and thus enable the exploitation of the rhizome, investigation should be made of the total non-structural carbohydrate (T.N.C.) which has been recorded in rhizomes of L. uliginosus cv. Maku by Sheath (1978). This study will give details of the possible role which accumulated starch plays in the winter survival of the plant and may indicate if rhizomes utilise all their accumulated starch for their own growth or if they can act as temporary sinks, returning to a source situation when energy is required by the plant. This latter situation would occur after grazing, for example, when dormant or new leaf buds would require a large carbohydrate supply for growth. Investigations into the effect of cutting, light and temperature regimes will therefore be required to obtain knowledge about T.N.C. accumulation and mobilisation under varying conditions. Management systems should be designed to allow maximum production of rhizome which in the long term will result in the extension of ground cover. A compromise between grazing, vegetative growth, extension of rhizomes, possible storage of carbohydrates for winter survival and fixation by the plant will have to be taken into account when formulating management practices.

20. APPENDIX IA. *Investigational technique for determining an acetylene reduction assay*Aim

The aim was to establish an assay system for measurement of acetylene (C_2H_2) reduced to ethylene (C_2H_4) by intact nodulated Lotus plants.

Materials & Methods

The equipment consisted of the following: acetylene cylinder, ethylene cylinder, wire netting, 1 ml and 50 ml B-D disposable plastic syringes, needles to fit these syringes, 10 ml B-D evacuated glass tubes (vacutainers), Pye Unicam 104 Gas Liquid Chromotograph and a Servoscribe chart recorder.

The incubation chambers were of three types, viz. (a) Polypropylene buckets, volume ten litres, each with a snap-on lid into which a rubber septum was inserted to facilitate addition and removal of gases by hypodermic syringe; (b) Glass jars, volume 4.6 litres, equipped with screw-on metal lids modified to allow insertion of a rubber septum and (c) Universal glass bottles, volume 33 ml, with rubber septum inserted in the lids. The appropriate type of incubation chamber was chosen with regard to sample volume. The chambers were tested to ensure that they were adequately gas tight and no gas leakage was apparent.

Pieces of wire netting were placed on the bottom of the incubation chambers and the plants, either shaken free from their growth medium or still rooted in it, placed on top of this. This ensured that the gas present in the chambers would completely surround the plants. The lids were then fitted on the chambers and 5% of the volume of air present in the chambers was removed, through the rubber septum present

in the lid of each chamber, using a hypodermic syringe. An equivalent volume of C_2H_2 gas was then added, through the rubber septum. The syringe, while still inserted into the septum, was pumped a number of times to facilitate mixing of the gases or alternatively, after removal of the syringe, the incubation chambers were inverted a few times, care being taken not to damage the plants.

The chambers could then be placed under any environmental conditions and the plants incubated for any length of time ranging from minutes to days. Samples could be removed at any time during the incubation. One means of sample removal was by double ended needle i.e. one end of the needle pierced the septum inserted in the lid of an incubation chamber and the other pierced the septum inserted in an evacuated tube (vacutainer). There was a movement of gas from the incubation chamber into the vacutainer tube because the tube was evacuated. The needle was not removed from the vacutainer until after ten seconds to allow time for the gases in the incubation chamber and the vacutainer to equilibrate.

As a precaution against loss of sample, duplicate samples of gas were removed from each incubation chamber at any one time. The gas samples could be stored indefinitely in the vacutainers.

Alternatively, a sample was removed using a hypodermic syringe and the needle of the syringe then stabbed in a rubber stopper to prevent leakage. If this was done, the samples were analysed within two hours to avoid loss of gas by leakage.

The incubation was terminated by allowing the gas present in the chamber to mix with air.

To detect the presence of C_2H_4 which may be present in commercial C_2H_2 or emitted by plants and equipment, it was necessary to prepare controls. Incubation chambers were prepared as above but no plants were added. The chambers were incubated with C_2H_2 for a set time and gas samples removed and analysed. Any C_2H_4 detected in the samples

would therefore be present either in the C_2H_2 gas, the equipment or both. Emission of C_2H_4 by the plants in the absence of C_2H_2 can also be detected by incubating the plants for a set time in the absence of C_2H_2 and analysing the gas samples removed. Any C_2H_4 present was therefore released by the plant, the equipment or both. The quantity of C_2H_4 detected in the control was always deducted from the final calculation of C_2H_4 production by nodulated plants under the above assay conditions.

The gas samples were analysed using a Pye Unicam 104 gas liquid chromatograph. The column used was stainless steel, nine-feet long, had an 1/8 inch OD bore and was prepacked with Poropak R. When operating, the oven temperature was set at 60°C and the N_2 carrier gas flow rate was 20 ml/minute.

The volume of gas sample injected into the column was 0.4 ml. With a nine-foot column, methane (CH_4), an impurity in the commercial C_2H_2 , had the shortest retention time followed by C_2H_4 then C_2H_2 . The peaks produced by the gas samples were recorded on a Servoscribe chart recorder. Recorded as chart units, the peak heights correspond to the concentration of each gas present in the sample.

Using chart units obtained from samples analysed by the GLC, the following calculation was used to determine the μ moles of C_2H_4 produced from the reduction of C_2H_2 by the nodulated plants during a given incubation time:

$$\mu\text{mole } C_2H_4 = \frac{CV}{IV} \times K \text{ (SCU-CCU)}$$

where CV = volume of incubation chamber

IV = volume of gas injected into the GLC

SCU = sample chart units

CCU = control chart units

K = conversion factor

K is the number of μ moles C_2H_4 which produce one chart unit, calculated from the number of chart units obtained after injection of a standard

sample of C_2H_4 gas into the GLC.

$$K = \frac{V_2}{22400 \text{ ml}} \times 10^6 \text{ umoles} \div \text{CUS}$$

where $V_2 = V_1$ corrected for temperature and pressure at the time of gas analysis.

22400 ml = 1 mole of C_2H_2 at STP

CUS = chart units of standard sample of C_2H_4 gas

V_1 , the exact volume of standard gas injected into the GLC remained constant throughout all the assays carried out during work for this thesis. To a gas tight glass bottle of volume 619.7 ml, 1 ml of standard C_2H_4 gas was added via a rubber septum in the lid of the bottle. The gases were mixed by repeated plunging of a syringe. 0.4 ml of this gas mixture was removed and injected into the GLC and the chart units obtained were noted.

$$V_1 = \frac{1}{619.7} \times 0.4 \text{ ml} = 0.0006454 \text{ ml}$$

$$V_2 = \frac{P_1}{P_2} \cdot \frac{T_1}{T_2} \cdot V_1$$

where P_1 = Pressure at time of gas sample analysis

P_2 = Standard pressure; 760 mm Hg

T_1 = Standard temperature plus temperature at time of gas sample analysis

T_2 = Standard temperature; 273°K

B Calculation of vacutainer correction factor

The chart units obtained from samples drawn into vacutainers were always lower than those obtained from samples drawn by syringe and then immediately entered into the GLC; this occurred although the samples were drawn at the same time and whether or not the vacutainers were stored for a period before gas content analysis. It transpired that the above phenomenon occurred because the vacutainer tubes were not completely evacuated and when sample gas was drawn in, it then mixed with a small quantity of air. One method of compensating for this was to add 1 ml of water to the vacutainer just before removal of a sample of gas for injection into the GLC. By doing this the pressure in the tube was brought up to atmospheric. This procedure was inconvenient and since acetylene is soluble in water there was always risk of a slight loss of gas. Another method of correcting the discrepancy was to compare the draw exerted by a number of vacutainers with the actual volumes of the same vacutainers. This was done using water and a correction factor calculated as follows:

(Appendix I B 1)

$$\frac{V_t}{D_t} \times \text{SCU} = \text{CCU}$$

where V_t = volume of vacutainers in ml

D_t = volume of draw exerted by vacutainers in ml

SCU = sample chart unit from vacutainer

CCU = corrected chart unit for vacutainer

$$\frac{V_t}{D_t} = \frac{13.1}{9.7} + 0.4 \text{ ml (sample injected into GLC)}$$

$$\frac{V_t}{D_t} = \frac{13.5}{9.7}$$

The check on the validity of the correction factor comparisons were made between chart units obtained using the above factor. There was no significance difference between the results indicating that the

Appendix I : B : Table 1

Comparison between the draw exerted by a vacutainer and the total volume of the vacutainer

Draw (ml)	Total volume (ml)
9.88	13.39
9.58	13.05
9.64	12.97
9.73	13.31
9.49	13.04
9.52	13.14
9.44	13.19
9.56	12.98
9.76	13.00
9.65	13.11
9.84	13.20
9.80	13.16
9.59	13.00
9.50	12.98
<u>9.70</u>	<u>13.24</u>
9.65 ± .04	13.11 ± .04

Correction factor = $\frac{13.1}{9.7} + (0.4 \text{ ml sample Volume injected into GLC})$

Correction factor = $\frac{13.5}{9.7}$

Appendix I : B : Table 2

Student t tests for determining significant differences between chart units obtained from gas chromatograph analysis of gas samples stored in syringes and corrected chart units obtained from samples stored in vacutainers

	<u>GCL Chart Units</u>															Mean SE _t	
Vacutainers	28	36	32	43	30	19	19	19	17	17	22	27	19	20	10	15	23.31 ± 2.16
Corrected Vacutainers	39	50	46	60	42	26	26	26	24	24	31	38	26	28	14	21	32.56 ± 3.04
Syringe	42	53	51	60	38	28	29	27	24	27	32	32	27	29	15	20	33.38 ± 3.09

Vacutainer chart units versus syringe chart units t = 1.75 @ 15 df significant @ 10% level

Vacutainer corrected chart units versus syringe chart units t = 0.317 @ 15 df NS

Vacutainer corrected chart units versus vacutainer chart units t = 2.61 @ 15 df significant @ 2.5% level

correction factor calculated was valid when using 10 ml vacutainers.

(Appendix I B 2). Reliable comparison between field and laboratory work can therefore be made since use of syringes is confined to laboratory work and vacutainers used mainly for field work.

C *Determination of variability associated with the Acetylene
Reduction Technique*

An experiment was set up to determine whether variation in acetylene reduction results was due to the variability of the assay techniques in use.

To check this the acetylene reduction by two sets of L. uliginosus cv. Maku plants was measured using two assay techniques i.e.:

- (1) Placing the plants in air-tight glass jars, volume 4600 ml.
- (2) Placing the plants in air-tight polypropylene buckets,
volume 10 litres.

Both systems were subjected to 5% concentration of C_2H_2 gas and incubated for four hours.

One set of plants was subjected to assay (1) and the second set to assay (2). After incubation the plants were removed and enough time allowed to lapse to complete the dissipation of the C_2H_4 gas, i.e. one hour. The same plants were then again subjected to the same assay, i.e. either (1) or (2), and the two sets of results from each separate technique compared to establish that similar results were obtained.

A converse experiment was set up to compare the results obtained using different incubation chambers. Plants were either subjected to assay (1), incubated, removed and incubated under assay (2) or vice versa. The results from a set of plants incubated under two assay techniques were then compared and validity t tests were carried out on the data (Table 1). None of the results was significantly different. There is therefore no variation in the results obtained by using different incubation chambers for measuring acetylene reduced by plants. Results obtained using either jars or buckets can be used and the apparatus can be interchanged without affecting the final results obtained. Variation obtained in future assays is thus due to the condition of the plants and not the assay technique.

Appendix I : C : Table 1

Student t tests for determining significant differences between acetylene reduction ($\mu\text{moles C}_2\text{H}_4/\text{hour/pot}$) rates by L. uliginosus cv. Maku using different incubation chambers

System : Jars

<u>1st Reading</u>	0.920	0.837	0.954	2.121	1.837	2.051	1.076	1.022	1.129	2.178	1.259	1.288	1.206	2.683	0.997	0.922	1.095
	Mean = 1.40 \pm 0.14																
<u>2nd Reading</u>	1.405	1.044	1.035	2.727	3.347	2.819	1.464	1.166	0.771	3.353	2.881	1.749	1.556	1.001	1.379	1.208	0.973
	Mean = 1.76 \pm 0.22																
	t = 1.38 @ 16 df NS																

System : Buckets

<u>1st Reading</u>	0.88	1.01	1.16	1.77	1.85	2.28	1.12	1.13	1.38	1.84	2.09	1.12	1.25	1.54	2.52	1.25	1.51
	Mean = 1.51 \pm 0.12																
<u>2nd Reading</u>	1.68	1.42	1.04	3.08	0.26	2.28	1.60	1.37	1.26	1.84	2.22	1.64	1.51	1.32	1.37	1.19	1.72
	Mean = 1.58 \pm 0.15																
	t = 1.0 @ 16 df NS																

System : Jars and Buckets

<u>Jars : 1st Reading</u>	2.72	1.35	3.41	1.55	Mean = 2.26 \pm 0.49	<u>Buckets : 1st Reading</u>	1.61	1.91	2.68	Mean = 2.07 \pm 0.32
<u>Buckets : 2nd Reading</u>	2.79	1.46	2.90	1.33	Mean = 2.12 \pm 0.42	<u>Jars : 2nd Reading</u>	1.16	2.32	2.82	Mean = 2.10 \pm 0.49
	t = 0.96 @ 3 df NS									
	t = 0.13 @ 2 df NS									

Limited plant numbers necessitated the analysis of individual small plants at the start of the work. During the early growth period when the dried plant weighed approximately 1 mg, a spectrophotometric procedure using a sensitive indophenol blue technique was chosen for detecting the N content since conventional micro distillation procedures only detect a minimum of about 1 mg N.

The established technique used at the West of Scotland Agricultural College (W.S.A.C.) is based on the technique of Mitchell (1971) but uses a sulphuric acid hydrogen peroxide selenium digestion procedure instead of conventional catalysis. This was selected for modification to meet the above special requirement. This method gives a net extinction at 548 nm of 0.70 with 16 mg N and the sensitivity can be increased by using the peak absorbance filter of 620 nm, Beer's law being followed at both wave lengths. The modified method used was as follows:

- (1) Dry and weigh plant sample and add to a clean dry test tube.
- (2) Add 0.4 ml digestion mixture equivalent to 0.38 ml of 95% H_2SO_4 plus four anti bumping granules.
- (3) Place tube on heated rack and allow digestion to occur for forty minutes after liquid has cleared.
- (4) Remove tube from rack and allow contents to cool completely.
- (5) Add 5 ml of water, by automatic pipette, down the side of the tube.
- (6) Mix the contents of the tube, using a plunger, taking care to avoid cross contamination and then stopper tube.
- (7) Remove 3 x 1 ml aliquots from each tube, using a Fisons auto diluter, no longer than twenty minutes after plunging.

Flush each aliquot along with 3 ml of reagent I into a 30 ml glass vial.

- (8) Swirl two of the set of three aliquots to ensure adequate mixing of the sample and reagent. Add 10 ml of reagent II immediately, using an automatic pipette.
- (9) Stopper and shake the vials and leave for forty five minutes before measuring the indophenol blue colours in a 10 mm flow-through cell at 628 nm.
- (10) To the remaining aliquot add two drops of methyl red and titrate with N/10 H_2SO_4 to determine the alkali excess which is used in obtaining the correction factor.

Modified Reagents:

Reagent I Prepared in 1 litre deionised water

13.68 g NaOH

12.59 g phenol

0.064 g sodium nitroprusside

Reagent II Prepared in 1 litre deionised water

5.00 g NaOH

3.73 g Na_2HPO_4

31.80 g $Na_3PO_4 \cdot 12H_2O$

5 ml sodium hypochlorite

The first reagent added to the aliquot from the digested sample for N determination is calculated to neutralise the maximum theoretical acid present after digestion and the second raises the pH to 11. As the plants progressively aged their N content increased. Aliquots of the digests prepared as above were then diluted with 9% H_2SO_4 so avoiding changes in the aliquot removed for colour. The following is the procedure used to obtain the above method.

The established method uses 250 mg dry matter in a digest volume of 50 ml containing approximately 5 ml concentrated H_2SO_4 and requires an aliquot of 0.08 ml for N determination.

The 1 mg dried samples from the present work were digested in 0.4 ml H_2SO_4 and diluted to 5.4 ml using a total addition technique. The 5 ml of H_2O was added, down the side of the tube, on top of the acid remaining after digestion. Even allowing for this increased concentration of nitrogen present in the digest an aliquot, for colour development, of up to 2 mls was necessary to contain a nitrogen content equivalent to that present in the original method i.e. 16 mg N.

Problems, other than sample size, were encountered in the development of the method and are dealt with as below:

(1) Sulphate concentration:

The theoretical alignment of the modified and original methods at about 16 mg N was shown to have an additional hazard of precipitation of sodium sulphate during colour development. To overcome this a lower aliquot of 1 ml was adopted and the 50% reduction in sensitivity partly offset by the use of the more sensitive 620 nm filter.

(2) Homogeneity of small volume digests:

The technique, mixing by stoppering and inverting tubes, was found to cause differential loss. An alternative mixing technique by plunging, i.e. the use of a narrow clearance disc mounted on a rod plunged several times through the sample, was tested. Simulated digests of potassium dichromate and water were used to provide very stable colour levels, read at 420 nm. The results for the two techniques are exemplified in App. I : D : Table 1.

Appendix I : D : Table 1

Effect of plunging technique versus inversion technique on extinctions of a potassium dichromate, water mixture

	<u>Extinction</u>	
	Plunging	Inversion
	0.311	0.331
	0.309	0.337
	0.309	0.317
	0.310	0.321
Mean	0.310	0.327

The results showed that inversion gave high and erratic results due to retention of dilutant in the crevices between stopper and tube.

(3) Maintenance of homogeneity:

Time between plunging and aliquot removal was found to be critical due to the fact that nearly all of the sample was removed.

Appendix I : D : Table 2

Effect on extinctions relating to N content in an aliquot when the delay between plunging of the test tube contents and aliquot removal was one hour

N content	Gross extinctions	
	1st aliquot	2nd aliquot
0.00	0.032	0.032
0.04	0.495	0.483
0.04	0.535	0.515
0.02	0.283	0.268
0.02	0.277	0.255
0.02	0.277	0.255

Appendix I : D : Table 3

Effect on extinctions relating to N content in an aliquot when the delay between plunging of the test tube contents and aliquot removal was twenty minutes

N content	Gross extinctions	
	1st aliquot	2nd aliquot
0.00	0.040	0.040
0.04	0.502	0.504
0.04	0.498	0.498
0.02	0.273	0.275
0.02	0.275	0.277

App. I : D : Table 2 shows that delay in sampling resulted in a lower extinction for the second aliquot removed from a sample. This lowering of extinction was attributed to the fact that moisture absorption had taken place in the upper layer of the sample. The level of sample had fallen after removal of the first aliquot and the second aliquot was removed from the diluted top layer.

A delay of 20 minutes was adopted to allow silica to settle and give uniformity of standard, as shown in App. I : D : Table 3.

(4) Control of the alkalinity in colour development:

The theoretical acid concentration present in the diluted digest is 0.38% (0.4 ml of 95% H₂SO₄ digestion mixture plus 5 ml water). This is subject to variation due to difficulty in precise measurement of concentrated acid and by inconsistent acid losses during digestion. In practice estimates of acid concentration derived from titration were found to be in the range of 0.28-0.38 mls.

This varying quantity of acid in the digest has two effects:

(i) Due to the fact that a total volume of 5 ml is added after digestion, low residual acid present will lower the total digest volume. This will influence the N concentration in the digest by as much as 2% as calculated below:

Assumed N content of digest (µg)	Quantity of acid	Total volume	N content of aliquot
40	0.38	5.38	7.43
40	0.28	5.28	7.58

(ii) Since the alkali present in reagent I is calculated to neutralise the maximum theoretical acid present after digestion any acid losses during digestion will result in progressively increasing alkali excesses. This was found to have a marked depressing effect on sensitivity and to mask the inverse volumetric effect.

The alkali excess can also be influenced by changes in the setting of the diluter and composition of reagent I.

To study the above effects a colour matrix was prepared utilising simulated digests ranging in nitrogen and acid content as below App. I : D : Table 4. The alkali excess after addition of reagent I to an aliquot of a sample was measured by titration and tested as a correction factor.

Appendix I : D : Table 4

Effect of acid and alkali excess (B) on the extinctions (A) of varying N contents

Acid Conc	N contents mg/ml					
	0.00	0.01	0.02	0.03	0.04	
0.380	(A)	0.086	0.021	0.350	0.473	0.600
	(B)	0.8	0.8	0.9	0.9	1.10
0.361	(A)	0.086	0.207	0.370	0.471	0.595
	(B)	2.25	2.25	2.20	2.00	2.30
0.323	(A)	0.084	0.213	0.343	0.464	0.585
	(B)	0.084	0.213	0.343	0.464	0.585
0.285	(A)	0.077	0.199	0.325	0.449	0.570
	(B)	6.50	6.70	6.55	6.50	6.55

Linear regressions of extinction values (y) against alkali excess (x) at each level of N were calculated using the data from App. I : D : Table 4. The equations and coefficients of correlation for each N level are shown in App. I : D : Table 5.

Appendix I : D : Table 5

Regression equations and correlation coefficients calculated using extinction value (y) and alkali excess (x) at five N content levels (0.00, 0.01, 0.02, 0.03 and 0.04 mg N/ml)

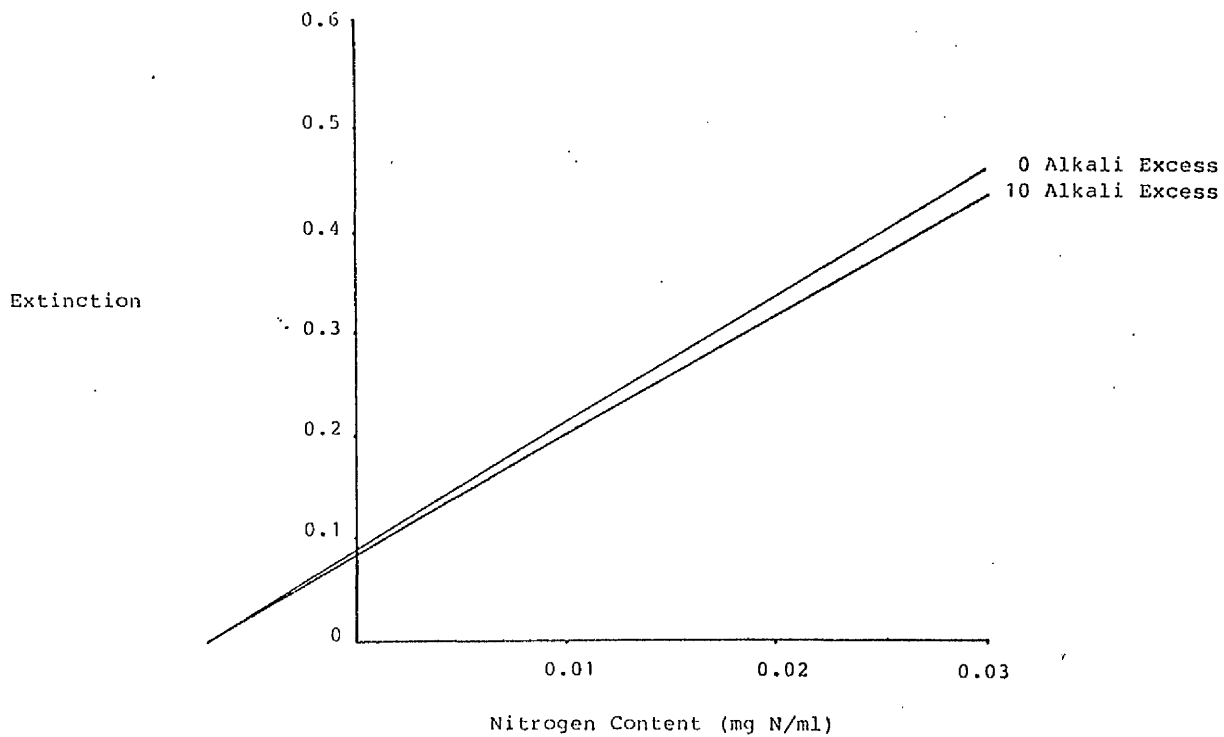
N content	Equation of line (y)	R
0.00	$Y = -0.001601x + 0.088940$	-0.92
0.01	$Y = -0.001740x + 0.213862$	-0.90
0.02	$Y = -0.004216x + 0.359860$	-0.93
0.03	$Y = -0.004257x + 0.478757$	-0.97
0.04	$Y = -0.005626x + 0.607504$	-0.99

The high correlation values obtained indicated that the relationships were linear and that correction would be possible.

App. I : D : Table 6 shows the theoretical extinctions derived, at the limits of 0 and 10 ml alkali excess, from the linear regressions in App. I : D : Table 5. From these the influence of alkali excess on method sensitivity was plotted in two graphs (see Figure I). The

Fig. I Influence of alkali excess (0 and 10 ml) on the sensitivity of extinctions obtained for varying N content levels (0, 0.01, 0.02, 0.03 and 0.04 mg N/ml)
Appendix I : D

0 Alkali Excess $y = 13.02x + 0.07860$ $r = 0.999$
10 ml Alkali Excess $y = 11.96x + 0.07475$ $r = 0.999$



equations and correlation coefficients were calculated as shown in

App. I : D : Table 6.

Appendix I : D : Table 6

0 ml Alkali excess		10 ml Alkali excess	
N content (x)	gross ext. (y)	N content (x)	gross ext. (y)
0.00	0.089	0.00	0.073
0.01	0.214	0.01	0.196
0.02	0.356	0.02	0.314
0.03	0.479	0.03	0.436
0.04	0.608	0.04	0.551
Equation of line (y) R		Equation of line (y) R	
Y = 13.02x + 0.0886 0.99		Y = 11.967x + 0.0745 0.99	

Methods of applying a correction for alkali excess were studied and the following approaches tested.

(a) Approximate correction:

$$\begin{aligned}
 \text{Ext. corrected to zero alkali} &= \text{Observed Ext.} + \frac{13.02}{11.967} \times \frac{\text{alkali (ml)}}{10} \\
 &= \text{Observed Ext.} + 0.088 \times \text{Ext. at 10 ml Alk excess} \\
 &\quad \times \frac{\text{Alk (ml)}}{10} \\
 &= \text{Observed Ext.} + 0.088 \times \text{Obs. Ext.} \frac{\text{Alk (ml)}}{10}
 \end{aligned}$$

(b) Exact correction:

$$\begin{aligned}
 \text{Ext. corrected to zero alkali} &= \text{Observed Ext.} \times \frac{13.02}{13.02} - (13.02 - \\
 &\quad 11.967) \times \frac{\text{Alk (ml)}}{10} \\
 &= \frac{\text{Observed Ext.}}{1 - 0.0088 \text{ Alk (ml)}}
 \end{aligned}$$

Both of these corrections were applied to the data from the colour matrix App. I : D : Table 4. Both were shown to reduce the coefficient of variance as below.

	CV %
Uncorrected	3.00
Approx. correction	1.99
Exact correction	1.87

In view of the magnitude of actual errors and the similarity of precision in practise the simpler approximation was applied in practise.

Appendix I : D : Table 7

Number and weight of nodules recorded throughout a fourteen week experimental period using L. uliginosus cv. Maku plants. (Experiment 6)

Week	Total dry weight (mg)	N present (mg)	Acetylene Reduction (umoles C ₂ H ₄ /hour)	Number of nodules	Fresh Wgt Nod (mg)
1	12.6	0.443	0	0	0
2	20.3	0.460	0	0	0
3	36.7	0.634	0.168	22	2.4
*					
5	67.1	2.100	0.087	57	7.8
6	107.8	2.040	0.156	106	24.4
7	165.4	3.760	0.883	158	57.6
8	245.8	5.550	1.130	227	80.1
9	452.2	10.980	2.270	286	112.9
10	532.8	12.590	1.890	254	128.3
11	1299.2	28.840	4.200	452	315.0
12	1490.0	32.380	4.410	538	305.3
13	2196.0	53.530	3.920	185	365.2
14	2320.0	54.950	3.640	167	448.5

* Week 4 missing

Aim

To investigate and find solutions for the problems concerned with the accurate measurement of nitrogen fixation in a sward containing an uneven distribution of Lotus plants. In its first few years of establishment Lotus does not form a close mat of material over the surface of the ground. Distinct Lotus plants can be detected and spaces between them are very evident. Turves removed at random from a plot may not contain any Lotus or only a small quantity, recording little fixation as measured by the acetylene reduction technique. A false representation of the potential of the species to fix N_2 is therefore obtained. Conversely a turf with overall Lotus coverage will record a high fixing potential which interpolated to the total area of the plot will give a false interpretation of the data. Total cover distribution over a plot gives an indication of the potential of Lotus but does not give an accurate measurement of its N_2 fixing potential. A technique for accurate measurement of acetylene reduction in a sward containing Lotus plants therefore had to be developed.

Preparatory investigations were made to determine an optimum size of turf to use with ten litre, polypropylene gas tight incubation chambers.

Materials & Methods

The turves were removed from plots sown, in 1974 at Sorn, with a mixture of L. uliginosus cv. Maku and perennial ryegrass. The plots were 2 m x 5 m but the turves were removed by spade from 0.5 m x 5 m discard strips remaining after a 1 m² x 5 m centre strip was cut for other purposes. Two parameters were varied within the turves: depth and width. In one instance while the depth remained at a constant 20 cm the width was cut back in sequence from 24 x 20 to 15 x 20 to 10 x 20, and after each cut

acetylene reduction was measured using the ten litre buckets as incubation chambers with an incubation time of twenty-four hours. Alternatively, while the width remained at a constant 20 x 20 cm the depth was cut down from 20 to 15 to 10, acetylene reduction again being measured after each cutting. The most convenient turf size to use with these buckets was 20 x 20 x 10 cm but, using this technique, high variability in the results was noted both within and between plots (Table 1) making it impossible to obtain any worthwhile conclusions from the data. An alternative technique had to be found which would take account of the uneven distribution of Lotus and reduce the variation present, particularly within plots.

Workers in New Zealand studying N_2 fixation by Lotus in the field utilised cores instead of turves (Charlton pers. comm.). Fourteen 2.5 cm diameter cores were incubated in one litre incubation jars for one hour. An investigation was set up to investigate the validity of modification of this technique using cores, and to determine if its use would reduce the variability in acetylene reduction results, within and between plots.

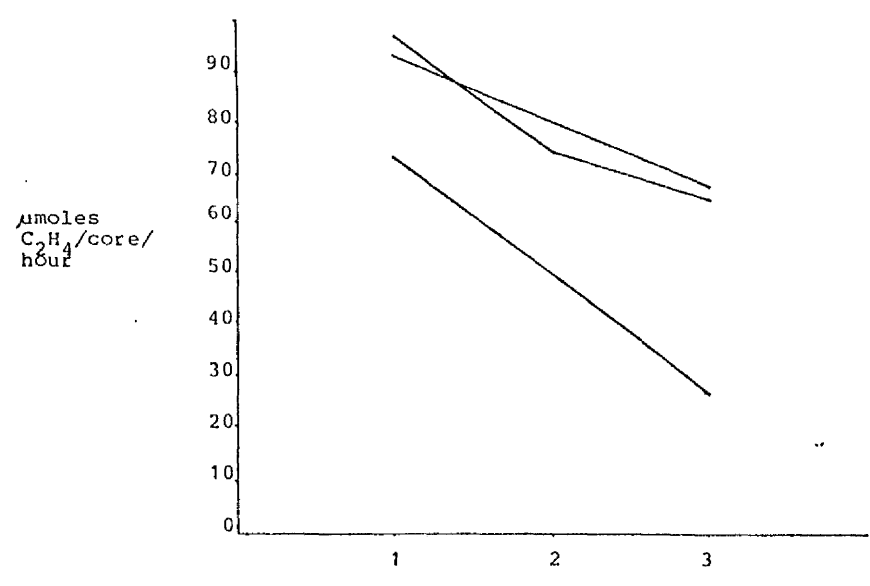
The site used was that at Sorn. The plots, comprising six replicates of the treatment, were sown in May 1977 with L. uliginosus cv. Maku at a rate of 3 kg/ha. The plots were 5 m x 3 m and each received lime at 7.5 tonnes/ha, 45 kg/ha ground mineral phosphate, 20 kg/ha superphosphate (20% P_2O_5) and 72 kg/ha muriate of potash (60% K_2O).

Two sets, each containing seven cores of 5 cm diameter and 7.5 cm depth, were removed from each plot. Each set of cores was placed into separate incubation jars of 4600 ml volume and incubated for four hours. Gas samples from 3 of the 12 jars were removed after each hour to determine a time course graph (Fig. 2).

Results

A decline in fixation was observed after an hour, indicating that to obtain accurate measurements incubations should be no longer than one hour.

Fig. II μ moles C_2H_4 produced by three sets of cores each set containing seven 5 cm diameter, 4.5 cm depth cores removed from plots sown with L. uliginosus cv. Maku. Gas samples were removed hourly throughout a four hour incubation. Appendix I : E.



Appendix I : E : Table 1

Effect of varying the dimensions of turves removed from a hill site (Sorn) on the variability of acetylene reduction rate measured as $\mu\text{moles C}_2\text{H}_4$ produced/hour recorded for each turf

Turf size (cm)						
(a) Constant area						
20 cm x 20 cm						
Replicates						
Depth cm	1	2	3	4	MEAN	SE
20	16.55	64.13	29.70	23.10	33.37	10.6
15	32.40	25.63	11.32	43.12	28.12	1.79
10	54.80	16.55	6.05	92.70	42.78	3.21
(b) Constant depth						
20 cm						
Area cm						
24 x 24	12.54	39.31	74.16	12.54	34.64	2.71
15 x 15	5.26	10.73	36.08	16.02	17.02	1.78
10 x 10	7.34	0.61	8.77	1.70	4.61	1.07

Appendix I : E : Table 2

Acetylene reduction rate measured as $\mu\text{moles C}_2\text{H}_4/\text{core}/\text{hour}$ from 5 cm diameter 7.5 cm depth cores removed from a hill site (Sorn) sown with *L. uliginosus* cv. Maku. Each core contained *Lotus* plant material. Six cores were removed from two plots and within plot and between plot variation noted

Core Replicates	Replicates						MEAN	SE
	1	2	3	4	5	6		
1	94.79	62.28	137.46	127.30	68.37	126.25	102.74	0.96
2	60.25	94.79	74.47	78.53	98.85	76.50	80.56	0.60

Although some variation was still present both within and between plots it was greatly reduced compared to that obtained using turves (Table 2).

Discussion

Even allowing for the fact that the volume of seven cores was smaller than that of a single turf, acetylene was nevertheless reduced in greater quantities by the cores. This is because the cores are removed from the vicinity of Lotus plants and will therefore contain a greater number of nodules. Gaseous diffusion in the incubation jar will also be less inhibited because of the smaller volume of soil present (Sinclair et. al. (1976). The rapid decline in fixation associated with cores is probably due to the fact that roots and nodules are damaged on removal of the cores from the soil. Defoliation also tends to take place and various workers have noted the rapid decline in fixation associated with defoliation, (Chu & Robertson, (1974); Butler et. al., 1959). Although it should be possible to increase the accuracy of this technique by increasing the number of samples removed, the constantly changing environmental factors will tend to alter the activity therefore a fairly high standard error will always be associated with the measurements.

The assay measured activity during the incubation period and will have a variable relationship to the mean activity for the full diurnal cycle. This can only be overcome by removing samples throughout a twenty-four hour incubation period, which is not practical. This along with the constantly changing environmental factors makes it impossible for acetylene reduced to be converted to absolute N_2 fixed in the sward. The results obtained are therefore only a guide as to the fixation occurring in the sward at a particular time.

Appendix II : A : Table 1

Data showing the effect of added trace elements on measurements
recorded at the termination of experiment 10

Variate: Dry Weight of Root

(b)

Species: LOTUS

Phosphate Level	0 kg P/ha		15 kg P/ha		30 kg P/ha	
	-	+	-	+	-	+
Add trace element						
Acid Level						
3.5	0.008	0.010	0.002	0.024	0.009	0.072
4.0	0.021	0.014	0.092	0.093	0.199	0.072
4.5	0.180	0.195	0.330	0.327	0.373	0.422
5.0	0.198	0.280	0.339	0.352	0.396	0.436
5.5	0.281	0.332	0.366	0.370	0.373	0.340
Within Table SED			0.044			

Species: CLOVER

Phosphate Level	0 kg P/ha		15 kg P/ha		30 kg P/ha	
	-	+	-	+	-	+
Add trace element						
Acid Level						
3.5	0.000	0.000	0.000	0.000	0.009	0.005
4.0	0.000	0.000	0.048	0.055	0.119	0.149
4.5	0.163	0.179	0.196	0.259	0.273	0.246
5.0	0.163	0.231	0.249	0.289	0.286	0.290
5.5	0.229	0.249	0.276	0.268	0.309	0.405
Within Table SED			0.042			

Appendix II : A : Table 1

Data showing the effect of added trace elements on measurements
recorded at the termination of experiment 10

Variate: Number of Effective Nodules

(c)

Species: LOTUS

Phosphate Level	0 kg P/ha		15 kg P/ha		30 kg P/ha	
	-	+	-	+	-	+
Add trace element						
Acid Level						
3.5	-0.0	0.0	0.0	0.0	7.0	5.0
4.0	0.0	0.0	7.0	11.3	10.7	14.7
4.5	54.7	60.7	44.0	41.0	35.7	36.0
5.0	39.7	49.3	54.7	71.7	64.3	45.3
5.5	59.3	76.0	39.3	66.0	40.7	55.7
Within Table SED			9.34			

Species: CLOVER

Phosphate Level	0 kg P/ha		15 kg P/ha		30 kg P/ha	
	-	+	-	+	-	+
Add trace element						
Acid Level						
3.5	0.0	0.0	0.0	0.0	0.0	0.0
4.0	0.0	0.0	0.0	0.0	11.7	27.7
4.5	61.0	76.0	46.3	79.3	79.3	47.7
5.0	67.3	65.3	103.0	92.0	118.0	84.7
5.5	107.0	84.3	106.0	113.7	134.7	90.7
Within Table SED			19.01			

Appendix II : A : Table 1

Data showing the effect of added trace elements on measurements recorded at the termination of experiment 10

Variate: Fresh Weight of Effective Nodules

(d)

Species: LOTUS

Phosphate Level	0 kg P/ha		15 kg P/ha		30 kg P/ha	
	-	+	-	+	-	+
Acid Level						
3.5	0.000	0.000	0.000	0.000	0.029	0.016
4.0	0.000	0.000	0.014	0.019	0.020	0.063
4.5	0.131	0.141	0.129	0.137	0.098	0.169
5.0	0.130	0.158	0.171	0.256	0.156	0.153
5.5	0.198	0.280	0.117	0.167	0.121	0.134
Within Table SED			0.037			

Species: CLOVER

Phosphate Level	0 kg P/ha		15 kg P/ha		30 kg P/ha	
	-	+	-	+	-	+
Acid Level						
3.5	0.000	0.000	0.000	0.000	0.000	0.000
4.0	0.000	0.000	0.000	0.000	0.026	0.121
4.5	0.154	0.288	0.183	0.282	0.335	0.268
5.0	0.202	0.417	0.177	0.268	0.182	0.303
5.5	0.187	0.228	0.206	0.268	0.220	0.147
Within Table SED			0.058			

Appendix II : A : Table 1

Data showing the effect of added trace elements on measurements recorded at the termination of experiment 10

Variate: Acetylene Reduction

(e)

Species: LOTUS

Phosphate Level	0 kg P/ha		15 kg P/ha		30 kg P/ha	
	-	+	-	+	-	+
Acid Level						
3.5	0.00	0.00	0.00	0.00	0.33	0.08
4.0	0.00	0.00	0.18	0.16	0.65	0.89
4.5	3.15	3.66	4.46	3.15	3.63	5.55
5.0	3.41	6.15	9.20	10.11	5.15	5.59
5.5	9.81	11.16	6.28	6.81	5.11	4.63
Within Table SED			1.62			

Species: CLOVER

Phosphate Level	0 kg P/ha		15 kg P/ha		30 kg P/ha	
	-	+	-	+	-	+
Acid Level						
3.5	0.00	0.00	0.00	0.00	0.00	0.00
4.0	0.00	0.00	0.15	0.09	0.25	2.60
4.5	7.77	11.04	5.41	9.74	10.83	12.28
5.0	6.17	12.84	10.43	10.92	8.82	15.09
5.5	12.66	12.03	13.23	10.80	12.76	15.14
Within Table SED			2.53			

Appendix II : B : Table 1

Dry weights (g) of crown and tap root for each of the cv. Maku plants removed between April and November during 1979 from the Sorn plots sown in 1977. The mean numbers are noted in experiment 13

Crown	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	SEPT.	SEPT.	OCT.	OCT.	NOV.
	1.34	1.59	0.73	0.62	1.48	0.43	1.70	1.76	2.33	0.35	3.13
	1.37	1.45	1.27	1.52	1.86	0.30	1.86	0.87	2.52	0.37	2.22
	0.40	1.33	0.39	0.44	2.00	3.30	2.65	5.05	3.63	0.46	0.43
	1.00	2.13	1.68	1.07	1.00	1.81	0.22	2.00	2.00	1.73	1.00
	3.87	0.89	2.68	0.49	0.32	0.29	*	1.62	2.08	3.67	0.92
	0.36	2.00	0.33	0.62	1.87	0.38	0.71	1.62	0.60	1.29	0.82
	3.21	2.09	0.30	0.44	2.00	0.65	0.66	*	0.54	0.94	0.42
	0.66	0.77	1.45	0.52	1.68	1.16	0.77	0.81	0.44	1.36	1.50
	1.00	2.48	0.58	1.48	4.00	1.81	0.21	0.51	5.00	1.39	0.24
	0.89	1.46	0.60	2.32	*	2.80	1.18	1.72	2.92	0.54	1.00
	1.28	0.82	0.94	2.28	1.13	1.66	1.56	0.42	0.33	4.31	2.51
	1.44	0.71	0.83	3.00	3.60	1.45	0.72	2.42	1.29	1.36	1.74
	0.35	0.41	0.62	1.18	0.92	0.67	4.27	1.35	2.82	5.17	3.16
	0.42	0.54	0.45	0.53	2.19	1.35	1.27	0.61	2.33	1.46	2.10
	1.13	*	0.14	1.27	0.94	0.44	1.26	0.72	1.01	3.50	0.89
	0.71	0.81	0.19	0.84	2.20	1.07	1.16	1.86	6.00	0.13	1.20
	0.71	1.25	1.95	*	0.70	1.90	1.18	0.28	0.69	0.52	0.62
	1.78	0.93	1.47	0.39	1.16	1.04	0.20	1.33	0.38	0.46	*
	1.30	0.41	2.00	0.57	0.38	0.32	1.25	1.35	*	0.69	*
	0.83	0.66	0.30	4.00	0.43	0.55	1.85	*	*	1.22	*
MEAN	1.20	1.20	0.94	1.19	1.63	1.17	1.30	1.46	2.05	1.55	1.41
SE	±0.20	±0.14	±0.16	±0.22	±0.23	±0.19	±0.21	±0.25	±0.38	±0.32	±0.22
Tap	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	SEPT.	SEPT.	OCT.	OCT.	NOV.
	1.12	0.13	0.52	0.35	0.38	0.58	1.50	1.51	0.88	0.40	1.00
	0.48	1.56	1.64	0.33	1.36	0.15	1.57	0.57	0.40	0.10	1.00
	0.45	1.34	0.70	1.11	0.72	1.00	2.05	1.74	0.74	0.37	0.17
	1.00	0.87	1.35	0.61	0.44	0.68	0.05	0.35	1.10	0.27	0.55
	1.72	1.04	1.96	0.49	0.36	0.25	0.04	0.62	0.89	1.08	0.25
	0.69	1.94	0.82	0.70	1.84	0.08	*	1.36	0.15	1.24	0.71
	1.27	0.62	0.45	0.56	1.40	0.39	0.28	0.03	0.20	0.18	0.67
	0.28	0.49	0.58	0.65	1.00	0.23	1.12	0.58	0.28	0.36	0.44
	1.18	1.36	0.61	1.16	1.68	0.37	0.21	0.43	0.65	0.25	0.07
	0.72	1.49	0.39	1.08	*	0.54	0.63	1.07	1.52	0.71	0.53
	0.43	1.00	0.76	0.80	0.50	0.71	0.23	0.07	0.51	1.55	1.92
	1.27	1.07	0.50	0.13	0.14	0.39	0.29	1.85	0.93	0.56	0.96
	0.20	0.49	0.72	0.78	1.19	0.29	1.56	0.67	0.93	*	3.31
	0.61	0.54	0.29	0.61	0.71	0.12	1.01	0.46	0.91	1.77	2.38
	1.77	0.91	0.21	0.38	0.17	0.83	0.39	0.43	0.51	1.35	0.16
	0.45	1.68	0.27	0.23	0.89	0.69	0.31	0.62	0.88	0.22	1.11
	0.74	0.87	0.86	0.55	0.78	1.35	0.55	0.14	0.58	0.33	0.45
	1.86	0.44	2.00	0.15	0.48	1.00	0.20	0.48	0.50	1.46	*
	0.95	1.06	1.55	0.20	0.40	0.18	0.69	1.19	*	0.49	*
	0.76	0.26	0.39	1.60	0.24	0.18	0.50	*	*	0.14	*
MEAN	0.90	0.96	0.83	0.62	0.77	0.50	0.69	0.75	0.70	0.62	0.92
SE	±0.11	±0.11	±0.13	±0.07	±0.12	±0.08	±0.13	±0.13	±0.18	±0.12	±0.21

Appendix II : B : Table 2

Dry weights (g) of new and old rhizome for each of the cv. Maku plants removed between April and November 1979 from the Sorn plots sown in 1977. The mean numbers are noted in experiment 13

New	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	SEPT.	OCT.	OCT.	OCT.	NOV.
	6.26	5.56	4.27	1.94	0.00	0.95	3.94	9.35	4.05	7.57	6.46
	5.83	17.91	7.83	4.00	0.06	1.54	3.25	6.00	8.43	5.47	4.06
	3.37	5.96	5.14	2.38	0.00	7.15	2.53	6.20	11.53	9.25	3.00
	1.53	6.40	4.87	4.40	0.29	0.70	3.81	1.81	3.67	5.02	3.11
	3.21	5.88	18.63	6.29	0.00	0.36	0.20	5.33	1.92	9.49	5.58
	1.22	5.94	1.32	1.74	0.65	1.00	3.42	5.21	4.25	3.00	7.51
	11.15	11.10	1.65	3.30	0.02	1.03	3.84	3.76	3.07	1.54	7.94
	4.52	4.81	4.13	3.67	0.38	1.75	3.18	3.04	4.26	2.12	6.55
	4.56	4.07	3.00	4.48	0.41	0.20	0.34	0.49	7.00	12.76	11.83
	2.39	13.07	4.75	4.45	0.04	2.11	6.30	15.35	7.03	7.37	5.71
	8.23	5.98	3.87	4.00	0.05	2.31	7.40	1.98	2.88	3.53	5.82
	6.16	3.43	5.14	4.73	0.07	1.69	8.97	7.20	3.90	2.17	8.99
	1.87	2.26	3.13	5.85	0.18	0.76	3.95	1.83	4.93	2.82	6.36
	7.69	3.63	3.53	1.75	0.61	0.15	3.65	1.45	4.19	3.81	2.27
	10.69	1.76	1.38	3.31	0.20	0.26	1.22	1.54	1.82	10.86	5.07
	6.49	2.72	2.47	4.07	0.00	0.83	4.49	3.00	5.59	4.81	10.05
	4.67	8.26	5.76	2.20	0.04	3.25	0.95	3.14	2.42	7.06	1.41
	11.00	4.42	4.24	3.06	0.12	1.00	5.09	6.69	5.99	1.91	*
	5.00	8.75	16.21	1.76	0.00	0.63	1.68	5.88	*	4.92	*
	8.54	5.16	9.71	16.26	0.06	0.36	0.36	*	*	7.14	*
MEAN	5.72	6.35	5.55	4.18	0.16	1.40	3.61	4.71	4.83	5.60	5.98
SE	±3.15	±0.88	±1.02	±0.70	±0.04	±0.35	±0.50	±0.81	±0.58	±0.79	±0.67
Old	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	SEPT.	SEPT.	OCT.	OCT.	NOV.
	3.05	1.28	0.74	0.47	5.93	3.48	13.52	11.65	13.89	10.76	12.00
	1.00	3.55	0.89	1.46	6.66	2.63	11.17	7.88	10.27	7.28	5.89
	1.03	6.17	1.48	0.95	6.70	31.14	24.68	20.89	22.29	7.89	5.00
	2.00	0.00	0.66	0.42	6.75	0.51	10.53	6.08	17.04	4.41	6.84
	1.73	0.33	6.16	0.18	1.00	1.85	7.96	9.87	5.57	14.20	5.36
	0.30	1.26	0.06	1.38	10.00	5.22	11.33	8.58	6.89	1.63	9.46
	5.85	0.51	0.27	0.35	10.54	1.99	1.38	5.86	9.74	2.35	9.00
	1.23	0.73	2.61	1.78	5.35	5.89	7.95	3.93	13.65	6.68	13.33
	3.48	0.51	0.00	0.53	12.52	3.62	6.22	2.55	22.18	19.43	6.59
	0.88	0.00	1.00	0.92	10.84	10.90	17.47	22.12	16.16	9.63	10.48
	3.18	0.91	0.20	0.18	8.00	0.65	10.23	7.28	10.65	2.94	18.36
	3.56	0.87	1.56	1.83	7.00	9.60	16.16	9.63	9.19	2.92	13.23
	0.00	0.07	0.00	2.36	5.10	4.38	23.13	7.00	8.99	11.70	12.45
	1.25	0.28	0.42	0.20	9.62	3.00	4.61	11.86	14.30	19.00	10.39
	2.83	0.20	0.81	0.69	4.58	0.87	3.83	15.41	7.91	8.99	7.44
	0.38	0.43	0.46	0.24	3.00	4.72	12.96	5.25	11.00	5.00	12.11
	0.58	1.68	0.00	1.34	5.67	13.00	4.49	7.29	3.34	12.55	1.94
	1.86	1.25	1.81	0.53	3.73	1.57	13.41	7.15	5.69	5.27	*
	0.39	0.81	6.13	0.24	3.62	*	4.37	7.82	*	8.18	*
	12.02	0.14	0.80	3.41	2.92	0.62	6.68	*	*	18.02	*
MEAN	2.33	1.05	1.30	0.97	6.48	5.28	10.60	9.37	11.62	8.94	9.40
SE	±0.62	±0.32	±0.45	±0.19	±0.68	±1.58	±1.40	±1.19	±1.27	±1.23	±0.97

Appendix II : B : Table 3

Dry weights (g) of shoot and woody tissue for each of the plants removed between April and November 1979 from the Sorn plots sown in 1977. The mean numbers are noted in experiment 13

Shoot	APRIL	MAY	JUNE	JULY	AUG.	SEPT.	SEPT.	SEPT.	OCT.	OCT.	NOV.
	0.57	0.29	2.32	10.43	23.00	16.66	55.66	49.66	31.99	27.13	20.28
	0.48	0.62	4.09	16.82	46.75	17.48	38.67	24.44	22.10	13.45	6.84
	0.19	0.36	3.91	10.64	47.00	55.12	50.07	49.13	50.92	8.45	20.05
	0.20	1.12	2.72	19.47	30.55	9.27	22.70	23.58	24.10	12.37	7.82
	0.53	0.75	11.03	14.93	14.48	5.60	29.37	20.58	15.00	36.07	2.23
	0.09	0.47	1.44	8.58	40.57	7.20	35.70	27.85	20.44	6.97	16.24
	0.70	1.62	1.42	17.16	32.37	17.00	20.88	20.64	20.16	5.54	19.15
	0.38	0.22	3.83	14.69	30.09	11.15	19.17	8.87	27.00	11.63	15.68
	0.70	0.43	1.50	17.30	71.82	13.06	23.34	7.99	59.00	20.88	5.87
	0.34	0.43	2.28	17.62	36.77	53.38	65.58	51.85	43.30	25.56	6.00
	0.62	0.57	3.09	14.29	35.10	22.87	29.04	72.29	15.94	9.60	23.81
	0.07	0.24	1.10	19.37	48.65	18.14	37.47	25.07	21.11	18.70	14.69
	1.19	0.21	1.00	8.43	46.34	15.20	22.89	43.00	37.46	18.10	26.49
	1.05	0.17	1.05	10.43	22.53	23.63	26.40	40.92	14.18	22.81	6.87
	0.48	0.15	1.34	4.72	17.20	24.81	19.14	30.50	24.62	12.91	36.44
	0.58	0.58	3.11	12.39	45.86	63.74	19.95	11.53	9.01	22.05	6.55
	0.53	0.33	1.89	9.81	25.75	35.33	33.45	21.96	13.51	9.59	*
	0.53	0.58	4.44	8.63	27.16	19.32	14.27	32.31	*	20.72	*
	0.70	0.29	4.18	56.21	15.00	11.00	19.30	*	*	59.87	*
MEAN	0.53	0.50	2.81	15.71	34.02	22.37	31.57	30.37	26.19	19.24	14.86
SE	±0.08	±0.08	±0.50	±2.37	±3.19	±3.72	±3.14	±3.88	±3.19	±2.73	±2.21

Woody Tissue	AUG.	SEPT.	SEPT.	SEPT.	OCT.	OCT.	NOV.
	0.15	1.35	5.26	2.21	2.44	3.12	2.99
	0.00	0.69	0.24	2.03	1.29	1.21	1.50
	0.00	1.43	2.31	6.20	6.86	0.87	1.00
	1.48	1.21	0.94	2.55	3.48	1.89	0.55
	0.42	0.65	0.38	2.81	1.56	8.77	0.94
	1.49	1.54	1.02	4.00	1.28	0.46	2.87
	2.61	1.39	1.03	2.76	2.55	0.25	3.51
	0.25	0.00	0.74	0.58	3.93	1.52	3.12
	0.00	1.10	0.77	0.69	2.34	1.14	1.90
	0.00	0.86	6.03	9.54	3.43	0.94	2.95
	1.50	0.00	6.01	3.94	1.00	2.77	2.62
	0.67	0.39	5.14	3.30	1.09	1.80	4.77
	0.93	1.29	1.86	3.05	4.36	3.02	2.99
	0.00	0.92	1.17	2.34	2.07	5.23	1.70
	0.39	5.71	3.19	12.17	1.14	4.00	0.53
	0.60	0.45	3.34	1.40	1.18	1.61	2.22
	2.89	3.40	1.64	1.00	1.54	5.21	1.42
	0.14	2.41	1.50	1.48	1.64	0.00	*
	0.46	2.48	0.99	1.46	*	2.00	*
	0.00	0.41	0.57	*	*	0.00	*
MEAN	0.70	1.38	2.21	3.34	2.40	2.29	2.21
SE	±0.19	±0.30	±0.43	±0.69	±0.63	±0.49	±0.28

Appendix II : B : Table 4

Dry weights (g) of crown and shoot for each of the cv. Maku plants removed between August and November 1978 from the Sorn plots sown in 1977.
The mean numbers are noted in experiment 13

Crown	AUGUST	SEPTEMBER			OCTOBER		NOVEMBER
		1.56	5.08	0.29	5.80	6.69	2.79
		2.09	2.47	0.10	6.46	5.00	*
		1.50	0.32	0.64	7.48	2.71	2.36
		0.45	0.72	1.62	4.40	1.00	1.31
		0.15	0.53	1.08	4.00		1.77
		0.58	0.28	1.77	3.49		1.11
		0.81	0.73	1.00	0.67		7.20
		3.31	0.36	0.36	3.35		0.55
		2.31	0.22	1.28	7.33		4.81
		0.70	0.29		8.00		2.25
		0.43	0.57		7.75		1.91
		0.89	0.12		5.00		3.54
		0.40	3.91		2.00		3.44
		0.91	1.31		0.62		2.00
		0.29	1.98		1.45		2.83
		0.22	0.70		5.92		2.90
		0.18	0.64		2.00		3.26
		1.00	3.82		2.91		1.30
		3.45	0.30		3.00		1.00
		4.96	0.34		1.42		
MEAN			1.21		4.10		2.57
SE			0.18		0.49		0.37
Shoot	AUGUST	SEPTEMBER			OCTOBER		NOVEMBER
	7.09	12.48	35.31	3.54	32.53	2.88	13.65
	12.88	10.90	27.66	3.45	32.64	29.66	7.00
	6.80	5.77	16.00	2.11	79.21	18.23	6.22
	2.13	2.56	2.12	2.20	22.45	8.11	13.45
	9.17	0.77	6.26	2.93	32.31	18.55	12.42
	5.11	3.00	3.48	0.23	15.00		34.87
	2.66	8.13	9.56	2.88	4.49		37.56
	1.48	20.79	6.74	2.43	16.23		7.00
	6.46	12.91	3.68	3.57	34.15		20.40
	1.07	4.29	0.72	9.40	27.86		3.77
	8.79	2.30	2.03	6.22	30.72		6.42
	3.66	4.04	4.75	10.52	16.47		14.14
	2.46	1.71	0.58	2.27	10.31		28.80
	3.82	4.00	28.13	3.57	3.14		7.37
	0.80	1.41	12.49		7.44		6.00
	1.28	1.00	3.16		25.30		21.00
	1.32	0.69	*		12.00		6.22
	0.78	2.34	15.32		14.26		5.57
	3.47	31.65	3.28		28.72		4.57
MEAN	4.27		7.20		18.55		13.50
SED+	0.77		1.16		3.32		2.39

Appendix II : B : Table 5

Dry weights (g) of new rhizomes for each of the cv. Maku plants removed between August and November from the Sorn plots sown in 1977. The mean numbers are noted in experiment 13

	AUGUST	SEPTEMBER			OCTOBER		NOVEMBER
	0.68	0.64	1.90	0.10	11.23	4.81	10.40
	0.57	1.35	2.06	0.18	16.36	9.20	8.04
	0.15	0.11	0.10	0.13	4.40	3.39	2.81
	0.14	0.14	0.12	0.13	12.58	8.70	3.24
	0.09	0.00	0.24	0.47	8.41		2.00
	0.00	0.13	0.24	0.00	8.61		4.61
	0.03	0.28	0.00	1.87	1.00		10.45
	0.00	1.83	0.13	2.36	4.00		2.69
	0.00	1.40	0.10	1.88	8.78		8.63
	0.00	0.23	0.16	3.82	3.51		7.49
	0.00	0.17	0.00	0.06	8.30		2.30
	0.00	0.15	0.04	0.19	4.00		5.81
	0.00	0.05	2.53		1.48		4.80
	0.00	0.84	1.00		0.26		2.17
	0.00	0.20	1.06		2.88		2.85
	0.04	0.00	1.42		8.00		5.34
	0.00	0.09	0.06		1.00		4.28
	0.01	0.54	0.12		5.13		2.00
	0.00	0.50	0.07		3.34		2.23
	0.00	2.17	0.05		2.04		
MEAN	0.09	0.64			5.89		4.85
SE ±	0.12	0.13			0.84		0.66

Appendix II : C : Table 1

Root and stem lengths (cm) for each plant removed between July and November 1979 from pipes sown at Auchincruive in 1979. The means of the figures are noted in experiment 14

Root	25th July	20th August	17th September	15th October	5th November
	7.5	14.0	11.3	17.5	19.5
	7.3	11.5	11.0	7.0	16.0
	6.2	21.0	12.0	27.0	20.0
	6.0	9.5	16.0	33.0	15.0
	7.2	11.5	7.5	13.5	15.5
	6.5	10.0	12.0	34.5	16.0
	6.0	9.5	12.0	17.5	27.0
	9.5	8.5	19.0	12.0	30.0
	6.6	13.5	19.5	17.0	11.5
	5.5	17.0	22.0	19.0	11.0
	8.5	11.3	15.5	22.0	14.0
	6.2	10.2	12.7	18.5	16.0
	7.0	9.2	14.0	14.0	12.2
	9.5	12.0	16.5	15.6	12.0
	7.7	8.0	14.8	6.5	26.0
	5.0	10.5	10.0	10.5	15.0
	5.4	10.0	12.7	11.0	18.3
	6.5	10.5	13.0	7.1	14.5
	7.0	13.0	14.5	*	13.5
	7.5	10.5	19.0	*	12.0
MEAN	6.90	11.60	14.25	16.84	16.75
SE	±0.27	±0.68	±0.80	±1.92	±1.20

Stem	25th July	20th August	17th September	15th October	5th November
	7.2	18.0	20.7	28.5	21.0
	5.2	15.5	9.0	24.0	17.5
	4.4	14.0	10.7	30.0	16.0
	4.9	8.5	9.0	38.0	9.5
	6.0	10.5	12.0	7.0	20.0
	6.4	6.4	13.0	34.0	17.5
	5.4	7.5	15.3	28.0	12.5
	7.0	8.2	21.0	15.5	18.0
	5.5	10.0	12.7	19.0	20.5
	5.0	10.0	13.0	19.5	15.0
	9.0	7.7	11.0	21.0	16.0
	4.5	11.6	14.0	35.0	15.0
	5.0	9.5	14.4	17.0	15.0
	6.0	13.5	14.0	26.0	13.0
	7.5	14.0	13.5	16.6	18.3
	5.3	10.0	11.5	17.0	17.5
	4.7	12.3	8.6	18.0	12.5
	5.0	10.0	12.0	12.5	11.7
	5.5	10.6	13.0	12.0	11.5
	5.6	11.0	9.8	14.5	9.5
MEAN	5.76	10.90	12.91	20.67	15.38
SE	±0.26	±0.65	±0.74	±1.93	±0.78

Appendix II : C : Table 2

Dry weight (mg) of root and shoot for each plant removed between July and November 1979 from pipes sown at Auchincruive in 1979. The means of the figures are noted in experiment 14.

Root	25th July	20th August	17th September	15th October	5th November
	6.30	86.00	154.6	240.0	153.0
	4.60	68.00	61.0	89.0	99.4
	6.6	127.0	56.5	101.2	254.2
	3.1	20.0	96.2	117.0	90.0
	5.1	34.0	48.4	114.9	88.7
	8.8	23.0	86.6	850.0	143.1
	3.7	24.0	70.6	414.9	80.9
	9.5	16.0	241.5	126.7	404.0
	7.8	80.0	95.3	166.1	117.9
	4.2	95.0	79.1	162.1	78.5
	7.7	14.0	98.1	241.0	113.6
	4.8	64.0	38.1	295.0	177.8
	4.4	68.0	83.5	123.9	134.4
	9.6	33.0	87.6	260.9	125.7
	10.4	23.0	105.5	44.3	172.7
	3.0	17.0	74.7	58.2	71.7
	2.7	53.0	20.5	36.3	71.9
	5.5	33.0	24.6	88.4	105.7
	6.4	57.0	62.6	*	125.3
	8.5	34.0	77.5	*	52.1
MEAN	6.14		83.13	252.38	134.53
SE	±1.28		±10.73	±22.30	±18.25

Shoot	25th July	20th August	17th September	15th October	5th November
	25.8	357.0	927.1	1130.0	512.8
	19.4	251.0	184.7	613.8	409.2
	19.10	365.0	107.1	958.4	637.1
	23.7	62.0	274.2	4040.0	81.7
	24.4	109.0	162.1	236.2	243.8
	36.9	66.0	354.5	4270.0	452.8
	21.6	64.0	250.8	1252.6	175.4
	44.5	108.0	753.2	552.7	684.1
	23.6	179.0	302.9	600.5	369.4
	15.8	395.0	161.7	743.2	180.3
	42.4	37.0	282.1	887.3	210.0
	16.9	195.0	248.8	789.5	269.6
	18.1	295.0	198.8	517.2	489.2
	37.7	183.0	394.1	539.8	192.3
	60.0	149.0	295.6	252.0	381.2
	14.0	79.0	188.3	229.9	159.6
	17.3	246.0	56.1	148.2	253.4
	26.3	150.0	134.8	332.1	366.3
	15.4	243.0	171.5	*	300.1
	24.9	142.0	31.7	*	1175.7
MEAN	26.39	184.0	274.0	1005.19	327.5
SE	±2.26		±48.41	±380.92	±36.8

Appendix II : C : Table 3

Seasonal N_2 fixation ($\mu\text{moles } C_2H_4/m^2/\text{hour}$) by *L. uliginosus* cv. Border, Beaver, 4704, 4703 and white clover at a grazed site (Eaglesham) throughout 1979

Date 1979	Cultivar				
	Border	Beaver	4704	4703	White Clover
1st June	34.9 ± 5.47	53.9 ± *	42.9 ± *	113.5 ± 7.72	366.1 ± *
12th June	333.3 ± *	210.3 ± 9.59	263.5 ± 11.16	219.8 ± 11.45	333.3 ± *
26th June	206.0 ± 3.08	248.2 ± 4.65	323.12 ± *	241.6 ± 6.17	181.94 ± *
9th July	027.8 ± 6.02	179.8 ± 3.48	287.5 ± 6.92	191.4 ± *	275.1 ± *
24th July	191.4 ± *	83.7 ± 5.99	203.8 ± 11.50	96.1 ± 6.92	395.2 ± 3.47
10th August	*	315.1 ± 8.51	179.8 ± 11.25	305.7 ± 7.36	125.9 ± *
24th August	234.3 ± 8.49	342.0 ± *	251.8 ± 8.51	296.9 ± 9.98	315.12 ± 7.96
7th September	301.3 ± 13.31	267.1 ± 2.42	255.4 ± 11.95	124.5 ± 8.95	243.8 ± 12.41
19th September	204.5 ± *	204.5 ± 3.42	227.1 ± 6.76	295.5 ± *	238.7 ± *
22nd October	147.7 ± 3.42	156.5 ± 5.34	61.9 ± 4.15	105.5 ± 3.37	56.8 ± 4.76
9th November	11.6 ± 0	17.5 ± 2.34	6.6 ± 2.26	8.7 ± 1.71	28.4 ± 4.10

*Only one sample reading

Appendix II : C : Table 4

Seasonal N₂ fixation ($\mu\text{moles C}_2\text{H}_4/\text{m}^2/\text{hour}$) by *L. uliginosus* cv. Maku at an ungrazed field site (Sorn) between July and December 1978 and March and November 1979

Date	$\mu\text{moles C}_2\text{H}_4/\text{m}^2/\text{hour}$
20th July 1978	6670.55 \pm 13.04
11th August 1978	7111.56 \pm 19.42
12th September 1978	1152.75 \pm 33.94
9th October 1978	1005.02 \pm 14.17
6th November 1978	43.66 \pm 2.11
5th December 1978	8.01 \pm 0.70
14th March 1979	24.02 \pm 1.25
6th April 1979	32.02 \pm 0.67
27th April 1979	32.02 \pm 1.59
8th May 1979	26.20 \pm 1.71
23rd May 1979	19.65 \pm 1.27
12th June 1979	885.67 \pm 6.89
26th June 1979	1824.47 \pm 11.64
9th July 1979	1964.92 \pm 11.49
24th July 1979	1654.17 \pm 12.86
9th August 1979	1084.35 \pm 9.14
24th August 1979	987.74 \pm 10.64
6th September 1979	539.26 \pm 5.14
19th September 1979	155.74 \pm 3.98
5th October 1979	52.40 \pm 2.26
19th October 1979	47.30 \pm 3.10
2nd November 1979	17.47 \pm 1.04

Appendix II : D : Table 1

Number and dry weight (mg) of effective nodules for each plant, cv. Maku, removed monthly between July and November 1979 from pipes sown at Auchincruive in 1979. The means of these figures are noted in experiment 16

Number	25th July	20th August	17th September	15th October	5th November
	0	1	125	22	46
	3	19	0	25	29
	0	49	16	2	49
	0	15	14	130	4
	0	11	48	1	9
	0	0	0	75	36
	0	23	18	22	20
	0	0	10	8	36
	0	0	13	35	0
	0	19	0	61	10
	6	4	18	19	3
	0	26	5	23	38
	0	9	7	15	13
	8	0	0	135	1
	0	2	7	0	3
	0	3	18	23	0
	0	0	0	38	14
	1	0	10	5	8
	1	8	1	*	8
	0	3	4	*	0

Weight	25th July	20th August	17th September	15th October	5th November
	0	0	42.6	33.6	36.4
	1.2	0	0	22.8	22.7
	0	15.0	11.1	0	59.5
	0	7.0	18.7	188.9	2.4
	0	2.0	19.6	0	1.6
	0	0	0	178.7	18.9
	0	8.0	12.8	15.7	17.2
	0	0	18.8	9.8	27.0
	0	0	17.6	14.4	0
	0	4.0	0	23.3	11.7
	1.0	4.0	11.8	22.3	2.5
	0	7.0	0	112.7	15.6
	0	3.0	4.9	44.5	2.5
	2.5	0	0	122.8	7.7
	0	0	8.9	24.9	0
	0	0	8.7	69.9	7.7
	0	0	0	0	9.7
	0	0	10.2	8.6	9.7
	0	2.0	0	8.0	16.0
	0	1.0	5.9	3.2	0
MEAN	0.235	2.650	9.580	45.210	13.44
SE \pm	0.142	0.888	0.640	13.193	3.309

Appendix II : D : Table 2

Number and dry weight (mg) of non-effective nodules for each plant, cv. Maku, removed monthly between July and November 1979 from pipes sown at Auchincruive in 1979. The means of these figures are noted in experiment 16

Weight	25th July	20th August	17th September	15th October	5th November
	0	0	3.2	9.1	3.6
	0	7.0	0	0	3.1
	0	1.0	4.4	0	6.0
	0	2.0	6.8	0	1.0
	0	4.0	1.3	23.6	1.5
	0	0	0	1.9	5.9
	0	2.0	7.5	1.4	1.7
	0	0	3.9	0	1.2
	0	0	4.6	1.8	0
	0	0	0	1.5	1.2
	0	1.0	7.1	9.4	0
	0	9.0	0	9.7	0
	0	6.0	3.5	20.7	27.3
	1.1	0	0	1.1	2.5
	0	0	2.7	1.3	0
	0	0	1.9	0.7	2.5
	0	0	3.0	7.3	1.6
	0.7	0	0	0.6	1.6
	0	2.0	3.6	0	3.1
	0	0	0	0	0
MEAN	0.09	1.70	2.86	4.51	3.19
SE \pm	0.06	0.61	0.57	1.54	1.34

Number	25th July	20th August	17th September	15th October	5th November
	0	0	11	31	24
	0	47	0	5	17
	0	11	43	0	18
	0	8	36	100	6
	0	24	11	6	5
	0	0	2	100	29
	0	11	22	13	7
	0	0	29	13	26
	0	0	21	6	2
	0	0	0	12	8
	0	6	9	14	0
	0	33	2	30	0
	0	29	12	31	14
	0	0	0	54	13
	5	0	7	6	23
	0	1	14	20	0
	0	0	8	15	12
	2	0	6	55	3
	0	13	5	9	13
	0	0	1	2	0
MEAN	0.35	9.15	11.95	26.10	11.00
SE \pm	0.27	3.07	2.78	6.66	2.11

Appendix II : D : Table 3

Number of stems/plants and the distance (cm) from the crown that the root starts to branch for each plant, cv. Maku, removed monthly between July and November 1979 from pipes sown at Auchincruive in 1979. The means of these figures are noted in experiment 16

Number	25th July	20th August	17th September	15th October	5th November
	3	5	8	3	5
	3	3	3	3	4
	3	3	3	3	7
	3	2	5	11	1
	3	3	4	5	1
	3	3	5	10	5
	2	4	3	7	3
	3	3	3	8	8
	1	4	3	7	5
	3	5	4	6	3
	1	1	3	6	3
	1	3	5	11	3
	3	5	3	8	5
	3	2	5	11	3
	2	3	4	7	6
	1	2	3	5	3
	3	3	3	3	5
	3	3	5	5	7
	2	4	5	4	6
	3	3	4	5	5
MEAN	2.45	3.20	4.05	5.89	4.4
SE \pm	0.19	0.24	0.29	0.56	0.44

Distance	25th July	20th August	17th September	15th October	5th November
	-	0.3	2.5	0.4	2.0
	0.7	0.4	-	1.0	1.0
	-	1.5	1.6	5.0	4.0
	-	1.0	0.6	1.0	3.0
	-	3.0	1.5	2.0	0.2
	-	-	5.1	2.0	3.0
	-	3.3	0.8	2.0	0.5
	-	-	1.3	1.3	1.3
	-	-	3.0	3.5	0.5
	-	6.0	-	3.0	2.5
	1.5	1.0	2.2	2.5	0.6
	-	3.0	5.0	0.2	0.5
	-	1.0	2.0	0.2	1.5
	1.7	-	-	5.0	0.7
	-	3.0	2.0	6.0	1.5
	-	2.4	1.2	0.3	-
	-	-	3.2	1.7	0.2
	3.0	-	6.7	1.0	5.0
	-	0.5	4.2	0.2	1.7
	-	1.5	4.0	1.5	-
MEAN	0.345	1.395	2.345	1.990	1.485
SE \pm	0.181	0.360	0.422	0.388	0.313

Appendix II : E : Table 1

Meteorological data for 1978 obtained from the
 Meteorological station situated at Auchincruive

		Soil Temp (°C) at 10 cm depth	Rainfall (mm)	Hour Sunshine
<u>Jan:</u>	1- 7	5.4	24.3	1.41
	8-14	4.6	20.1	2.54
	15-21	3.8	1.8	7.70
	22-28	3.7	20.0	1.50
<u>Feb:</u>	29- 4	3.2	24.6	1.76
	5-11	3.6	0.6	3.63
	12-18	1.9	0.0	5.94
	19-25	1.6	16.3	1.44
<u>Mar:</u>	26- 4	4.5	8.4	2.70
	5-11	5.2	27.9	1.91
	12-18	6.0	25.7	2.48
	19-25	5.3	53.6	2.21
<u>Apr:</u>	26- 1	5.9	19.8	3.28
	2- 8	6.8	1.4	5.50
	9-15	6.0	15.1	4.47
	16-22	7.2	0.1	4.73
	23-29	7.9	2.3	3.97
<u>May:</u>	30- 6	7.8	1.0	3.17
	7-13	9.3	5.0	5.79
	14-20	10.1	0.0	19.0
	21-27	11.4	1.1	5.34
<u>June:</u>	28- 3	13.7	13.05	25.4
	4-10	13.9	17.6	5.74
	11-17	12.8	0.0	8.36
	18-24	13.3	13.7	4.47
<u>July:</u>	25- 1	13.0	14.0	2.41
	2- 8	12.9	15.0	5.14
	9-15	13.9	0.0	11.11
	16-22	14.0	13.1	3.60
	23-29	14.1	13.3	5.1
<u>Aug:</u>	30- 5	14.6	15.9	2.7
	6-12	14.6	25.7	2.34
	13-19	14.6	28.4	1.90
	20-26	14.5	18.8	5.34
<u>Sept:</u>	27- 2	14.1	6.2	3.70
	3- 9	13.7	43.9	2.29
	10-16	13.6	38.7	2.47
	17-23	13.1	12.5	3.44
	24-30	12.7	52.8	2.51
<u>Oct:</u>	1- 7	12.0	13.3	5.04
	8-14	12.5	5.0	4.07
	15-21	11.4	13.9	1.98
	22-28	11.1	26.0	1.12
<u>Nov:</u>	29- 4	11.0	23.9	2.29
	5-11	10.9	2.9	1.61
	12-18	10.0	64.1	0.41
	19-25	9.4	38.9	0.80
<u>Dec:</u>	26- 2	5.6	6.7	2.74
	3- 9	5.4	30.7	0.49
	10-16	6.4	4.2	1.50
	17-23	3.9	4.1	2.56
	24-30	4.5	21.4	0.60

Appendix II : E : Table 2

Meteorological data for 1979 obtained from the Meteorological station situated at Auchincruive

	Soil Temp (°C) at 10 cm depth	Rainfall (mm)	Hour Sunshine
<u>Jan:</u>	31- 6	2.6	29.6
	7-13	2.7	26.0
	14-20	2.5	21.4
	21-27	2.2	1.8
<u>Feb:</u>	28- 3	1.8	5.8
	4-10	1.6	0.0
	11-17	1.3	0.5
	18-24	1.4	4.3
<u>Mar:</u>	25- 3	3.3	25.6
	4-10	4.3	41.9
	11-17	4.2	4.4
	18-24	3.2	8.5
	25-31	4.0	22.5
<u>Apr:</u>	1- 7	5.0	2.7
	8-14	5.6	33.0
	15-21	7.6	6.7
	22-28	8.1	12.6
<u>May:</u>	29- 5	7.6	5.7
	6-12	8.3	6.3
	13-19	10.3	13.7
	20-26	10.3	9.3
<u>June:</u>	27- 2	11.0	9.8
	3- 9	12.9	19.3
	10-16	13.5	8.2
	17-23	13.9	6.1
	24-30	13.3	12.3
<u>July:</u>	1- 7	13.3	3.0
	8-14	13.9	11.4
	15-21	13.6	16.2
	22-28	14.1	35.6
<u>Aug:</u>	29- 4	15.3	17.1
	5-11	14.8	64.7
	12-18	15.0	55.7
	19-25	14.2	19.7
<u>Sept:</u>	26- 1	13.9	12.6
	2- 8	14.4	13.0
	9-15	13.8	7.0
	16-22	12.8	24.1
	23-29	11.8	14.1
<u>Oct:</u>	30- 6	11.7	17.3
	7-13	12.2	15.8
	14-20	11.7	33.5
	21-27	10.0	8.3
<u>Nov:</u>	28- 3	9.3	53.7
	4-10	8.6	40.5
	11-17	5.9	40.0
	18-24	6.5	39.4
<u>Dec:</u>	25- 1	7.8	43.5
	2- 8	8.2	46.3
	9-15	7.1	16.5

Appendix III : A : Table 1

Statistical analysis relating to experiment 7

Variate: Total Dry Weight

N Source: NH_4NO_3

Variate: Total Dry Weight

Source of Variation	DF	Variance Ratio
Species	1	2.395
Nitrogen Level	3	3.895
Species - N Level	3	1.071
Residual Mean Square	13(1)	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
Lotus	12.7	40.8	60.0	73.7	46.8	0.92
Clover	24.0	29.5	32.0	46.4	33.0	
Mean	18.4	35.1	46.0	60.1		
SED	12.62					
SED Within Table			17.85			

N Source: NaNO_3

Variate: Total Dry Weight

Source of Variation	DF	Variance Ratio
Species	1	7.180
Nitrogen Level	3	2.999
Species - N Level	3	2.102
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
Lotus	8.8	35.8	102.0	82.9	57.4	13.43
Clover	15.9	21.3	25.3	23.1	21.4	
Mean	12.3	28.5	63.6	53.0		
SED	18.99					
SED Within Table			26.86			

N Source: KNO_3

Variate: Total Dry Weight

Source of Variation	DF	Variance Ratio
Species	1	0.090
Nitrogen Level	3	2.416
Species - N Level	3	3.484
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
Lotus	12.67	59.70	85.18	95.79	63.33	10.41
Clover	49.15	62.99	64.77	133.08	77.49	
Mean	30.91	61.34	74.97	114.43		
SED	14.73					
SED Within Table			20.83			

Appendix III : A : Table 2

Statistical analysis relating to experiment 7

Variate: Dry Weight of Shoot

N Source: NH_4NO_3

Variate: Dry Weight of Shoot

Source of Variation	DF	Variance Ratio
Species	1	1.687
N Level	3	4.011
Species - N Level	3	1.622
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	5.2	31.1	49.3	62.0	36.9	7.62
<u>Clover</u>	21.2	25.3	24.5	37.1	27.0	
Mean	13.2	28.2	36.9	49.5		
SED	10.77					
SED Within Table			15.23			

N Source: NaNO_3

Variate: Dry Weight of Shoot

Source of Variation	DF	Variance Ratio
Species	1	6.525
N Level	3	3.876
Species - N Level	3	2.103
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	6.3	27.1	81.9	72.3	46.9	11.28
<u>Clover</u>	12.4	18.8	21.8	19.1	18.1	
Mean	9.4	23.0	51.8	45.7		
SED	15.96					
SED Within Table			22.57			

N Source: KNO_3

Variate: Dry Weight of Shoot

Source of Variation	DF	Variance Ratio
Species	1	289.260
Nitrogen Level	3	560.239
Species.N Level	3	47.269
Residual Mean Square	3(11)	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	8.61	45.67	66.76	74.74	48.94	0.256
<u>Clover</u>	42.40	53.95	53.63	115.07	66.26	
Mean	25.50	49.81	60.19	94.90		
SED	0.362					
SED Within Table			0.512			

Appendix III : A : Table 3

Statistical analysis relating to experiment 7

Variate: Dry Weight of Root

N Source: NH_4NO_3

Variate: Dry Weight of Root

Source of Variation	DF	Variance Ratio
Species	1	3.156
Nitrogen Level	3	5.555
Species - N Level	3	0.706
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	2.27	9.66	10.74	11.75	8.60	1.476
<u>Clover</u>	2.86	4.26	7.49	9.32	5.98	
Mean	2.57	6.96	9.12	10.54		
SED	2.088					
SED Within Table			2.952			

N Source: NaNO_3

Variate: Dry Weight of Root

Source of Variation	DF	Variance Ratio
Species	1	9.925
Nitrogen Level	3	3.655
Species - N Level	3	2.243
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	MEAN	SED
Species						
<u>Lotus</u>	2.5	8.7	20.1	10.7	10.5	2.25
<u>Clover</u>	2.5	3.5	3.9	3.8	3.4	
Mean	2.5	6.1	12.0	7.2		
SED	3.18					
SED Within Table			4.49			

Appendix III : A : Table 4

Statistical analysis relating to experiment 7

Variate: Shoot Length

N Source: NH_4NO_3
Variate: Shoot Length

Source of Variation	DF	Variance Ratio
Species	1	1.596
N Level	3	9.331
Species - N Level	3	9.573
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	2.61	7.29	11.07	12.94	8.48	0.854
<u>Clover</u>	8.35	9.94	10.65	9.28	9.56	
Mean	5.48	8.61	10.86	11.11		
SED	1.208					
SED Within Table			1.709			

N Source: NaNO_3
Variate: Shoot Length

Source of Variation	DF	Variance Ratio
Species	1	2.558
N Level	3	14.025
Species - N Level	3	6.039
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	3.75	8.95	14.49	12.61	9.95	0.752
<u>Clover</u>	7.40	8.63	10.14	8.81	8.74	
Mean	5.58	8.79	12.31	10.71		
SED	1.064					
SED Within Table			1.505			

N Source: KNO_3
Variate: Shoot Length

Source of Variation	DF	Variance Ratio
Species	1	10.554
N Level	3	3.351
Species - N Level	3	13.510
Residual Mean Square	3(11)	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	3.91	9.85	12.93	14.11	10.20	0.698
<u>Clover</u>	11.21	13.53	13.95	14.43	13.28	
Mean	7.56	11.69	13.44	14.27		
SED	0.987					
SED Within Table			1.396			

Appendix III : A : Table 5

Statistical analysis relating to experiment 7.

Variate: Root Length

N Source: NH_4NO_3
Variate: Root Length

Source of Variation	DF	Variance Ratio
Species	1	1.455
N Level	3	0.700
Species - N Level	3	0.811
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	9.08	11.29	12.57	9.82	10.69	0.907
Clover	9.94	9.40	9.69	9.36	9.60	
Mean	9.51	10.34	11.13	9.59		
SED	1.282					
SED Within Table			1.813			

N Source: NaNO_3
Variate: Root Length

Source of Variation	DF	Variance Ratio
Species	1	1.031
N Level	3	2.372
Species - N Level	3	0.813
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	11.96	12.64	7.99	9.96	10.64	0.973
Clover	9.20	11.47	9.42	8.51	9.65	
Mean	10.58	12.06	8.71	9.24		
SED	1.376					
SED Within Table			1.945			

N Source: KNO_3
Variate: Root Length

Source of Variation	DF	Variance Ratio
Species	1	0.471
N Level	3	25.844
Species - N Level	3(11)	0.450
Residual Mean Square		

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	12.92	19.93	16.6	16.17	16.18	0.876
Clover	11.23	13.44	13.94	16.46	13.76	
Mean	11.62	16.68	15.27	16.31		
SED	1.239					
SED Within Table			1.752			

Appendix III : A : Table 6

Statistical analysis relating to experiment 7

Variate: Fresh Weight of Effective Nodules

N Source: NH_4NO_3

Variate: Fresh Weight of Effective Nodules

Source of Variation	DF	Variance Ratio
Species	1	1.079
N Level	3	1.700
Species x N Level	3	1.515
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	2.4	24.7	13.5	12.6	13.3	3.58
<u>Clover</u>	9.3	9.9	9.4	9.7	9.6	
Mean	5.9	17.3	11.4	11.2		
SED	5.07					
SED Within Table			7.17			

N Source: NaNO_3

Variate: Fresh Weight of Effective Nodules

Source of Variation	DF	Variance Ratio
Species	1	43.213
N Level	3	2.325
Species x N Level	3	3.694
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	2.78	7.69	1.79	1.83	3.52	1.64
<u>Clover</u>	3.08	3.21	1.75	1.33	2.34	
Mean	2.93	5.45	1.77	1.58		
SED	2.56					
SED Within Table			2.32			

N Source: KNO_3

Variate: Fresh Weight of Effective Nodules

Source of Variation	DF	Variance Ratio
Species	1	168.169
N Level	3	16.537
Species x N Level	3	8.438
Residual Mean Square	3(11)	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	4.32	7.00	4.57	6.26	5.54	0.501
<u>Clover</u>	7.55	11.86	13.49	15.24	12.04	
Mean	5.94	9.43	9.03	10.75		
SED	0.709					
SED Within Table			1.002			

Appendix III : A : Table 7

Statistical analysis relating to experiment 7

Variate: Number of Effective Nodules

N Source: NH_4NO_3

Variate: Number of Effective Nodules

Source of Variation	DF	Variance Ratio
Species	1	0.122
N Level	3	1.153
Species x N Level	3	0.944
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	6.67	5.33	5.67	6.33	6.00	1.191
<u>Clover</u>	7.67	7.33	2.67	4.67	5.58	
Mean	7.17	6.33	4.17	5.50		
SED	1.685					
SED Within Table			2.383			

N Source: NaNO_3

Variate: Number of Effective Nodules

Source of Variation	DF	Variance Ratio
Species	1	0.194
N Level	3	3.187
Species x N Level	3	0.647
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	7.00	6.00	8.67	1.67	5.83	1.325
<u>Clover</u>	6.67	7.00	5.00	2.33	5.25	
Mean	6.83	6.50	6.83	2.00		
SED	1.875					
SED Within Table			2.651			

N Source: KNO_3

Variate: Number of Effective Nodules

Source of Variation	DF	Variance Ratio
Species	1	18.274
N Level	3	20.271
Species x N Level	3	53.664
Residual Mean Square	3(11)	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	8	11	7	5	8	0.528
<u>Clover</u>	12	15	13	11	13	
Mean	10	13	10	8		
SED	0.747					
SED Within Table			1.057			

Appendix III : A : Table 8

Statistical analysis relating to experiment 7

Variate: Acetylene Reduction

N Source: NH_4NO_3

Variate: Acetylene Reduction

Source of Variation	DF	Variance Ratio
Species	1	2.530
N Level	3	0.232
Species x N Level	3	0.339
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	0.0093	0.0083	0.0090	0.0080	0.0087	0.00283
<u>Clover</u>	0.0123	0.0173	0.0103	0.0127	0.0132	
Mean	0.0108	0.0128	0.0097	0.0103		
SED 0.00400						
SED Within Table			0.00566			

N Source: NaNO_3

Variate: Acetylene Reduction

Source of Variation	DF	Variance Ratio
Species	1	7.433
N Level	3	0.076
Species x N Level	3	0.530
Residual Mean Square	14	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	0.0073	0.0097	0.0030	0.0017	0.0054	0.00639
<u>Clover</u>	0.0220	0.0167	0.0217	0.0310	0.0228	
Mean	0.0147	0.0132	0.0123	0.0163		
SED 0.00903						
SED Within Table			0.01278			

N Source: KNO_3

Variate: Acetylene Reduction

Source of Variation	DF	Variance Ratio
Species	1	122.399
N Level	3	39.369
Species x N Level	3	31.012
Residual Mean Square	3(11)	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	0.00367	0.00288	0.00467	0.00433	0.00389	0.000576
<u>Clover</u>	0.00567	0.00400	0.00833	0.00688	0.00622	
Mean	0.00467	0.00344	0.00650	0.00561		
SED 0.000815						
SED Within Table			0.001630			

APPENDIX III : A : Table 9

Statistical analysis relating to experiment 7

Species: LotusN Source: NH_4NO_3

Variate: Total Plant Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	4.392
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Total Dry Weight	17.9	40.8	60.0	73.7
SED	16.35			

Species: Clover

N Source: NH_4NO_3

Variate: Total Plant Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.505
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Total Dry Weight	24	29.5	32	46.4
SED	37.96			

Species: LotusN Source: NaNO_3

Variate: Total Plant Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	2.24
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Total Dry Weight	8.80	35.76	101.99	82.84
SED	40.36			

Species: Clover

N Source: NaNO_3

Variate: Total Plant Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	4.157
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Total Dry Weight	15.9	21.3	25.3	23.06
SED	3.404			

APPENDIX III : A : Table 10
 Statistical analysis relating to experiment 7

Species: Lotus

N Source: KNO_3

Variate: Total Plant Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	17.10
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Total Dry Weight	12.19	56.70	81.34	89.00
SED	11.92			

Species: Clover

N Source: KNO_3

Variate: Total Plant Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	16.77
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Total Dry Weight	53.61	50.26	84.89	130.29
SED	12.32			

Species: Lotus

N Source: "Enmag"

Variate: Total Plant Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	2.594
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
Total Dry Weight	14.73	76.32	121.36	74.98
SED	38.42			

Species: Clover

N Source: "Enmag"

Variate: Total Plant Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	7.98
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
Total Dry Weight	48.22	94.24	110.12	16.06
SED	11.52			

APPENDIX III : A : Table 11

Statistical analysis relating to experiment 7

Species: LotusN Source: NH_4NO_3

Variate: Root Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	3.27
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Root Dry Weight	2.27	9.66	10.74	11.75
SED	5.68			

Species: CloverN Source: NH_4NO_3

Variate: Root Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	2.362
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Root Dry Weight	2.86	4.26	7.49	9.32
SED	5.434			

Species: LotusN Source: NaNO_3

Variate: Root Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	2.91
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Root Dry Weight	2.5	8.7	20.1	10.7
SED	13.6			

Species: CloverN Source: NaNO_3

Variate: Root Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	1.741
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Root Dry Weight	2.61	3.82	3.65	4.22
SED	0.734			

APPENDIX III : A : Table 12

Statistical analysis relating to experiment 7

Species: LotusN Source: KNO₃

Variate: Root Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	124.79
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Root Dry Weight	4.06	14.63	18.42	21.05
SED	0.74			

Species: Clover

N Source: KNO₃

Variate: Root Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	20.26
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Root Dry Weight	6.75	9.04	11.14	18.01
SED	0.516			

Species: Lotus

N Source: "Enmag"

Variate: Root Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	2.03
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
Root Dry Weight	3.99	23.99	34.06	23.51
SED	12.48			

Species: Clover

N Source: "Enmag"

Variate: Root Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	9.14
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
Root Dry Weight	5.13	8.22	13.42	2.68
SED	2.14			

APPENDIX III : A : Table 13

Statistical analysis relating to experiment 7

Species: LotusN Source: NH_4NO_3

Variate: Shoot Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	6.44
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Shoot Dry Weight	5.25	31.13	49.28	61.95
SED	13.70			

Species: Clover

N Source: NH_4NO_3

Variate: Shoot Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.329
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Shoot Dry Weight	21.2	25.3	24.5	37.1
SED	34.24			

Species: LotusN Source: NaNO_3

Variate: Shoot Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	2.297
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Shoot Dry Weight	6	27	82	72
SED	67.4			

Species: Clover

N Source: NaNO_3

Variate: Shoot Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	2.011
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Shoot Dry Weight	12.44	18.83	21.82	19.14
SED	3.96			

APPENDIX III : A : Table 14

Statistical analysis relating to experiment 7

Species: LotusN Source: KNO_3

Variate: Shoot Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	13.248
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Shoot Dry Weight	8.61	45.67	66.76	74.74
SED	11.43			

Species: CloverN Source: KNO_3

Variate: Shoot Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	5.545
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Shoot Dry Weight	42.40	53.95	53.65	115.07
SED	19.80			

Species: Lotus

N Source: "Enmag"

Variate: Shoot Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	2.43
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
Shoot Dry Weight	10.90	66.47	100.97	63.05
SED	33.72			

Species: Clover

N Source: "Enmag"

Variate: Shoot Dry Weight

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	10.59
Residual Mean Square			2(4)	
N Level mg N/pot	0	30	70	120
Shoot Dry Weight	42	82.3	97.7	7
SED	17.82			

APPENDIX III : A : Table 15

Statistical analysis relating to experiment 7

Species: LotusN Source: NH_4NO_3

Variate: Root Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	1.09
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Root Length	9.08	11.29	12.57	9.82
SED	2.10			

Species: CloverN Source: NH_4NO_3

Variate: Root Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.072
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Root Length	9.94	9.40	9.69	9.36
SED	2.86			

Species: LotusN Source: NaNO_3

Variate: Root Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	1.354
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Root Length	11.96	12.64	7.99	9.96
SED	5.10			

Species: CloverN Source: NaNO_3

Variate: Root Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	6.859
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Root Length	9.20	11.47	9.42	8.51
SED	0.68			

APPENDIX III : A : Table 16

Statistical analysis relating to experiment 7

Species: Lotus
 N Source: KNO₃
 Variate: Root Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	22.37
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Root Length	12.02	19.93	16.60	16.17
SED	0.97			

Species: Clover
 N Source: KNO₃
 Variate: Root Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	4.469
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Root Length	11.23	13.44	13.94	16.46
SED	0.434			

Species: Lotus
 N Source: "Enmag"
 Variate: Root Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.68
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
Root Length	12.59	15.20	15.45	9.39
SED	4.86			

Species: Clover
 N Source: "Enmag"
 Variate: Root Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	5.83
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
Root Length	10.7	13.38	14.97	5
SED	2.56			

APPENDIX III : A : Table 17

Statistical analysis relating to experiment 7

Species: LotusN Source: NH_4NO_3

Variate: Shoot Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	13.17
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Shoot Length	2.61	7.29	11.07	12.94
SED	1.78			

Species: Clover

N Source: NH_4NO_3

Variate: Shoot Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.549
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Shoot Length	8.35	9.94	10.65	9.28
SED	3.734			

Species: LotusN Source: NaNO_3

Variate: Shoot Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	11.882
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Shoot Length	3.75	8.95	14.49	12.61
SED	3.47			

Species: Clover

N Source: NaNO_3

Variate: Shoot Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	3.13
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Shoot Length	7.40	8.63	10.14	8.81
SED	0.894			

APPENDIX III : A : Table 18
 Statistical analysis relating to experiment 7

Species: Lotus
 N Source: KNO₃
 Variate: Shoot Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	12.83
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Shoot Length	3.91	9.85	12.93	14.11
SED	1.62			

Species: Clover
 N Source: KNO₃
 Variate: Shoot Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	36
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Shoot Length	11.21	13.53	13.95	14.43
SED	0.336			

Species: Lotus
 N Source: "Enmag"
 Variate: Shoot Length

Source of Variation			DF	Variance Ratio
Nitrogen Level				1.94
Residual Mean Square				
N Level mg N/pot	0	30	70	120
Shoot Length	4.03	9.84	14.17	6.74
SED	4.42			

Species: Clover
 N Source: "Enmag"
 Variate: Shoot Length

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	28.37
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
Shoot Length	12.43	13.29	12.45	3.83
SED	1.34			

APPENDIX III : A : Table 19

Statistical analysis relating to experiment 7

Species: LotusN Source: NH_4NO_3

Variate: Fresh Weight of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	1.67
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Weight of Nodules	2.44	24.66	13.47	12.63
SED	9.92			

Species: Clover

N Source: NH_4NO_3

Variate: Fresh Weight of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	8.424
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Weight of Nodules	8.51	10.01	13.12	11.52
SED	1.95			

Species: LotusN Source: NaNO_3

Variate: Fresh Weight of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	2.326
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Weight of Nodules	2.78	7.69	1.79	1.83
SED	5.216			

Species: Clover

N Source: NaNO_3

Variate: Fresh Weight of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.12
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Weight of Nodules	10.25	10.08	10.96	10.65
SED	1.63			

APPENDIX III : A : Table 20

Statistical analysis relating to experiment 7

Species: LotusN Source: KNO_3

Variate: Fresh Weight of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	3.39
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Weight of Nodules	3.44	6.82	4.46	5.59
SED	1.116			

Species: Clover

N Source: KNO_3

Variate: Fresh Weight of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	9.25
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Weight of Nodules	8.72	11.42	8.97	16.88
SED	1.78			

Species: Lotus

N Source: "Enmag"

Variate: Fresh Weight of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level				4.875
Residual Mean Square				
N Level mg N/pot	0	30	70	120
Weight of Nodules	3.74	9.44	11.50	5.83
SED	2.24			

Species: Clover

N Source: "Enmag"

Variate: Fresh Weight of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	6.98
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
Weight of Nodules	11.02	21.02	14.95	0.83
SED	4.54			

APPENDIX III : A : Table 21

Statistical analysis relating to experiment 7

Species: LotusN Source: NH_4NO_3

Variate: Number of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.37
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Number of Nodules	7	5	6	6
SED	1.42			

Species: Clover

N Source: NH_4NO_3

Variate: Number of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	1.218
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Number of Nodules	8	7	3	5
SED	6.06			

Species: LotusN Source: NaNO_3

Variate: Number of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	1.234
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Number of Nodules	7	6	9	2
SED	7.6			

Species: Clover

N Source: NaNO_3

Variate: Number of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	6.92
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
Number of Nodules	7	7	5	2
SED	1.146			

APPENDIX III : A : Table 22

Statistical analysis relating to experiment 7

Species: LotusN Source: KNO_3

Variate: Number of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	8.28
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Number of Nodules	8	11	7	5
SED	1.146			

Species: Clover

N Source: KNO_3

Variate: Number of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.806
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
Number of Nodules	12	15	13	11
SED	2.512			

Species: Lotus

N Source: "Enmag"

Variate: Number of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level				0.05
Residual Mean Square				
N Level mg N/pot	0	30	70	120
Number of Nodules	9	6	10	10
SED	5.52			

Species: Clover

N Source: "Enmag"

Variate: Number of Effective Nodules

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	3.95
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
Number of Nodules	8	11	16	0
SED	4.70			

APPENDIX III : A : Table 23

Statistical analysis relating to experiment 7

Species: LotusN Source: NH_4NO_3

Variate: Acetylene Reduction

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.084
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
A.R.	0.009	0.008	0.009	0.008
SED	0.00176			

Species: CloverN Source: NH_4NO_3

Variate: Acetylene Reduction

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.537
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
A.R.	0.0123	0.0173	0.0103	0.0127
SED	0.0057			

Species: LotusN Source: NaNO_3

Variate: Acetylene Reduction

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	3.795
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
A.R.	0.00733	0.00967	0.003	0.00167
SED	0.0027			

Species: CloverN Source: NaNO_3

Variate: Acetylene Reduction

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.18
Residual Mean Square			6	
N Level mg N/pot	0	30	70	120
A.R.	0.022	0.016	0.021	0.031
SED	0.0091			

APPENDIX III : A : Table 24

Statistical analysis relating to experiment 7

Species: LotusN Source: KNO₃

Variate: Acetylene Reduction

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	0.89
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
A.R.	0.003	0.003	0.004	0.004
SED	0.66			

Species: CloverN Source: KNO₃

Variate: Acetylene Reduction

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	4.74
Residual Mean Square			5(1)	
N Level mg N/pot	0	30	70	120
A.R.	0.005	0.004	0.008	0.007
SED	7.16			

Species: Lotus

N Source: "Enmag"

Variate: Acetylene Reduction

Source of Variation			DF	Variance Ratio
Nitrogen Level			2	2.387
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
A.R.	0.006	0.003	0.002	0.0003
SED	19.21			

Species: Clover

N Source: "Enmag"

Variate: Acetylene Reduction

Source of Variation			DF	Variance Ratio
Nitrogen Level			3	4.09
Residual Mean Square			3(3)	
N Level mg N/pot	0	30	70	120
A.R.	0.003	0.004	0.010	0.001
SED	13.66			

APPENDIX III : B : Table 1

Statistical analysis relating to experiment 8

Variate: Total Plant Dry Weight

Species: LotusN Source: 30 mg N/pot KNO₃

Source of Variation DF Variance Ratio

Time 6 39.58

Residual Mean Square 12

(Days after transplanting)	Time	0	6	12	18	24	30	Control (No N added)
Total Dry weight		87.09	91.41	24.26	61.14	39.19	39.76	42.71
SED		.34						

Species: Clover

N Source: 30 mg N/pot KNO₃

Source of Variation DF Variance Ratio

Time 6 35.98

Residual Mean Square 12

(Days after transplanting)	Time	0	6	12	18	24	30	Control (No N added)
Total Dry Weight		85.52	81.44	25.26	47.27	41.25	35.66	39.25
SED		2.08						

Species: LotusN Source: 70 mg N/pot KNO₃

Source of Variation DF Variance Ratio

Time 6 51.40

Residual Mean Square 12

(Days after transplanting)	Time	0	6	12	18	24	30	Control (No N added)
Total Dry Weight		99.38	70.17	53.21	67.06	46.73	31.12	42.71
SED		2.08						

Species: Clover

N Source: 70 mg N/pot KNO₃

Source of Variation DF Variance Ratio

Time 6 7.59

Residual Mean Square 12

(Days after transplanting)	Time	0	6	12	18	24	30	Control (No N added)
Total Dry Weight		87.69	99.57	35.76	44.60	45.80	42.21	39.25
SED		5.02						

APPENDIX III : B : Table 2

Statistical analysis relating to experiment 8

Variate: Dry Weight of Shoot

Species: Lotus

Source of Variation	DF	Variance Ratio
Nitrogen	1	0.085
Time	5	33.950
Nitrogen.Time	5	4.621
Residual	26	

Time	0	6	12	18	24	30	Control
30 mg N	64.0	68.6	37.5	45.1	20.9	31.1	8.4
70 mg N	71.5	50.5	38.0	49.7	37.6	23.9	
SED	5.63						

Species: Clover

Source of Variation	DF	Variance Ratio
Nitrogen	1	1.959
Time	5	47.529
Nitrogen.Time	5	0.360
Residual	25(1)	

Time	0	6	12	18	24	30	Control
30 mg N	71.5	68.8	20.3	39.4	34.0	29.7	32.1
70 mg N	76.0	70.2	27.9	36.7	38.3	35.1	
SED	6.01						

Source of Variation	DF	Variance Ratio
Species	1	2.283
Residual	56(1)	

<u>Lotus</u>	Clover	SED
42.07	44.64	1.702

APPENDIX III : B : Table 3

Statistical analysis relating to experiment 8
 Variate: Dry Weight of Root

Species: Lotus

Source of Variation		DF	Variance Ratio					
Nitrogen		1	0.916					
Time		5	8.952					
Nitrogen.Time		5	1.525					
Residual		26						
Time	0	6	12	18	24	30	Control	
30 mg N	19.76	19.62	14.51	14.09	16.85	7.66	4.28	
70 mg N	24.11	17.66	13.16	15.39	8.20	5.77		
SED	3.500							

Species: Clover

Source of Variation		DF	Variance Ratio					
Nitrogen		1	1.366					
Time		5	2.171					
Nitrogen.Time		5	0.837					
Residual		25(1)						
Time	0	6	12	18	24	30	Control	
30 mg N	10.5	9.9	4.0	6.0	5.6	4.8	5.4	
70 mg N	10.8	26.5	5.6	6.5	6.0	5.6		
SED	7.06							

Source of Variation		DF	Variance Ratio	
Species		1	13.581	
Residual		56(1)		

<u>Lotus</u>	Clover	SED
13.93	8.25	1.54

APPENDIX III : B : Table 4

Statistical analysis relating to experiment 8
 Variate: Fresh Weight of Effective Nodules

Species: Lotus

Source of Variation					DF		Variance Ratio
Nitrogen					1		0.979
Time					5		14.968
Nitrogen.Time					5		0.825
Residual					26		
Time	0	6	12	18	24	30	Control
30 mg N	9.62	9.40	6.63	5.50	4.83	2.63	3.61
70 mg N	11.91	8.51	5.85	4.45	2.61	1.15	
SED	1.709						

Species: Clover

Source of Variation							Variance Ratio
Nitrogen					1		6.922
Time					5		31.777
Nitrogen.Time					5		1.396
Residual					25(1)		
Time	0	6	12	18	24	30	Control
30 mg N	16.12	13.76	4.86	8.55	6.31	5.02	7.89
70 mg N	13.73	10.12	6.00	5.75	6.00	3.44	
SED	1.486						

Source of Variation					DF		Variance Ratio
Species					1		27.622
Residual					50(1)		

<u>Lotus</u>	Clover	SED
5.90	8.27	0.452

Appendix III : B : Table 5

Statistical analysis relating to experiment B
 Variate: Number of Effective Nodules

Source of Variation	DF	Variance Ratio
Species	1	34.124
Control VS N Treated	1	5.046
Species Control VS N Treated	1	1.192
Nitrogen	1	0.768
Time	5	13.533
Species.Nitrogen	1	0.192
Species.Time	5	1.462
Nitrogen.Time	5	0.473
Residual Mean Square	4.630 [56 (1) df]	

MEANS

Species	<u>Lotus</u>		Clover		SED			
	7.97		10.82		0.487			
Nitrogen	30KNO ₃	70KNO ₃	MEAN		SED			
Species								
<u>Lotus</u>	8.39	7.72	8.06		0.507			
Clover	11.17	10.94	11.06					
MEAN	9.78	9.33						
SED			0.507					
SED Within Table			0.717					
Time	0	6	12	18	24	30	MEAN	SED
Nitrogen								
30KNO ₃	12.23	12.17	7.50	8.33	10.00	7.83	9.28	0.507
70KNO ₃	12.50	11.50	8.17	8.50	9.33	6.00	9.33	
MEAN	12.67	11.83	7.83	8.42	9.67	6.92		
SED					0.878			
SED Within Table					1.242			

Species: Lotus

Source of Variation	DF	Variance Ratio					
Nitrogen	1	1.592					
Time	5	9.993					
Nitrogen.Time	5	2.945					
Residual	26						
Time	0	6	12	18	24	30	Control
30 mg N	11.33	8.67	8.67	6.33	9.80	6.33	7.00
70 mg N	11.33	10.00	6.00	8.67	6.00	4.33	
SED	1.294						

Appendix III : B : Table 6
 Statistical analysis relating to experiment 8
 Variate: Acetylene Reduction

Source of Variation	DF	Variance Ratio
Species	1	20.710
Control VS N Treated	1	2.096
Species Control VS N Treated	1	0.265
Nitrogen	1	5.714
Time	5	3.958
Species.Nitrogen	1	0.196
Species.Time	5	1.312
Nitrogen.Time	5	1.688
Residual Mean Square	0.000001772 [56 (1) df]	

MEANS

Species	Lotus	Clover	SED
	0.00215	0.00353	0.000301

Species	Nitrogen		MEAN	SED
	30KNO ₃	70KNO ₃		
Lotus	0.00189	0.00250	0.00219	0.00314
Clover	0.00317	0.00406	0.00361	
MEAN	0.00253	0.00328		
SED			0.00314	
SED Within Table			0.000769	

Time	0	6	12	18	24	30	MEAN	SED
	Nitrogen							
30KNO ₃	0.00233	0.00250	0.00267	0.00217	0.00233	0.00317	0.00253	0.00314
70KNO ₃	0.00267	0.00250	0.00267	0.00233	0.00400	0.00550	0.00328	
MEAN	0.00250	0.00250	0.00267	0.00225	0.00317	0.00433		
SED				0.00543				
SED Within Table				0.000769				

Time	0	6	12	18	24	30	MEAN	SED
	Species							
Lotus	0.00233	0.00183	0.00217	0.00150	0.00250	0.00283	0.00219	0.00314
Clover	0.00267	0.00317	0.00317	0.00300	0.00383	0.00583	0.00361	
Mean	0.00250	0.00250	0.00267	0.00225	0.00317	0.00433		
SED				0.000543				
SED Within Table				0.000769				

Appendix III : C : Table 1

Statistical analysis relating to experiment 9

Variate: Total Dry Weight

N Source: ENMAG

Variate: Total Dry Weight

Source of Variation	DF	Variance Ratio
Species	1	19.360
Nitrogen Level	3	8.006
Species.N Level	3	32.630
Residual Mean Square	5(9)	

Nitrogen Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	17.3	90.5	136.7	178.2	105.7	9.24
<u>Clover</u>	92.5	112.8	9.1	45.6	65.0	
Mean	54.9	101.7	72.9	111.9		
SED 13.87						
SED Within Table			18.48			

Appendix III : C : Table 2

Statistical analysis relating to experiment 9

Variate: Dry Weight of Shoot

N Source: ENMAG

Variate: Dry Weight of Shoot

Source of Variation	DF	Variance Ratio
Species	1	0.043
N Level	3	5.226
Species.N Level	3	1.670
Residual Mean Square	7(7)	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	12.3	66.5	102.4	64.5	61.4	15.01
<u>Clover</u>	43.0	84.0	99.2	7.1	58.3	
Mean	27.6	75.3	100.8	35.8		
SED 21.22						
SED Within Table			30.01			

Appendix III : C : Table 3

Statistical analysis relating to experiment 9

Variate: Dry Weight of Root

N Source: ENMAG

Variate: Dry Weight of Root

Source of Variation	DF	Variance Ratio
Species	1	0.919
N Level	3	1.620
Species.N Level	3	0.418
Residual Mean Square	7(7)	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	1.52	1.52	1.43	0.63	1.27	0.299
<u>Clover</u>	2.12	1.65	1.24	1.23	1.56	
Mean	1.82	1.58	1.33	0.93		
SED	0.422					
SED Within Table						0.597

Appendix III : C : Table 4

Statistical analysis relating to experiment 9

Variate: Fresh Weight of Effective Nodules

N Source: ENMAG

Variate: Fresh Weight of Effective Nodules

Source of Variation	DF	Variance Ratio
Species	1	3.290
N Level	3	6.970
Species.N Level	3	3.289
Residual Mean Square	7(7)	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	0.64	1.94	2.76	1.53	1.72	0.462
<u>Clover</u>	2.19	4.38	3.60	0.06	2.56	
Mean	1.41	3.16	3.18	0.80		
SED	0.653					
SED Within Table						0.924

Appendix III : C : Table 5

Statistical analysis relating to experiment 9

Variate: Number of Effective Nodules

N Source: ENMAG

Variate: Number of Effective Nodules

Source of Variation	DF	Variance Ratio
Species	1	59.178
N Level	3	7.916
Species.N Level	3	6.471
Residual Mean Square	7(7)	

N Level mg N/pot	0	30	70	120	Mean	SED
Species						
<u>Lotus</u>	2.80	19.00	10.30	6.30	9.60	1.306
<u>Clover</u>	1.39	0.20	0.20	0.00	0.45	
Mean	0.71	9.40	5.05	3.15		

SED 1.847

SED Within Table

2.612

Appendix III : D : Table 1

Statistical analysis relating to experiment 10

Variate: Total Plant Dry Weight

Species: LOTUS

Source of Variation	DF	Variance Ratio
Acid Level	4	101.212
Phosphate Level	2	44.601
Add trace element	1	1.908
Acid Level.Phosphate Level	8	4.464
Acid Level.Add Trace Element	4	0.784
Phosphate Level.Add Trace Element	2	0.352
Acid L.Phosphate L.Add Trace Element	8	0.910
Residual Mean Square	58	

Phosphate Level Add Trace Element	0 kg P/ha		15 kg P/ha		30 kg P/ha	
	-	+	-	+	-	+
Acid Level						
3.5	0.025	0.030	0.006	0.078	0.239	0.228
4.0	0.061	0.040	0.256	0.511	0.559	0.773
4.5	0.561	0.539	1.005	1.011	1.230	1.406
5.0	0.559	0.690	1.312	1.310	1.228	1.506
5.5	1.001	1.209	1.453	1.197	1.283	1.150
Within Table SED			0.168			

Species: CLOVER

Source of Variation	DF	Variance Ratio
Acid Level	4	69.056
Phosphate Level	2	11.516
Add Trace Element	1	4.102
Acid Level.Phosphate Level	8	1.730
Acid Level.Add Trace Element	4	0.945
Phosphate Level.Add Trace Element	2	0.159
Acid L.Phosphate L.Add Trace Element	8	0.563
Residual Mean Square	58	

Phosphate Level Add Trace Element	0 kg P/ha		15 kg P/ha		30 kg P/ha	
	-	+	-	+	-	+
Acid Level						
3.5	0.000	0.000	0.000	0.000	0.021	0.010
4.0	0.000	0.000	0.088	0.091	0.289	0.428
4.5	0.657	1.040	0.818	1.438	1.134	1.157
5.0	0.551	0.990	1.204	1.479	1.631	1.856
5.5	1.225	1.459	1.607	1.314	1.779	2.017
Within Table SED			0.290			

Appendix III : D : Table 2

Statistical analysis relating to experiment 10

Variate: Dry Weight of Shoot

Source	DF	Variance Ratio
Species	1	12.512
Acid Level	4	113.976
Phosphate Level	2	22.811
Add Trace Element	1	5.502
Species.Acid L	4	6.913
Species.Phosphate L	2	0.524
Acid L.Phosphate L	8	2.057
Species.Add TE	1	2.633
Acid L.Add TE	4	1.904
Phosphate L.Add TE	2	0.942
Species.Acid L.Phosphate L	8	1.379
Species.Acid L.Add TE	4	0.699
Species.Phosphate L.Add TE	2	0.476
Acid L.Phosphate L.Add TE	8	0.772
Residual Mean Square	0.06946 (126 df)	

MEANS

Species	<u>Lotus</u>		Clover		SED		
	0.518		0.657		0.0393		
Acid Level	3.5	4.0	4.5	5.0	5.5	SED	
	0.037	0.147	0.743	0.936	1.075	0.0621	
Phosphate Level	0	15	30			SED	
	0.419	0.599	0.744			0.0481	
Add Trace Element		+		-		SED	
		0.633		0.541		0.0393	
Acid Level	3.5	4.0	4.5	5.0	5.5	MEAN	SED
Species							
<u>Lotus</u>	0.070	0.207	0.664	0.776	0.872	0.518	0.0393
Clover	0.003	0.087	0.821	1.095	1.277	0.657	
MEAN	0.037	0.147	0.743	0.936	1.075		
SED				0.0621			
SED Within Table				0.0879			

Appendix III : D : Table 3

Statistical analysis relating to experiment 10

Variate: Dry Weight of Root

Source	DF	Variance Ratio
Species	1	56.303
Acid Level	4	228.870
Phosphate Level	2	74.819
Add Trace Element	1	5.731
Species.Acid L	4	1.509
Species.Phosphate L	2	5.118
Acid L.Phosphate L	8	4.356
Species.Add TE	1	0.032
Acid L.Add TE	4	0.348
Phosphate L.Add TE	2	0.115
Species.Acid L.Phosphate L	8	2.010
Species.Acid L.Add TE	4	0.214
Species.Phosphate L.Add TE	2	0.125
Acid L. Phosphate L.Add TE	8	0.432
Residual Mean Square	0.002795 (126 df)	

MEANS

Species	<u>Lotus</u>		Clover		SED		
	0.2240		0.1649		0.00788		
Acid Level	3.5	4.0	4.5	5.0	5.5	SED	
	0.0166	0.0888	0.2621	0.2882	0.3166	0.012466	
Phosphate Level		0	15		30	SED	
		0.1343	0.1968		0.2523	0.00788	
Add Trace Element		+		-		SED	
		0.2039		0.1850		0.00788	
Acid Level	3.5	4.0	4.5	5.0	5.5	MEAN	SED
Species							
<u>Lotus</u>	0.0309	0.1156	0.3046	0.3251	0.3438	0.2240	
<u>Clover</u>	0.0023	0.0619	0.2195	0.2513	0.2893	0.1649	0.00788
MEAN	0.0166	0.0888	0.2621	0.2882	0.3166		
SED				0.01246			
SED Within Table				0.01762			

Appendix III : D : Table 4

Statistical analysis relating to experiment 10
 Variate: Number of Effective Nodules

Source	DF	Variance Ratio
Species	1	51.506
Acid Level	4	137.977
Phosphate Level	2	1.300
Add Trace Element	1	0.001
Species.Acid L	4	12.679
Species.Phosphate L	2	2.629
Acid L.Phosphate L	8	2.304
Species.Add TE	1	3.100
Acid L.Add TE	4	0.434
Phosphate L.Add TE	2	3.166
Species.Acid L.Phosphate L	8	0.760
Species.Acid L.Add TE	4	2.190
Species.Phosphate L.Add TE	8	0.999
Acid L.Phosphate L.Add TE	2	0.626
Residual Mean Square	346.5 (126 df)	

MEANS

Species		<u>Lotus</u>		Clover		SED
		33.0		53.2		2.81
Acid Level	3.5	4.0	4.5	5.0	5.5	SED
	1.0	6.9	55.1	71.3	81.1	4.45
Phosphate Level		0	15		30	SED
		40.0	43.8		45.5	3.45
Add Trace Element			+		-	SED
			43.1		43.0	2.81
Acid Level	3.5	4.0	4.5	5.0	5.5	MEAN
Species						SED
<u>Lotus</u>	2.0	7.3	45.3	54.2	56.2	33.0
<u>Clover</u>	0.0	6.6	64.9	88.4	106.1	53.2
MEAN	1.0	6.9	55.1	71.3	81.1	
SED				4.45		
SED Within Table				6.29		

Appendix III : D : Table 5

Statistical analysis relating to experiment 10

Variate: Number of Non Effective Nodules

Source	DF	Variance Ratio
Species	1	12.396
Acid Level	4	35.783
Phosphate Level	2	1.806
Add Trace Element	1	0.565
Species.Acid L	4	3.652
Species.Phosphate L	2	0.916
Acid L.Phosphate L	8	0.703
Species.Add TE	1	2.535
Acid L.Add TE	4	0.261
Phosphate L.Add TE	2	0.709
Species.Acid L.Phosphate L	8	0.774
Species.Acid L.Add TE	4	0.940
Species.Phosphate L.Add TE	2	1.175
Acid L.Phosphate L.Add TE	8	0.257
Residual Mean Square	908.8 (126 df)	

MEANS

Species		<u>Lotus</u>		Clover		SED
		45.5		29.6		4.49
Acid Level	3.5	4.0	4.5	5.0	5.5	SED
	0.5	9.2	57.1	59.4	61.6	7.11
Phosphate Level		0	15		30	SED
		34.9	34.2		43.6	5.50
Add Trace Element			+		-	SED
			35.9		39.2	4.49
Acid Level	3.5	4.0	4.5	5.0	5.5	MEAN
Species						SED
<u>Lotus</u>	1.1	14.0	74.9	77.7	59.6	45.5
Clover	0.0	4.3	39.3	41.1	63.6	29.6
MEAN	0.5	9.2	57.1	59.4	61.6	
SED				7.11		
SED Within Table				10.05		

Appendix III : D : Table 6

Statistical analysis relating to experiment 10
 Variate: Fresh Weight of Effective Nodules $\times 10^2$

Source of Variation	DF	Variance Ratio
Species	1	13.381
Acid Level	4	79.486
Phosphate Level	2	2.379
Add Trace Element	1	11.985
Species.Acid L	4	4.027
Species.Phosphate L	2	0.265
Acid L.Phosphate L	8	1.933
Species.Add TE	1	0.398
Acid L.Add TE	4	2.403
Phosphate L.Add TE	2	1.338
Species.Acid L.Phosphate L	8	1.532
Species.Acid L.Add TE	4	0.678
Species.Phosphate L.Add TE	2	0.759
Acid L.Phosphate L.Add TE	8	0.804

Residual Mean Square 0.0001480 (126 df)

MEANS

Species	<u>Lotus</u>					Clover	SED
	0.01819					0.02482	0.001813
Acid Level	3.5	4.0	4.5	5.0	5.5		SED
	0.00064	0.00317	0.03122	0.03797	0.03453		0.002867
Phosphate Level		0		15		30	SED
		0.01882		0.02352		0.02218	0.002221
Add Trace Element			+			-	SED
			0.02464			0.01837	0.001813
Acid Level	3.5	4.0	4.5	5.0	5.5	MEAN	SED
Species							
<u>Lotus</u>	0.00128	0.00328	0.02200	0.03272	0.03167	0.01819	
Clover	0.00000	0.00306	0.04044	0.04322	0.03739	0.02482	0.001813
MEAN	0.00064	0.00317	0.03122	0.03797	0.03453		
SED				0.002867			
SED Within Table				0.004055			

Appendix III : D : Table 7

Statistical analysis relating to experiment 10

Variate: Dry Weight of Non Effective Nodules

Source of Variation	DF	Variance Ratio
Species	1	0.000
Acid Level	4	22.859
Phosphate Level	2	6.444
Add Trace Element	1	1.159
Species.Acid L	4	2.615
Species.Phosphate L	2	1.583
Acid L.Phosphate L	8	0.471
Species.Add TE	1	1.117
Acid L.Add TE	4	0.157
Phosphate L.Add TE	2	0.663
Species.Acid L.Phosphate L	8	0.592
Species.Acid L.Add TE	4	0.162
Species.Phosphate L.Add TE	2	1.196
Acid L.Phosphate L.Add TE	8	0.265
Residual Mean Square	0.00005589 (126 df)	

MEANS

Species	<u>Lotus</u>		Clover			SED
	0.00781		0.00781			0.001114
Acid Level	3.5	4.0	4.5	5.0	5.5	SED
	0.00014	0.00261	0.01244	0.01250	0.0036	0.001762
Phosphate Level	0		15	30		SED
	0.00537		0.00780	0.01027		0.001365
Add Trace Element	+		-			SED
	0.00841		0.00721			0.01114
Acid Level	3.5	4.0	4.5	5.0	5.5	MEAN
Species						SED
Lotus	0.00028	0.00400	0.01300	0.01389	0.00789	0.0078
Clover	0.00000	0.00122	0.01189	0.01111	0.01483	0.00781
MEAN	0.00014	0.00261	0.01244	0.01250	0.01136	
SED	0.001762					
SED Within Table	0.002492					

Appendix III : D : Table 8

Statistical analysis relating to experiment 10

Variate: Acetylene Reduction

Source of Variation	DF	Variance Ratio
Species	1	62.784
Acid Level	4	117.108
Phosphate Level	2	0.596
Add Trace Element	1	7.007
Species.Acid L	4	10.477
Species.Phosphate L	2	4.191
Acid L.Phosphate L	8	2.340
Species.Add TE	1	2.287
Acid L.Add TE	4	2.132
Phosphate L.Add TE	2	1.018
Species.Acid L.Phosphate L	8	1.622
Species.Acid L.Add TE	4	0.908
Species.Phosphate L.Add TE	2	0.414
Acid L.Phosphate L.Add TE	8	0.385
Residual Mean Square	6.705 (126 df)	

MEANS

Species	<u>Lotus</u>		Clover		SED		
	3.64		6.70		0.386		
Acid Level	3.5	4.0	4.5	5.0	5.5	SED	
	0.03	0.41	6.72	8.66	10.04	0.610	
Phosphate Level		0	15		30	SED	
		4.99	5.06		5.47	0.473	
Add Trace Element		+		-		SED	
		5.68		4.66		0.386	
Acid Level	3.5	4.0	4.5	5.0	5.5	MEAN	SED
Species							
<u>Lotus</u>	0.07	0.31	4.5	6.60	7.30	3.64	
<u>Clover</u>	0.00	0.52	9.51	10.71	12.77	6.70	0.386
MEAN	0.03	0.41	6.72	8.66	10.04		
SED				0.610			
SED Within Table				0.863			

Appendix III : E : Table 1

Statistical analysis relating to experiment 12

Variate: Acetylene reduction by *L. uliginosus* cv. Maku. Cut oneSpecies: LOTUS

Source of Variation	DF	Variance Ratio
Days after Harvest	5	2.720
Light	1	69.699
Temperature	1	28.985
Days after Harvest.Light	5	2.898
Days after Harvest.Temperature	5	5.358
Light.Temperature	1	0.021
Residual Mean Square	0.2100 [93 (2) df]	

MEANS

Days after Harvest	1	3-4	6-7	10-11	17-18	20	SED
	0.580	0.771	0.908	1.397	1.959	2.208	0.162
Light		12/12		16/8			SED
		0.913		1.694			0.0936
Temperature		21/18		18/15			SED
		1.556		1.052			0.0936
Days after Harvest	1	3-4	6-7	10-11	17-18	20	SED
Temperature							
21/18	0.709	0.821	0.982	1.630	2.217	2.974	1.556
18/15	0.450	0.720	0.835	1.164	1.700	1.442	1.052
MEAN	0.580	0.771	0.980	1.397	1.959	2.208	
SED	0.162						
SED Within Table	0.2292						

Days after Harvest	1	3-4	6-7	10-11	17-18	20	MEAN	SED
Light								
12/12	0.437	0.586	0.531	0.885	1.319	1.721	0.913	0.0936
16/8	0.722	0.955	1.285	1.909	2.599	2.695	1.694	
MEAN	0.580	0.771	0.908	1.397	1.959	2.208		
SED	0.162							
SED Within Table	0.2292							

Appendix III : E : Table 2

Statistical analysis relating to experiment 12
 Variate: Acetylene reduction by T. repens cv. Huia. Cut one

Species: CLOVER

Source of Variation	DF	Variance Ratio
Days after Harvest	5	4.160
Light	1	2.554
Temperature	1	0.207
Days after Harvest.Light	5	0.146
Days after Harvest.Temperature	5	0.479
Light.Temperature	1	2.481
Residual Mean Square	1.279 [64 (1) df]	

MEANS

Days after Harvest	1	3-4	6-7	10-11	17-18	20	SED	
	0.65	0.83	1.00	2.01	2.38	3.38	0.482	
Light		12/12		16/8			SED	
		1.46		1.91			0.267	
Temperature		21/18		18/15			SED	
		1.77		1.64			0.267	
Days after Harvest	1	3-4	6-7	10-11	17-18	20	MEAN	SED
Temperature								
21/18	0.57	0.75	0.95	1.93	2.60	3.80	1.77	
18/15	0.74	0.92	1.07	2.12	2.11	2.87	1.64	0.267
MEAN	0.65	0.83	1.00	2.01	2.38	3.38		
SED	0.482							
SED Within Table	0.800							

Days after Harvest	1	3-4	6-7	10-11	17-18	20	MEAN	SED
Light								
12/12	0.66	0.63	0.75	1.67	2.04	3.02	1.46	
16/8	1.63	0.99	1.21	2.30	2.66	3.68	1.91	0.267
MEAN	0.65	0.83	1.00	2.01	2.38	3.38		
SED	0.482							
SED Within Table	0.730							
*Number of missing values								

Appendix III : E : Table 3

Statistical analysis relating to experiment 12

Variate: Acetylene reduction by *L. uliginosus* cv. Maku. Cut TwoSpecies: LOTUS

Source of Variation	DF	Variance Ratio
Days after Harvest	6	1.935
Light	1	41.912
Temperature	1	35.041
Days after Harvest.Light	6	0.526
Days after Harvest.Temperature	6	0.969
Light.Temperature	1	6.794
Residual Mean Square	0.7193 [108 (3) df]	

MEANS

Days after Harvest	1	3-4	6-7	10-11	14	17-18	20	SED
	1.461	1.461	1.534	1.811	2.214	2.592	2.861	0.2999

Light	12/12	16/8	SED
	1.473	2.510	0.1603

Temperature	21/18	18/15	SED
	2.466	1.517	0.1603

Days after Harvest	1	3-4	6-7	10-11	14	17-18	20	MEAN	SED
Light									
21/18	1.691	1.704	1.874	2.357	2.746	3.310	3.579	2.466	0.1603
18/15	1.230	1.231	1.195	1.264	1.682	1.870	2.144	1.517	
MEAN	1.461	1.467	1.534	1.811	2.214	2.592	2.861		

SED 0.2999

SED Within Table 0.4241

Days after Harvest	1	3-4	6-7	10-11	14	17-18	20	MEAN	SED
Light									
12/12	1.026	1.031	1.184	1.394	1.660	1.932	3.579	1.473	0.1603
16/8	1.895	1.904	1.885	2.227	2.769	3.251	2.144	2.510	
MEAN	1.461	1.461	1.534	1.811	2.214	2.592	2.861		

SED 0.2999

SED Within Table 0.4241

Light	12/12	16/8	MEAN	SED
Temperature				
21/18	1.738	3.194	2.466	0.1603
18/15	1.207	1.827	1.517	
MEAN	1.473	2.510		

SED 0.1603

SED Within Table 0.2267

Appendix III : E : Table 5

Statistical analysis relating to experiment 12

Variate: Acetylene reduction by L. uliginosus cv. Maku. Cut threeSpecies: LOTUS

Source of Variation	DF	Variance Ratio
Days after Harvest	5	0.783
Light	1	28.676
Temperature	1	22.311
Days after Harvest.Light	5	0.439
Days after Harvest.Temperature	5	0.471
Light.Temperature	1	1.936
Residual Mean Square	1.179 [88 (7) df]	

MEANS

Days after Harvest	1	3-4	6-7	10-11	14	17-18	SED	
	1.778	1.913	2.017	2.269	2.759	3.151	0.3839	
Light		12/12		16/8			SED	
		1.721		2.908			0.2216	
Temperature		21/18		18/15			SED	
		2.838		1.791			0.2216	
Days after Harvest	1	3-4	6-7	10-11	14	17-18	MEAN	SED
Temperature								
21/18	2.050	2.295	2.529	2.784	3.436	3.932	2.838	0.2216
18/15	1.505	1.530	1.506	1.754	2.082	2.369	1.791	
MEAN	1.778	1.913	2.017	2.269	2.759	3.151		
SED	0.3839							
SED Within Table	0.5429							

Days after Harvest	1	3-4	6-7	10-11	14	17-18	MEAN	SED
Light								
12/12	1.345	1.442	1.464	1.785	1.868	2.422	1.721	0.2216
16/8	2.210	2.384	2.571	2.752	3.651	3.829	2.908	
MEAN	1.778	1.913	2.017	2.269	2.759	3.151		
SED	0.3839							
SED Within Table	0.5429							

Appendix III : E : Table 6

Statistical analysis relating to experiment 12

Variate: Acetylene reduction by *T. repens* cv. Huia. Cut Three

Species: CLOVER

Source of Variation	DF	Variance Ratio
Days after Harvest	5	0.962
Light	1	4.832
Temperature	1	3.777
Days after Harvest.Light	5	0.003
Days after Harvest.Temperature	5	0.075
Light.Temperature	1	3.514
Residual Mean Square	2.993 [64 (1) df]	

MEANS

Days after Harvest

Light	12/12	16/8	SED
	1.54	2.48	0.408
Temperature	21/18	18/15	SED
	2.43	1.60	0.409

Days after Harvest	1	3-4	6-7	10-11	14	17-18	MEAN	SED
Temperature								
21/18	1.13	1.62	2.23	2.71	3.19	3.71	2.43	0.409
18/15	0.74	0.99	1.12	1.85	2.33	2.56	1.60	
MEAN	0.95	1.33	1.72	2.32	2.80	3.19		
SED	0.738							
SED Within Table	1.001							

Days after Harvest	1	3-4	6-7	10-11	14	17-18	MEAN	SED
Light								
12/12	0.64	0.93	1.25	1.68	2.12	2.61	1.54	0.408
16/8	1.20	1.67	2.12	2.85	3.36	3.67	2.48	
MEAN	0.95	1.33	1.72	2.32	2.80	3.19		
SED	0.738							
SED Within Table	1.117							

Light	12/12	16/8	MEAN	SED
Temperature				
21/18	1.56	3.22	2.43	0.409
18/15	1.62	1.66	1.60	
MEAN	1.54	2.48		
SED	0.408			
SED Within Table	0.500			

Appendix III : E : Table 7

Statistical analysis relating to experiment 12
 Variate: L. uliginosus cv. Maku dry weight of stem

Species: LOTUS

Source of Variation	DF	Variance Ratio
Light	1	11.960
Temperature	1	11.398
Harvest	2	9.213
Light.Temperature	1	1.329
Light.Harvest	2	0.558
Temperature.Harvest	2	1.535
Residual Mean Square	0.01106 [39 (2) df]	

MEANS

Light	12/12	16/8	SED	
	0.270	0.375	0.0304	
Temperature	21/8	18/15	SED	
	0.374	0.272	0.0304	
Harvest	1	2	3	SED
	0.415	0.274	0.280	0.0372

Appendix III : E : Table 8

Statistical analysis relating to experiment 12
 Variate: T. repens cv. Huia dry weight of stem

Species: CLOVER

Source of Variation	DF	Variance Ratio
Light	1	0.717
Temperature	1	0.367
Harvest	2	7.512
Light.Temperature	1	0.002
Light.Harvest	2	0.013
Temperature.Harvest	2	0.179
Residual Mean Square	0.04991 [24 (2) df]	

MEANS

Light	12/12	16/8	SED	
	0.373	0.439	0.0745	
Temperature	21/18	18/15	SED	
	0.436	0.387	0.0758	
Harvest	1	2	3	SED
	0.617	0.346	0.265	0.0953

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