THE NITROGEN ECONOMY OF PERENNIAL RYEGRASS - WHITE CLOVER ASSOCIATIONS

By

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THESIS SUMMARY

An experiment was conducted over the period 1963-65 with New Zealand cultivars of *Lolium perenne* (L) and *Trifolium repens* (L). They were sown in alternate rows, six inches apart and half the plots in the trial area were established to maintain root segregation between the species using a double layer of 500 gauge black polythene. Liberal quantities of phosphate and potash fertilisers were applied each year but the grass-legume association relied upon soil mineralisation, fixation by free-living organisms, rainfall and symbiotic fixation for its nitrogen supply.

Variations in defoliation frequency of two, four and six cuts per annum had little effect on the overall dry matter yield which amounted to 5,300, 6,100 and 6,000 pounds per acre per annum respectively. However, the average yearly production of nitrogen during the experimental period was 112, 166 and 217 pounds of nitrogen per acre which suggested a 48% increase by doubling the cutting frequency and a 98% increase when three times the number of defoliations were employed.

Root segregation of perennial ryegrass and white clover when grown in close association reduced the dry matter yield by 18% in the establishment year and by 6% in 1964 and it is suggested that

root check and root restriction, particularly in respect of the grass component, were mainly responsible. In 1965 the dry matter yield was 26% lower where root barriers had been introduced and from above ground appearance of the grass and from the yield data this was clearly the direct effect of eliminating underground nitrogen transference from the clover.

The nitrogen economy of this grass-legume association has been studied over a three year period and only in the final year was it possible to demonstrate above ground the results of underground nitrogen transfer. Clover contributed 30.79 pounds of nitrogen to its grass partner in 1965 and this figure is compared with predicted values using the theory of Walker, Orchiston and Adams and also data computed from supplementary grassland observation plots.

Micro-climatic temperatures recorded at ground level partially corroborate the findings of Johnstone-Wallace who showed lower diurnal fluctuations of temperature with a grass and clover sward compared with grass alone.

Seed of the same New Zealand cultivar of Trifolium repens (L) was inoculated with an effective strain of rhizobium (R.157 originating from Sydney, Australia) and compared with a non-inoculated control. From the limited results of this trial and from field observations it would appear that the indigenous strain of rhizobium at Auchincruive was an effective one.

The physical effects of the black polythene used for root segregation were examined through yield data in a special trial and laboratory and field tests were carried out on the permeability of this material as used in the experiments.

Dry matter production, nitrogen yields and herbage quality from the perennial ryegrass-white clover association are reported, discussed and compared with data from New Zealand and Holland.

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THE NITROGEN ECONOMY OF PERENNIAL RYEGRASS - WHITE CLOVER ASSOCIATIONS:-

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THE NITROGEN ECONOMY OF PERENNIAL RYEGRASS - WHITE CLOVER ASSOCIATIONS

1. SECTION I. INTRODUCTION

1.1 HISTORIAL INTRODUCTION

The value of legumes in agriculture has long been appreciated and Fred, Baldwin and McCoy (1932) have given us a brief historical outline of this subject. Their value as crops was well known to the Egyptians six thousand years ago (Hartman, 1923). The philosopher Theophrastus (370-285 B.C.) in his "Enquiry into Plants", spoke of their "reinvigorating" properties. The practice of growing mixed crops in agriculture is widespread throughout the world according to Nicol (1935), and many of these crop combinations are associations of legume and non-legume. Carrier (1923) in his book "The Beginnings of Agriculture in America", reproduces a picture (ascribed to Le Moyne, 1564) showing Indians planting corn and beans in the same field. Smith (1907) writing about the Indians and maize planting in Virginia indicated the sowing method to be four cereal grains and two beans in the same hole or "hill". Mollison (1901) in his treatise on agriculture in the Province of Bombay cites many other examples of legume/non-legume associations in crop production. The reasons for such crop combinations in primitive agriculture appear to be one or more of the following factors. Economical land use, a form of crop rotation, a method of obtaining some measure of weed control, the convenience of having the crops together and finally the suitability of one crop with another.

In early British agriculture associations of legume and non-legume appear from the literature to be rare and combinations of this kind were confined to mashlum, oats and beans or oats and tares. Here convenience and insurance against a single crop failure are the two principal reasons for such practices. This position was soon to be reversed following the introduction into Britain of the rotational grasses and clover during the early part of the 17th century, Weston being accredited with the introduction of red clover (Davies 1952). From then onwards came the development of ley farming using grass together with both white and red clover. Lisle (1713) writing at the beginning of the 18th century states that wild white clover was being sown in Hampshire and its value was well recognised in the County. However the true worth of this plant was not widely appreciated and recognised until this century according to Davies (1952). Warlidge (1668) in the mid-17th century refers to the use of lucerne and sainfoin and North a century later talks of sainfoin growing in long leys (Davies, 1952). During this period ryegrass or "ray-grass" as it was termed, the first of the rotational grasses, was being sown mixed with white clover and "non-such clover" (Medicago lupulina) for leys of six to ten years duration.

From the middle of the 18th century onwards the value of the ley became increasingly evident. This association of rotational grass and clover with its effect on general soil fertility when land was brought again into cereal or other crop production was slowly recognised. North in his "Account of the Different kinds of grasses propagated in England for the Improvement of Corn and Pasture land" (1759) and William Ellis writing in 1774 on the value of "resting the ground with some grasses" are two of the earliest in a spate of British authors on this topic (Davies 1952).

But in spite of the numerous writers on the benefits of white clover in the 17th, 18th and 19th centuries it was not universally acclaimed by the British farming community until the 20th century. Davis and Cooper (1951) pointed this out in 1951 when they classed "the appreciation of strain differences, selection and introduction of vigorous types of white clover" as one of the oustanding features of 20th century farming. Gilchrist in England (1909) and Findlay (1918) and Cruickshank (1936) in Scotland had pioneered the way for the extensive use of this legume and the Welsh Plant Breeding Station gave British Agriculture the right cultivars.

Similar appreciations were being made in other parts of the world. Bordley (1801) writing about New England made the following statements:- "Clover plowed in, together with the remains of grain stubble, year after year will gradually meliorate the soil." and "Clover is the best preparative for a crop of wheat ..... wheat on clover has the best grain and fullest crop."

On the European continent Thaer (1856) was advocating almost dictatorially the extensive use of the Leguminosae, his recommended rotation being:- Beans, Autumn corn, Clover for mowing, Spring grains, Peas, Autumn grains and Pasturage with white clover and grasses. Later in the 19th century Schultz-Lupitz, a fellow countryman of Thaer's, reinforced his early recommendations and he persuaded the

German farmers to grow lupins extensively on the light soils of their country. In 1881 he stated in a paper that such plants as clovers, lupins and peas are able to utilise nitrogen in some form other than that required by non-leguminous plants - calling legumes the accumulators and non-legumes the consumers.

The value which farmers and agriculturists put on the members of the Leguminosae in respect of their soil improving properties was not explained by scientists until the 19th century. Several distinguished scientists were enquiring into the source of N available to green plants and it was Boussingault (1838) who showed and eventually appreciated a difference between wheat on one hand and peas and clover on the other in their N uptake. He concluded that the source of N must have been the atmospheric ammonia and several eminent workers of the day supported this view (Liebig 1852 : Sachs 1860-61 : Schloesing 1874 and Mayer 1874). Berthelot (1885) reported a direct fixation of atmospheric N by clays soils and it was suggested that bacteria might be concerned with this phenomena. Bacteria had been "associated" with the nitrification process a few years earlier by Schloesing and Müntz (1877 and 1878) and Warington (1878, 1879 and 1884). During the period 1886 to 1889 Hellriegel working alone and then aided by Wilforth, produced his classical papers which put an end to all speculations on this subject. Briefly his findings were as follows:-

(1) Leguminoseae and Gramineae are fundamentally different in the way they absorb nitrogen for their nutrition.

(2) The Gramineae depend solely on assimilable nitrogen compounds in the soil and their subsequent growth and development is directly related to the amount of nitrogen available.

(3) A second source of nitrogen, other than the soil N is available to the Leguminoseae (i.e. the free N from the air).

(4) The legumes themselves do not possess the ability to assimilate free atmospheric N but require the active participation of micro-organisms in the soil.

(5) For the assimilation to work the micro-organisms from the soil have to enter into a symbiotic relationship with the legumes.

(6) The root nodules of the legumes are responsible for the assimilation of the free nitrogen.

Confirmation of these findings followed a few years later via the work of Lawes and Gilbert (1889 and 1891) in England, Atwater and Woods in America (1890, 1891 and 1892), Schloesing and Laurent (1892) in France also Alpe and Menozzi (1892) in Italy.

As a result of Hellriegel's finding and their confirmation, botanists, chemists, bacteriologists, biochemists and agronomists throughout the world have been working on every conceivable aspect associated with legume symbiosis. Thus in a brief historical account it is necessary to concentrate on those scientific aspects which are more relevant to the practical application and exploitation of this phenomena.

1.2 20th CENTURY BACKGROUND

A quarter of a century ago Virtanen and Hausen (1937) in their classical series of experiments showed a marked beneficial effect to Graminaceous plants when associated with legumes. They were able to show a consistent nitrogen [N] excretion by the legume and a subsequent increase in the uptake of N by the non-legume, the amount of N excreted on occasions equalling half the total amount fixed. In a further series of investigation Virtanen and Laine (1939), & Virtanen, Linkola, Hakala and Rantanen (1946) showed by means of Rhizobium-inoculated pea plants under sterile conditions, that nitrogenous compounds were excreted into the root medium in quantities which were sometimes as large as the amount of N assimilated by the plants. The excretion products under these circumstances were identified as L-aspartic acid, β-alanine, glutamic acid and ammonia and the concentration of aspartic acid was much in excess of the other products. Many other workers concentrating on similar lines were not able to substantiate these findings (Trumble and Strong, 1937; Trumble and Shapter, 1937; Bond and Boyes, 1939; Wilson, 1939; Wyss and Wilson, 1941; Ludwig and Allison, 1940; Myers, 1945 and Butler and Bathurst, 1956). Of the papers quoted, the contribution against the "Virtanen" school of thought from Trumble and Strong is particularly relevant to this thesis and their conclusions were briefly as follows:-

(1) Early and marked transfer of N from a growing legume to an associated grass is hypothetical.

(2) In pot cultures no evidence was obtained to show that grasses were capable of deriving N from associated legumes during the vegetative stages of the latter when grown during a winter period in a semi-arid climate.

(3) N is available, however, as a result of nodule break-down and root decomposition during senescence.

(4) N transference from perennial legumes to associated non-legumes has been shown to take place 12 weeks after seeding, but the amount is relatively small. This evidence was obtained with artificial watering in spring and early summer.

(5) Factors which determine whether or not a release of N and subsequent transfer takes place were discussed - species, strain, rhizobial strain and external environment including legume water supply were considered important.

(6) Under South Australian conditions there was no foundation for the belief that N could be transferred from clover to grass over the same growth interval, but previous growth and N accumulation is however likely to be of great importance.

Trumble and Shapter (1937) showed that at the end of the growing season of an annual legume, nitrogen was available in the root medium and a perennial grass may derive significant quantities of this N.

Using Medicago denticulata (burr trefoil) and Phalaris tuberosa they were able to obtain 30% of the N present in the root medium in a period of 8 weeks after the harvest of the legume. They also showed that an annual legume like Trifolium subterraneum (subterranean clover) would effect a greater soil N enrichment than burr trefoil on account of its greater growth capacity, extended growth period and a larger proportion of total N present in its root system. Butler and Bathhurst (1956) enumerate and discuss five factors concerned with N transfer, i.e. rate of N fixation, carbohydrate status, soluble nitrogenous constituents of the nodules, nitrogen content of the root and finally the root environment itself. They conclude that since the excretion of N is dependent on these five "extraordinarily specific set of circumstances" and that they have to be favourable simultaneously, it is not surprising to find so many workers unable to verify the "Virtanen School" findings. They were unable to produce the same meteorological conditions, thus affecting photosynthetic rate, N fixation and carbohydrate status to name some of the variables.

In respect of root environment it has been stated that the rate of secretion seemed to be higher, the higher the ability of the solid material to absorb the excreted compounds from the root medium, in other words concentration gradients favourable to excretion have to be maintained (Butler and Bathhurst 1956). It is significant that the soil micro-flora and grass roots in a grass-clover association can be considered an aid to the maintenance of this gradient.

1.3 RHIZOBIA, NODULATION, AND NUTRITION

1.31 RHIZOBIA

The secretions which take place from the legume root are responsible for a multiplication of the rhizobia in the root medium (West, 1939), these in turn are said to produce I.A.A. (indole-acetic acid) from the tryptophan (Kelford, Brockwell and Zwar 1960), which causes curling of the root hairs of the legume resulting in a condition for entry by the bacteria. Bieberdorf (1938) and Fred, Baldwin and McCoy (1932) suggest the root hairs as a point of entry: Bieberdorf (1938) and McCoy (1929) include also broken epidermal and cortical cells and Allen and Allen (1940), Arora (1954) and McCoy (1929) suggest the ruptured tissue at the site of rootlet emergence. During the symbiosis which follows a "tumour-inducing-principle" (T.I.P.) is thought to operate (Klein, D.T. and Klein, R.M., 1953), and a metamorphosis of cortical root tissue occurs giving rise to a nodule (Ward, 1887 and Bond, 1948). This is bacteria-filled tissue which lives on the plant and together they assimilate atmospheric nitrogen.

The rhizobia are gram negative, aerobic, heterotrophic rods 0.5-0.9 x 1.2-3.0 micra (Allen, E.K. and Allen, O.A., 1940). There are six species, R. meliloti, R. leguminosarum, R. phaseoli, R. japonicum, R. lupini and R. trifolii, the last named being of concern in this thesis. It is thought that R. trifolii embraces several strains some effective in nodulation others not. Chen and Thornton (1940) growing red clover plants in agar demonstrated this, showing the initiation and final decay in 7 and 15 days with ineffective strains and in 4 and 8 weeks respectively for the effective ones. With ineffective strains nitrogen fixation and the amount of nodule tissue formed is small and senescence begins very early.

1.32 Nodulation

The subject of optimum conditions for the entry of the bacteria, their multiplication and resultant symbiosis via the nodulation is a complex one. However in general an environment which is suitable for good clover establishment and growth is required e.g. high pH, sufficient quantities of available Ca and other inorganic nutrients including the trace elements, with the probable exception of high levels of N in soil and plant. Perkins (1924), Chailakhian and Megrabian (1945), Thornton (1935) and Purchase and Nutman (1957) have shown that nodule number is asymptotically related to bacterial density and the number of rhizobia present in the rhizosphere greatly exceeds that required for nodulation in the case of clover and lucerne. Their experimental results can be fitted to a compound Mitscherlich exponential.

Nutman (1958) has suggested that the clover host plant also governs the amount of nodulation and that it does so independently of the bacteria. This is likely to be at the higher soil levels of rhizobia. Also poor root development in the host is responsible for sparse nodulation. Whilst a much branched root system can acquire high nodule numbers. A self-regulating mechanism in the host plant has been suggested by Nutman (1958) which controls infection rate and nodule size - thus controlling the total nodule volume available for symbiotic fixation. Pate (1958) has shown nodule formation to be synchronised with leaf production and N accumulation thus corroborating Nutman's findings.

Nodulation is reported to be poor in seasons characterized by short days or low light intensity and similar conditions to these are said to operate in shade or where pastures of grasses and clovers are lightly grazed (Whyte, Nilsson-Leissner and Trumble, 1953). Both the infection of the clovers by the bacteria and the effectiveness of the resulting nodulation in terms of 'N' fixation improve usually as the source of light increases. Also the nitrogen-carbohydrate balance in the host plant appears important within limits; very high and very low ratios may reduce 'N' fixation. Thus as the rate of carbon assimilation is a critical factor so in turn do light and temperature have a critical part to play in the growth and nitrogen content of legumes (Whyte et al 1953).

1.33 Nutrient levels and their effect on host and bacteria

Hewitt (1958) suggests that at least seventeen mineral elements are thought to be involved in the nutrition of some higher plants or micro-organisms. He goes on to say that from evidence available there is no indication that the symbiotic relationship requires any other element which is not normally needed for the higher plants in general. Vanadium and tungsten, not normally associated with the growth of higher plants are required by micro-organisms and may influence nitrogen fixation. Of the trace elements, iron, cobalt and molybdenum are probably the most important.

Iron is required for healthy growth of symbiotic nitrogen-fixing systems and it has been established that more of this element is required when the symbiosis is entirely dependent on free nitrogen (Stewart, 1966). It is involved in enzyme functions, nitrate reduction, haemoglobin synthesis and possibly connected with nitrogen fixation and the young nodules are thought to act as an iron reservoir for the synthesis of haemoglobin.

Cobalt is essential for growth in legumes and rhizobia (Stewart, 1966). It is necessary for the synthesis of vitamin B_{12} and in nodulated legumes an increase in nitrogen fixation is associated with an increase in the vitamin B_{12} and haemoglobin content of nodules. There also appears to be connection between cobalt and the vitamin B_{12} co-enzyme which is found in the nodules (Kleiwer and Evans 1963).

Molybdenum is another element essential for healthy growth of legumes and since higher concentrations are required when free nitrogen is the sole source of nitrogen it has been suggested that the additional requirement is associated with fixation (Hewitt 1959). Crops like clover, which have small seeds, rely on soil molybdenum for their nutrition because the seed reserves are inadequate compared with large seeded legumes where the seed supply is sufficient for the healthy growth of two generations (Hewitt, 1958; Hewitt, Bolle-Jones and Miles, 1954; and Hewitt and Miles, 1952). Molybdenum-deficient plants have small nodules, very widely scattered over the root system rather than a few large ones closely congregating on the roots of plants well supplied with molybdenum. It is needed for nitrate reduction in plants and where deficiency occurs, much of the molybdenum present accumulates in the nodule and particularly in the bacteriod tissue (Jensen, 1946, and 1947; Jensen and Betty, 1943; Mulder, 1948; Hewitt, 1948). It may be significant that molybdenum is used as a catalyst in commercial chemical nitrogen fixation (Hewitt, 1948).

Anderson (1949) has reviewed the adverse effects of nutrient deficiency on symbiotic nitrogen fixation relative to the following four points:-

(a) growth restriction of the host plant

(b) development of unfavourable conditions in the host plant

(c) inhibition of the N-fixing reactions

(d) restriction of the development of rhizobium species in the soil.

There is still a great void in our knowledge on the subject but it can be said that for good clover growth and nitrogen fixation fairly large quantities of lime, phosphate and potash should be present with smaller quantities at hand of the other elements required for growth. The one element which does give concern is nitrogen itself.

Gïobel (1926) showed that nitrogen fixation proceeded best in plants which were well supplied with combined nitrogen during the early stages of growth. This added nitrogen is said to be required during the period when the nitrogen supply from the seed has been exhausted and there is still no symbiotic nitrogen available. This level of combined nitrogen is almost impossible to assess quantitatively. It will vary between species and is certain to be fairly small as there is plenty of evidence of the deleterious effect of large quantities of combined nitrogen both on clover growth and on nodulation. Thornton's work is interesting in this connection. Using N^{15} and experimenting with nodulated soy beans he showed that this crop depends on fixation for half of its nitrogen and that the amount assimilated could be related inversely with the amount of available combined nitrogen in the soil. In respect of clovers, Dutch white clover showed an adverse effect from applied N whereas red clover showed a response in growth rate.

1.4 MODE OF N TRANSFERENCE

When grass and clover are grown together there is a transference of N from the legume to the associated grass. Five ways in which this transference can come about have been suggested by Butler and Bathhurst (1956) namely:-

(1) By the excretion of nitrogenous compound by the growing legume.

(2) In the release of nitrogenous substances in root decay.

(3) From nitrogenous substances released when nodules disintegrate.

(4) Leaching of nitrogen compounds from the leaves of the legume.

(5) Release of nitrogen from fallen leaves and petioles.

The five known factors which are likely to affect No. 1 have already been listed and perhaps the most important of these is the rate of N-fixation in relation to the amount of N actually required by the legume itself - its own requirements must first be met. Also the rate of photosynthesis must be sufficiently large to ensure a good rate of N-fixation; but it must not be excessive since surplus carbohydrate in the plant immediately 'ties-up' the N as it is fixed.

Butler and Bathhurst (1956) have demonstrated that white clover roots contain on average 1.5% N and thus in root decay there is likely to be a small amount of nitrogen released, this had been suggested earlier by Lyon and Bizzell (1911). The total nitrogen content of white clover nodules taken from the field has averaged 6.3% of the dry weight and there has been little variation with nodule size

(Wilson, 1942). If the carbohydrate supply to the nodule is restricted N-fixation ceases resulting in senescence and sloughing off. Many factors can cause the nodules to part from the host plant, these include plant maturity (seeding or fruiting), extremes of temperature or soil moisture (Wilson, 1931), plant defoliation (Wilson, 1942) and pronounced plant shading (Strong and Trumble, 1939). All these factors are responsible for curtailing the carbohydrate supply thus accelerating the nodule drop. These factors are likely to operate continually in the field and thus it would seem that this nodule drop and resultant N release could be responsible for a large proportion of the total amount of N which is transferred. Young (1958) working on a perennial rye-grass - white clover sward showed that where nitrogen had been given to the sward 16% and 33% of the nodules were disintegrated in June and September respectively. Disintegrating nodules were found at all times during the season and recoverable decaying nodules amounted to 12½-17% of all nodules in absence of nitrogen applications. Leaching of nitrogen from the leaves and the amount released from fallen leaves and petioles are likely to be relatively small.

1.5 AMOUNT OF NITROGEN FIXED AND AMOUNT TRANSFERRED

1.51 Positive experimental evidence

The total amount of plant nitrogen in nodulated legumes is made up of two parts, namely that assimilated through the roots from the soil and the other fraction which is the symbiotically fixed free N from the air.

Wahhab and Muhammad (1954) using a range of leguminous plants estimated that 43-50% of the nitrogen taken up by plants on soils rich in combined N was from the air, whereas on soils poor in combined N, it was estimated that 53-70% of the total plant nitrogen was considered to be fixed.

Hopkins (1902) working both in greenhouses and in the field showed that with properly inoculated legumes two thirds of the plant N is obtained from the air and one third from the soil.

Nowotny-Mieczynska and Russkowska (1954) working with lucerne reported that 50% of the nitrogen was symbiotically fixed.

The level of N-fixation will vary between species and in support of this Erdman (1959) collecting data from various sources related to American conditions listed the average fixation of N in lb per acre by several different legumes as being:- Alfalfa 194, Ladino clover 179, Sweetclover 119, Alsike clover 119, Red clover 114, legumes in pasture 106, White clover 103, Crimson clover 94, Vetch 80, Peas 72, Soybeans 58 and Beans 40.

Further American data in respect of biennial and perennial legumes estimates that N-fixation over several million acres ranges from 30-150 lb of N per acre (Lipman and Conybeare, 1936), and experiments over 10 years conducted by Lyon and Bizzell (1934) on lucerne showed an annual net gain (compared with cereals) of 251 lb of N via symbiotic fixation. Also work by Wagner (1954) using Ladino white clover and tall fescue indicates that the amount of N fixed by the clover in the mixed sward to be equivalent to 169 lb of N/acre/annum.

In the North Island of New Zealand, where clover growth is active for 9-10 months in the year, Sears (1950) has obtained figures for N fixation of approximately 500 lb per acre per year.

Russel (1950) quotes figures of 100-200 lb of nitrogen fixed and harvested by some legumes and Yankovitch (1940) working with annual legumes such as beans and lentils puts the figure at about 450 lb of N per acre.

Under field conditions in Sweden, Bjälfe (1955) has estimated the maximum amount of fixation to be in the region of 267-357 lb N/acre/annum for clover and lucerne and 134-178 lb N/acre/annum for peas and vetches.

In Finland, Virtanen (1956) suggests 178-267 lb of N as being the amount fixed by a good red clover sward and with peas a figure of approximately 90 lb N. He goes on further to suggest that where the summer is longer than in Finland and the weather favourable, 357-446 lb of N should be fixed by red clover and even higher figures for blue lucerne.

Chapman, Liebig and Rayner (1949), working with purple vetch and sweet clover as winter cover crops in California, indicate a N fixation level of around 150 lb per acre and Karraker, Bortner and Fergus (1950) in Kentucky suggest the following:- white clover 148 lb, red clover 171 lb, lespedesa 206 lb and lucerne 225 lb of nitrogen per acre per annum.

In regard to N transference, Allen and Allen (1958) and Walker, Orchiston and Adams (1954) both emphasise in their reviews the fact that not all experiments conducted to assess N transference from legume to non-legume have shown any benefit. Wilson (1939) in his book depicts timothy/alsike clover, Canadian bluegrass/white clover and Kentucky bluegrass/white clover combinations quite depressed in their growth compared with the single species. Russel (1950) using oats and vetches and Trumble and Strong (1954) experimenting with lucerne/subterranean clover/perennial ryegrass and with lucerne/subterranean clover/phalaris tuberosa mixtures both were unable to show clear cut evidence of N benefit to the non-legume. Strong and Trumble (1939) showed from their pot experiments with oats and inoculated peas, grown together in pure sand, that by reducing the day length they could obtain a significantly higher nitrogen content in the cereal component. Some exacting conditions may thus be required before the transfer of nitrogen from legume to non-legume takes place. It would appear therefore that the potential for benefiting the non-legume is present under most circumstances but the physical conditions of the environment are sometimes acting as a screen. Butler and Bathhurst (1956) suggest that the stimulation to the growth of associated grasses by clover can be observed six months after sowing, which suggests that this benefit occurs before root decay is likely.

Johnstone-Wallace (1937) reporting on the growth of grasses alone and in conjunction with wild white clover at Cornell where lime, phosphate and potash had been used as a basal fertiliser dressing showed that perennial ryegrass grown alone yielded 1,678 lb DM per acre with a protein content of 22.8%. In association with clover the total yield went up to 3,360 lbs. DM per acre and the protein content increased to 30.2%. This higher protein content was due partly to the presence of clover itself which has a naturally higher nitrogen content than grass and at the same time the protein level in the grass, grown in association, was also higher. Johnstone-Wallace ascribes the higher protein content in the grass to the N transferred by the clover and to more favourable growth conditions recorded where the two were grown together (increase in the water absorption and smaller diurnal fluctuations in temperature viz. 47-68°F compared with 40-73°F when grass was grown alone).

Several workers have grown grasses with and without clover and assessed the benefit of the association and a summary of their findings appear in Table A.

Other workers have evaluated this N-transference from clover to associated grass in terms of the level of combined fertilizer nitrogen which is required by grass alone to give the same production.

TABLE A. The value of clover in mixed stands

Author and country	Grass species used	Clover species used	Average increased D.M. production from the mixture lb D.M. per acre/annum c.f. grass alone	Total increased N production from the mixture lb N per acre/annum
Johnstone-Wallace 1937 (b) U.S.A.	Lolium perenne	Trifolium repens wild	1682	101
" 1937 (a) U.S.A.	Poa pratensis	Trifolium repens wild	-	68*
Melville and Sears 1953 New Zealand	Mixed grasses	(Trifolium repens and (" pratense mixed	-	40 1st harvest year * 150 3rd " " *
Sears, Lambert and Thurston 1953 New Zealand	Mixed grasses	Trifolium repens	-	226 (55 additional in grass)
" " " " " "	Mixed grasses	Trifolium pratense	-	219 (23 additional in grass)
Cowling 1961 England	Dactylis glomerata	Trifolium repens	-	60 (106 max.) *
Holmes and Maclusky 1955 Scotland	Various	Trifolium repens	2450	91
Herriot and Wells 1955-57 Scotland	Dactylis glomerata	Trifolium repens ‡	4090	113
" " " " "	Lolium perenne	Trifolium repens ‡	4688	153
Wagner 1954 (a) and (b) U.S.A.	Dactylis glomerata	Ladino clover	5473	148
" " " " "	Tall Fescue	Ladino clover	4566	169

* Benefit here is reckoned as additional lb of N in the grass

‡ Mean values for the 3 years given for the 1 lb/acre clover seeding rate.

TABLE B

Amount of nitrogen fertilizer required (lb/acre N) by grass alone to replace the gross effect of clover

Author	Associated Grass	Equity on D.M. basis	Equity on N yield basis
Wilman 1959	Ryegrass (3 years)	191	260-278
Holmes and MacLusky 1955	Average of 12 grasses (5 years)	121	212
Cowling and Green 1956	Cocksfoot (Short periods)	162	238
Wagner 1954 (a) and (b)	Cocksfoot (2 years) Tall Fescue (2 years)	240+ 160+	Approx. 200 160+

Under New Zealand conditions Sears (1953) showed a 500% increase in the total amount of herbage on the inclusion of white clover to seeds mixtures and under the circumstances quoted the yield of the grass component had more than doubled. Reporting on trials at two centres he estimated that a total of 230 lb and 500 lb of nitrogen were fixed per acre per annum of which 55 lb and 140 lb respectively were transferred to grass.

Cowling and Green (1954) working with a cocksfoot and white clover sward, which contained 30-40% of clover, reported a yield of 170 lb of nitrogen per acre and they were able to show that this was 30 lb higher than a no-clover sward receiving 157.5 lb of fertiliser nitrogen. Of the 170 lb of nitrogen in a mixed sward 120 lb were supplied by the clover component : 75 lb being contributed directly and 45 lb indirectly.

1.52 Non-confirmatory evidence

So far the evidence has been presented for nitrogen fixation, nitrogen "exudation" and nitrogen transfer from legume to non-legume grown in association.

Working with soy beans grown in originally sterilised sand cultures Bond (1938) however revealed no evidence of any appreciable amounts of nitrogenous excretions in the root medium, neither was there any evidence to show that barley when introduced into the pots showed any uptake of nitrogen.

Bjälfve (1940) in Sweden, working with peas in association with potatoes or maize grown in pot experiments concluded that there were no special N benefits to be derived from the legume by the non-legume. The commonly accepted view at that period of a passage or diffusion of nitrogenous substances from legume nodules through the soil to associated non-legume species could not be confirmed.

Ennik (1960) working at Wageningen with Perennial Ryegrass and white clover, at $16^{\circ}C$ and with low and high light intensities (3 and 6×10^4 ergs/cm^{-2}/sec^{-1} respectively) has helped to interpret the "competition factor" existing between these species when grown together. His results indicate that low light intensities do not limit grass growth, the limiting factor is almost certainly low nitrogen levels. In these experiments there was no evidence of a direct transfer of nitrogen from the clover to the grass and also clover did not hamper nitrogen uptake by the grass. This is likely to be so when the rate of penetration, the density or the activity of the clover roots are less than that of grass. At high light intensity the clover:grass ratio increased until the grass vanished whereas at low intensity an equilibrium was reached clover:grass - 3:2.

Laissus and Teilhard de Chardin (1962) working with perennial ryegrass and several varieties and types of white clover and cutting their plots four times per season showed little change in dry matter yields between grass alone and grass plus clover on the application of fertiliser N. An extract of their data, relating to perennial ryegrass with New Zealand white clover (Certified Mother Seed) or S.100 appears below:-

D.M. Yields. Tons/Acre

	Grass + N.Z. Cert. white clover	Grass + S.100 white clover	Grass alone
No nitrogen fertilizer	1.84	1.96	1.70
134 lb N per acre	3.48	3.60	3.43
Fertilizer effect	1.64	1.64	1.73

Practically the same response to 134 lb N was obtained whether or not clover was present. From these data it would be reasonable to infer that transference of nitrogen from the clover to its companion grass species was either extremely small or non-existant. Recent trials, involving lucerne and meadow fescue reported by Ellis Davies (1964) have not been able to confirm the clover-grass picture in relation to N transference and consequent benefit to the grass species. In this experiment meadow fescue grown alone and relying entirely on mineralisation of the soil for its N took up more N per acre than when in association with lucerne - either broadcast or drilled.

Annual uptake of N (lb/acre)

Meadow fescue alone	Meadow fescue + Broadcast lucerne	Meadow fescue + Drilled lucerne
30.1	20.3	24.1

It must however be recorded that the lucerne in the mixed swards took up 205 lbs. of N per annum, and when these mixed stands were ploughed in, an indicator crop of rape showed an up-take of 29 lb of N per acre compared with only 11 lb when grown after the meadow fescue. Reasons put forward for this inability to show any N transference from lucerne to meadow fescue were that the lucerne was extremely competitive for space, water and nutrient uptake thus dominating the non-aggressive grass species.

From the evidence presented and from other experimental data, on balance, it would appear that the concept of "N-fixation, N-transference and associated benefit to a non-legume" can be accepted in principle. Under the specific circumstances of using white clover and perennial grasses this principle appears to have few exceptions particularly when grazing animals are used in defoliation. Here the major contribution to N transference undoubtedly comes from dung and urinary nitrogen and their acceleration of the relatively slow nitrogen cycle. Where cutting and the removal of herbage is the method of defoliation it is questionable whether the principle outlined above applies. The object of this thesis is to study the nitrogen economy of perennial ryegrass and white clover under a range of cutting frequencies in order to test the concept in the absence of as many external influencing factors as is possible.

In nearly all the experimental findings so far quoted on the amount of 'N' transference from legume to associated grasses such evidence is by inference - grass and clover yield minus grass yield when grown alone being the method of assessment. One recently published paper by Bakhuis and Kleter (1965) has attempted to rectify this situation by examining grass and clover growing singly and then comparing these observations with grass and clover together with and without root separation of the species. In a two year field experiment these two Dutch workers studied pure stands of white clover, perennial ryegrass and cocksfoot with alternate rows of clover and grass species and with separated and unseparated root systems on a nitrogen deficient soil - 91% sand, 7% clay and 2% humus. They measured the dry matter yields and protein content of the herbage and predicted the protein yields (since the clover was not weighed) by applying varying competition models derived from De Wit's competition theory (De Wit 1960; De Wit, Ennik, Van Den Bergh and Sonneveld 1960; and De Wit and Van Den Bergh 1965). The main conclusions in this pertinent paper were as follows:-

(1) The general picture of dry matter yield was similar for grass in association with clover compared with grass alone with and without root segregation.

(2) Below ground barriers were responsible for lower yields of dry matter.

(3) Crude protein yields of grass plus clover compared with grass alone indicated a similar picture to the dry matter yield data.

(4) The crude protein contents in the dry matter of grass in mixed stands were higher than those in pure grass stands.

(5) In the first two cuts of ryegrass and first three cuts of cocksfoot, grass alone yielded more than grass in association with clover. During the later cuts, grass benefited increasingly and significantly from the clover. From the sowing and harvesting dates reported this means that a significant contribution by clover to associated grass species was demonstrated 92 days after sowing. This was considered to be an above-ground influence and the direct result of nitrogen which had leached from decaying clover leaves and petioles. Perennial ryegrass appeared to be more effective in taking up this nitrogen.

(6) A significant underground nitrogen effect was observed after 62 days in respect of perennial ryegrass and after 88 days in respect of cocksfoot, both resulting from the association with clover.

(7) The crude protein contents of the dry matter of clover in mixed stands were mostly slightly lower than those of clover only.

(8) Mixed stands of grass and clover in the year of establishment yielded considerably more than pure grass stands but less than pure clover stands. Also in the establishment year the quantity of nitrogen fixed by clover in above-ground and underground parts of the plants amounted to about 134-143 lb nitrogen per acre.

These results, the circumstances under which they were obtained and their application in relation to those obtained under field conditions in Scotland will be discussed later. (Sect. III).

1.6 POSSIBLE SOURCES OF NITROGEN OTHER THAN FROM THE LEGUME-RHIZOBIA SYMBIOSIS.

1.61 Soil organic matter

The annual decomposition of organic matter, under aerobic conditions, will liberate nitrogen in forms available for plant growth and the amount released, under a given set of climatic and edaphic conditions, will be directly proportional to the level of organic matter which the soil contains. Total nitrogen analyses of the soil from the plots in the trial area at the beginning of the experiment (Table I Appendix B) indicated a range of 0.25 to 0.29% nitrogen, with an average of 0.267%. According to Walker, Orchiston and Adams (1954) this would suggest an annual contribution of approximately 45 lb of nitrogen for plant growth from the disintegration of soil organic matter.

1.62 Rainfall

Miller (1905), working at Rothamsted, where the average annual rainfall is 26½ inches Meteorological Office Data (1958), reported a yearly contribution of 4 lb of nitrogen, in the form of ammonia and nitrates, per acre per annum from the rainwater. In America, Leland (1952) has estimated this figure to be 5 lb nitrogen per acre per annum at Cornell and Erikson (1952) in a survey quotes a range of 2-20 lb of nitrogen from rainfall in a season using data from various parts of the world. Goldschmidt (1954) estimated this range to be of the order 4-10 lb of nitrogen per acre whereas Drover and Barrett-Lenard (1956) restricting their observations to the wheat belt of Western Australia recorded 0.6-3.7 lb of nitrogen during a season.

This work in Australia, carried out over the four years 1952-1955, indicated that ammonia was the principal nitrogenous constituent of the rainfall for the inland centres whereas nitrate formed the major part at the coastal station and that the magnitude of soil nitrogen gain could not be related to either total rainfall or locality. However, on close examination of their data, the highest recorded figure for total amount of nitrogen at each of the six centres was recorded in the season with the highest total rainfall. It is perhaps, worthy of note that the total average annual rainfall for Perth (Western Australia) - namely 36 inches (Molnar 1961) is very similar to that experienced at Auchincruive (see meteorological data in Appendix) - 37 inches, although the distribution and density patterns are dissimilar.

1.63 Bacteria

1.631 Genus Azotobacter

Within this genus the three species *chroococcum*, *beijerinckii* and *vinelandii* are considered common in soils - Russell (1961). These aerobic bacteria are usually confined to soils rich in phosphate and at pH values above 5.8 according to Jensen (1950a) and Kaila (1954). McKnight (1949) however, reported a few in soils with a pH between 5.5 and 6.0 and Tschan (1953a) and Jensen (1955) have also recorded their presence under acid conditions. Although Starkey and De (1939) were able to demonstrate that *Azotobacter indicum*, when isolated from Indian soils, was capable of nitrogen fixation under acid conditions, the presence of this species in soils of Western Europe is considered rather doubtful.

In spite of the fact that Azotobacter species are common in soils, Meiklejohn, working at Rothamsted (quoted by Russell 1961), Jensen (1950b) in Denmark and Anderson (1958) in America all report low cell numbers per gram of soil, thus suggesting low levels of nitrogen fixation. An estimate of this value was propounded by Albrecht et al in 1956 who put forward the range 26-37 lb of nitrogen fixed per acre per season. Since the average pH on the experimental site, before application of lime, stood at 5.34 and bearing in mind the earlier references to pH and occurrence of bacteria together with the low bacteria counts reported by three authorities, it would be unreasonable to expect a large contribution of nitrogen to the soil from the azotobacter species present.

1.632 <u>Genus Beijerinckia</u>

Originally classified as Azotobacter, but differing in morphology and nutritional requirement, the three recognised species of this genus are acid tolerant and are assumed to belong to a tropical genus Tschan (1953b). Partial confirmation of this hypothesis has been offered by Kluyver and Becking (1955) who failed to record significant quantities in temperate soils and although they are proven nitrogen fixers, further discussion of this genus is deemed unnecessary.

1.633 <u>Genus Clostridium</u>

These anaerobic bacteria occur widely in soils according to Russell (1961), but from the results of early laboratory tests have almost been discarded as significant nitrogen fixing organisms on the ground of inefficiency. Rosenblum and Wilson (1949; 1950) however, using N^{15}, were able to show that out of the fifteen clostridia tested only three failed consistently to fix nitrogen, and later they demonstrated that on an absolute basis clostridia were approximately half as efficient as azotobacter in fixing nitrogen. Working with Clostridium butyricum and using glucose as an energy source, Parker (1954) reports that 27 mg nitrogen were fixed per gram of carbohydrate dissimilated. This is high compared with the nitrogen fixing potential of many of the azotobacter species quoted by Nutman (1959) in his review. Under field conditions Albrecht and his co-workers (1956) suggest that clostridia are capable of fixing between 2 and 4 lb of nitrogen per acre per season.

1.634 Genus Pseudomonas

This is the second most widely distributed genus in soils, containing species associated with the rhizosphere of plants and some of these species are able to utilise molecular nitrogen Anderson (1955), Voets and Debacker (1955) and Krasil'nikov (1958). Wilson and Proctor (1958) have suggested that species of Pseudomonas can fix atmospheric nitrogen to a limited extent under aerobic conditions and to a greater extent when the oxygen supply is restricted. Surveying prairie soils, Paul and Newton (1961) report the presence of two azotobacter species and Pseudomonas azotogensis but on isolation and growth on carbohydrate and mannitol respectively they demonstrate a low fixation of nitrogen by the pseudomonas compared with the azotobacter. This inference of low nitrogen-fixing efficiency does not confirm the rate earlier quoted by Roy and Mukherjee (1957), namely 17 mg nitrogen fixed, when grown in pure culture, per gram of mannitol dissimilated, which compares favourable with some of the figures for azotobacter species cited in Nutman's review (1959).

1.635 Aerobacter and Achromobacter genera

Wilson and Burris (1953) examining several strains of *Aerobacter aerogenes* demonstrated that one was capable of fixing small but significant quantities of nitrogen. This was measured via an isotopic technique as the normal Kjeldahl determination was not sensitive enough. Two years later this was confirmed by Wilson and Hamilton (1955) and Jensen in 1956 showed that this species fixed 4 mg of nitrogen per gram of sucrose when grown as a pure culture.

Wilson and Proctor (1958) studying Achromobacter and Pseudomonas together were able to demonstrate that species from these genera could fix atmospheric nitrogen to a limited extent under aerobic conditions and better still when the oxygen supply was restricted.

1.636 Genera of Photosynthetic Bacteria

During the period 1949-52 several authors reported nitrogen fixation by photosynthetic bacteria viz:-

Rhodospirillum - Kamen and Gest (1949) and (1952).

Chromatium and *Chlorobacterium* - Linstrom, Tove and Wilson (1950).

Rhodopseudomonas and *Rhodomicrobium* - Lindstrom, Lewis and Pinsky (1951).

These, however will not contribute significantly to soil nitrogen supply in cultivated land since they are obligate anaerobes with a natural habitat below water, in mud or under algae (Garrett 1963).

Reviewing non-symbiotic bacterial nitrogen fixation Remy (1909) suggested a maximum of 10 mg of nitrogen per gram of nutrient consumed whilst Hutchinson (1918) showed that 6 mg of nitrogen were fixed per gram of plant residue under laboratory conditions rising to 9 mg nitrogen in pot experiments. Where conditions are favourable for these free living bacteria, namely a proper source of energy, sufficient neutralising lime, adequate available phosphates, soil aeration and correct soil temperatures Zipfel (1912), Waksman (1927) envisaged a range of 15-40 lb of available nitrogen fixed per acre per annum and usually not more than 10 lb would be the figure for average field conditions. This range or postulated average figure may well be on the low side when one considers the many organisms now accepted as nitrogen fixers which were not considered in the mid 1920's.

1.64 Actinomycetes

This group of heterotrophic organisms resemble both fungi and bacteria, connection with the former being in mode of development and with the latter in their intolerance of acidity. According to Krasil'nikov (1958) there are species belonging to the Genus Mycobacterium which can utilise atmospheric nitrogen and this was demonstrated earlier by Novak and Dvorakova (1955) who showed a fixing of 15 mg of nitrogen per gram of sucrose dissimilated. Nocardia species have also been recorded as nitrogen fixers by Metcalfe and Brown (1957) being higher when the carbohydrate substrate was cellulose compared with a mannitol energy source. In spite of the claims by Russell (1961) that actinomycetes are very active under grassland and may be the dominant micro-organism in the top few inches of soil in the environment, they still have to be proved as significant contributors to the soil nitrogen supply.

1.65 Algae

These autotrophic organisms occur in large numbers on the surface and within the top few inches of soils and where they can be traced to lower depths the agents of motivation have been rainfall, cultural operations, soil fauna or combinations of these (Garrett, 1963; Russell, 1961; Tchan and Whitehouse, 1953). It has also been suggested that their existence at lower depths is somewhat ephemeral (Garrett, 1963). Of the four groups of algae, blue-green (Cyanophyceae), yellow-green (Xanthophyceae), diatoms (Bacillariaceae), and green algae (Chlorophyceae) only the first one is concerned with the fixation of atmospheric nitrogen. The nitrogen fixing genera belonging to Cyanophyceae are associated with rice cultivations (Singh, 1942; Watanabe, Nishigaki and Konishi, 1951; Williams and Burris, 1952) and in this environment are able to contribute very significantly to the soil nitrogen status. De and Mandal (1956) indicated an average annual contribution of 41lb and a range between 14 and 71lb of nitrogen per acre from the blue-green algae under rice cultivation. These micro-organisms appear to require small quantities of molybdenum for efficient functioning (Bortels, 1940), and their optimum pH is on the alkaline side of neutrality, a feature which may mean they are precluded from the soils in these experiments. Russell (1961) in his survey of soil micro-organisms states that there is no positive evidence that algae contribute significantly to the soil nitrogen status in temperate regions in spite of their widespread occurrence.

1.66 Yeasts

Within this group of organisms, a Rhodotorula and a Saccharomycete are known to be nitrogen fixers. They were isolated from the A1 horizon below a betula-calluna heath by Metcalfe and Chayen (1954) and when grown on mannitol plates exhibited nitrogen fixation equal to 4 mg of nitrogen per gram of mannitol expended. Roberts and Wilson (1954) working with these yeasts extracted by Chayen and using N^{15}, showed their nitrogen fixing capacity as $^{1}/_{10}$th and $^{1}/_{50}$th of that of azotobacter. Since they were only found under very acid conditions (pH 4.5), were only isolated in small numbers and appear very inefficient fixers by other standards, it would be reasonable to assume little or no contribution to soil nitrogen in the experiments described in this thesis.

1.67 Summary of possible sources of nitrogen and estimates of their contribution

	Source	Amount of nitrogen lbs./acre/annum
1.	Soil organic matter	43-50
2.	Rainfall	5
3.	Azotobacter species	26-37
4.	Beijerinckia species	Probably nil.
5.	Clostridia	2-4+
6.	Pseudomonas	Very small if organism is present in the soil
7.	Aerobacter and Achromobacter	Very small
8.	Photosynthetic bacteria	Probably nil.
9.	Actinomycetes	Small?
10.	Algae	?
11.	Yeasts	Probably nil, even at very low pH contribution very small
		75-96+

In round figures, 80-100 lbs. of nitrogen could be available per season from all sources.

2. SECTION II EXPERIMENTS.

2. SECTION II. EXPERIMENTS

2.1 OBJECTIVES, EXPERIMENTS AND TECHNIQUES

2.11 Objectives and trial layouts

From the literature it is evident that little or no attempt has been made at a direct evaluation of nitrogen transference from clover to grass under field conditions. Under controlled conditions in the laboratory and in green house studies the transference has been assessed with other species and with grass and clover in the field indirect or predicted values have been calculated. The following objectives and questions have therefore been posed appertaining to the nitrogen economy of New Zealand perennial ryegrass - New Zealand white clover associations:-

(a) In the absence of fertilizer nitrogen, to study the nitrogen and dry-matter yields that can be obtained over a three year period:-

(i) Under normal root conditions in the field

(ii) Under field conditions where the root systems are kept separate using black polythene.

(b) To ascertain the effect, if any, of different cutting frequencies (2, 4 and 6 cuts per annum) on the nitrogen economy of the association.

(c) To test the theory of Walker et al (1954) regarding the soil N level and predictable clover contribution towards the grass nitrogen.

(d) To ascertain through observation plots the approximate level of fertilizer N that has to be applied to a pure stand of New

Zealand perennial ryegrass in order to obtain:-

(i) The same D.M. yield as that obtained from grass and clover combined without fertilizer N.

(ii) The same 'N' yield as that obtained from grass and clover combined without fertilizer N.

(e) To test the findings of Johnstone-Wallace in respect of diurnal fluctuations in micro-climatic temperature at ground level in grass versus grass plus clover plots.

(f) To measure the diurnal fluctuation in micro-climatic temperature at ground level under the different cutting frequencies established in the trial.

(g) To compare the effectivity of the natural strain of Rhizobia in the site against one of the most effective known.

The main trial designed to answer most of the questions is a split plot layout with four replications.

Main treatments (3)	2 - Twice during the season
(cutting frequency)	4 - Four times " " "
	6 - Six times " " "
Subsidiary treatments (2)	a - Root systems combined
	b - Root systems separated.

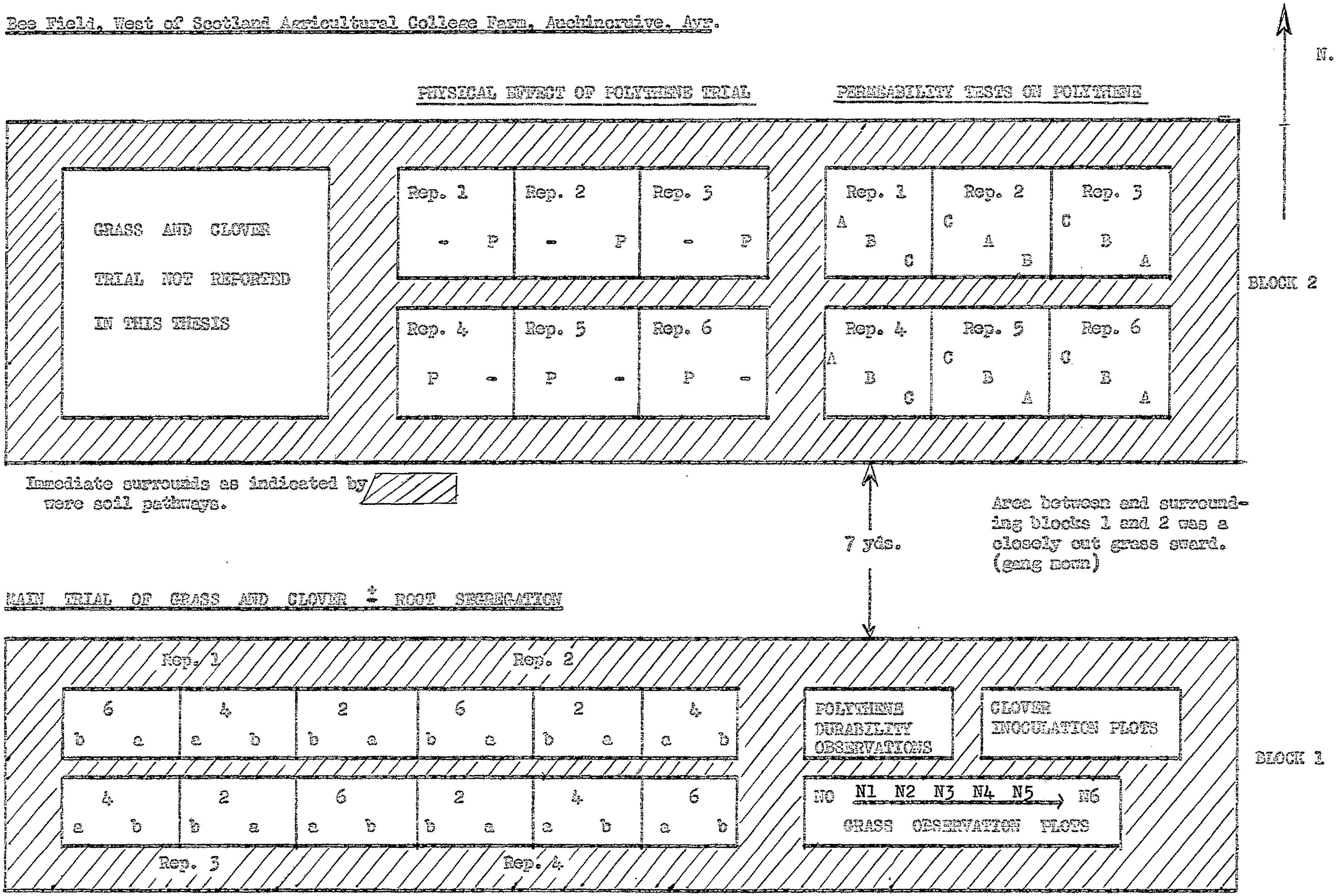
A series of grass plots was also established at the side of the trial in order to cover (d) and the question posed in (g) are covered by a small randomised block trial, referred to as the "clover inoculation trial."

In addition, it was thought necessary to test the permeability of the black polythene used as barrier material. This was done both in the laboratory and under field conditions the results being discussed in the appendix.

Also a measure of the physical effect of the polythene was obtained in a randomised block trial in the field. This was established in a manner similar to that described for the main trial except that the polythene was placed at right angles to the rows instead of being parallel to them. Again the results from this trial are discussed in the appendix.

FIELD PLAN OF THE EXPERIMENTS AND OBSERVATION PLOTS

Bee Field, West of Scotland Agricultural College Farm, Auchincruive, Ayr.



Key to symbols used in the master plan of trials and observation plots.

Main grass and clover trial

2 - Main treatment two cuts per annum

4 - Main treatment four cuts per annum

6 - Main treatment six cuts per annum

a - grass and clover with integrated root systems.

b - grass and clover with their root systems segregated.

Rep 1 - Replicate number one. (for all replicated experiments)

Rep 2 - Replicate number two, etc.

Physical effect of the polythene trial

P - grass and clover plots with polythene at right angles to the line of drilling.

\- control plot, grass and clover growing normally without the introduction of polythene.

Permeability tests on the polythene

A - Grass + fertilizer nitrogen (4 x 8 cwts./acre 21% Nitro-chalk)

B - Grass in close proximity to nitrogen applied in A, but separated by polythene

C - Grass distant from fertilizer N.

Grassland observation plots

N0 - Control plot without fertilizer nitrogen.

N1 - 1 cwt. of nitro-chalk per cut, equal to 94.08 lb N/acre/annum.

N2 - 2 cwts. of nitro-chalk per cut, equal to 188.16 lb N/acre/annum.

N3 - 3 cwts. of nitro-chalk per cut, equal to 282.24 lb N/acre/annum.

N4 - 4 cwts. of nitro-chalk per cut, equal to 373.32 lb N/acre/annum.

N5 - 5 cwts. of nitro-chalk per cut, equal to 470.40 lb N/acre/annum.

N6 - 6 cwts. of nitro-chalk per cut, equal to 564.48 lb N/acre/annum.

2.13 Experimental sites, establishment and methods used

2.131 Details of experimental sites

Geographic location

Field:- Bee Field.

Farm:- West of Scotland Agricultural College, Auchincruive, Ayrshire.

(National Grid Ref. N.S. 387236)

Lat. 55° 29' N.

Long. 4° 33' W.

Soil type:-

Sandy Loam over red-brown sandy clay with coal measures below.

Aspect:-

4° Slope in the direct of the S.W. with conifer wood to the north, the trial area itself being surrounded immediately on all sides by a ryegrass ley.

Previous cropping and manuring

1962	Potatoes.	10 cwt./acre	12% N:	12% P_2O_5:	18% K_2O.
1961	Peas and beans	4 cwt./acre	0% N:	20% P_2O_5:	20% K_2O.

Preparation of the experimental site

During the autumn of 1962 and spring of 1963 the trial area was hand dug to remove weeds in particular Agropyrum repens. In March 1963 the land was marked out plot by plot and soil analysis done - (for methods and details of analyses see appendix). As a result the whole area received a dressing of lime on 26th March which was designed to correct the pH from its previous level to a pH of 6.25.

It was considered unwise to correct to neutrality due to the risk of rendering several important trace elements unavailable and it was also considered unwise to attempt a plot by plot correction. This is condemned on the basis of introducing additional parameters to the specific treatments. Phosphate and potash levels were low and medium respectively and to mask any plot variations and to give sufficient fertilizer for establishment and growth during the maiden year the whole area received a dressing of 100 units of each. (i.e. 112 lb of P_2O_5 and 112 lb K_2O per acre). This was applied in granular form on 26th March and was worked into the top soil. Half the experimental site was excavated to a depth of 15 inches taking care to keep the various soil horizons separate. A double layer of 500 gauge black polythene was laid every 6" on those plots designated for root separation, and the soil was put back carefully in its correct order. This took place between March 28th and April 4th. The other half of the site was dug over to simulate the soil loosening which took place on those plots which had been excavated.

2.132 Establishment of the main trial

The seeding rate for the trial was 20 lb per acre of New Zealand certified mother strain perennial ryegrass and 2 lb per acre of New Zealand certified mother strain white clover.

Each plot contained 8 rows, alternately grass and clover, and the seed was measured out for these (volumetrically) on an individual row basis. Having marked out the rows they were opened up with a rake and the seed was hand sown and then covered. Seeding and brairding dates were as follows:-

SOWING DATES	BRAIRDING DATES
Split plot trial (1963)	
April 5th and 6th	Clover 18th April
	Grass 21st April
Grass observation plots (1963)	
April 8th	April 24th
Clover inoculation trial (1963)	
April 19th	April 29th
Polythene effect trial (1964)	
23rd and 24th March	April 29th
Permeability of polythene (1964)	
6th July	July 27th

2.133 Illustrations of method used in establishment

Plates 1-6

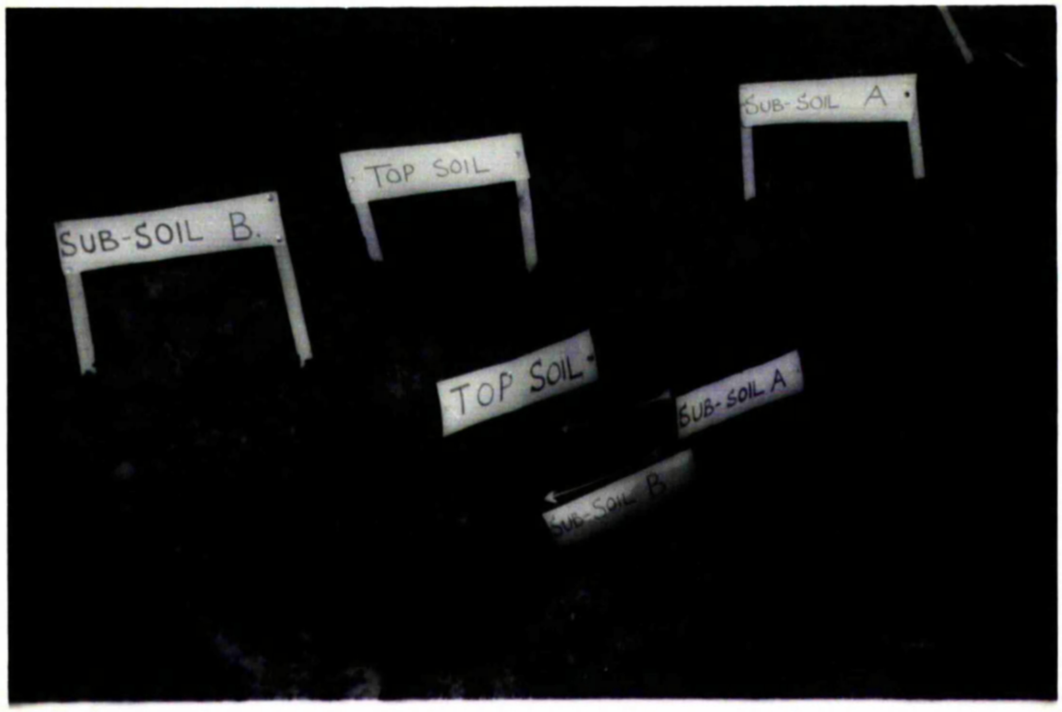
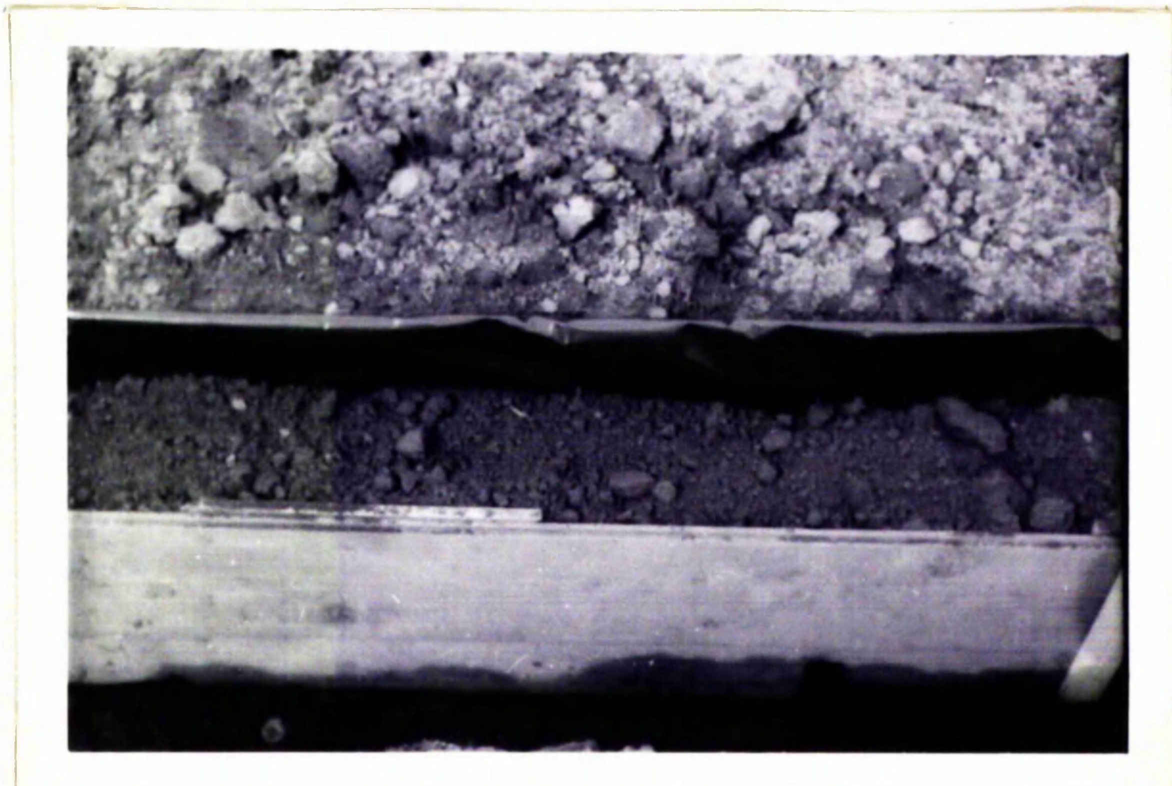


PLATE 1. Excavation and separation of top-soil, sub-soil A and sub-soil B.

PLATE 2. Introduction of polythene barrier supported by light wooden frames between layers.

<u>PLATE 3</u>. Illustration of method of filling using wooden retainer board.



<u>PLATE 4</u>. Back filling with both sub-soil layers complete.

PLATE 5. Back filling with both sub-soil layers complete on three sections.

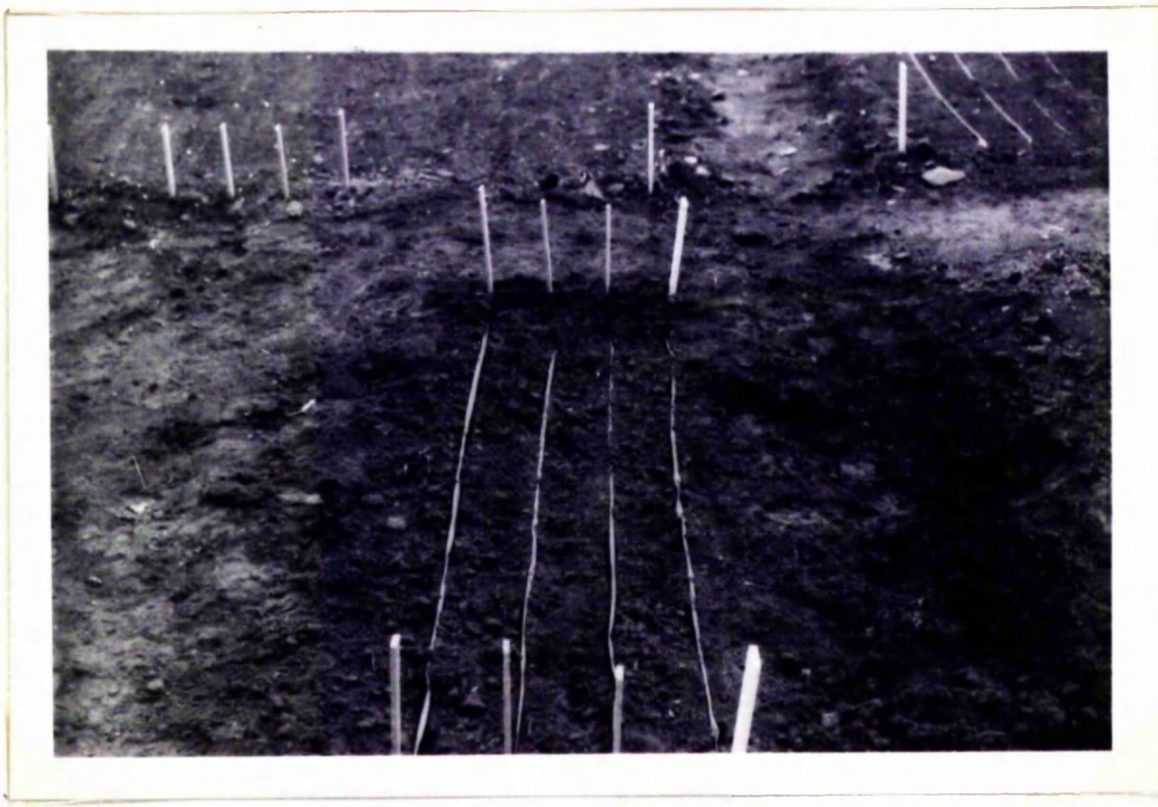


PLATE 6. Final layer of top soil added.

2.2 RESULTS FROM THE MAIN TRIAL IN ITS ESTABLISHMENT YEAR 1963

2.21 Preliminary details

During the establishment year of this trial it was considered impractical to apply the full treatment effects in respect of cutting frequency and a modified set were drawn up as follows:-

	2 Cuts/Annum	4 Cuts/Annum	6 Cuts/Annum
Establishment Cut	July 9th	July 9th	July 9th
1st Cut	Sept. 30th	Aug. 15th	Aug. 15th
2nd Cut	-	Sept. 30th	Sept. 16th
3rd Cut	-	-	Oct. 16th

Between the time of sowing (5/6th April) and the establishment cut (July 9th) several hand weedings were carried out over the trial area. Most of the quick growing annual weeds were removed so as to obtain a uniform "take" of grass and clover on all the plots.

The initial braird was in fact uniform and an even growth rate of both grass and clover was recorded during the first six weeks after emergence. However, towards the end of June there had appeared slight differences between the subsidiary treatments (a) root systems combined and (b) root system separated). Where the roots were combined (a) and therefore unrestricted in movement a slightly taller, more vigorous perennial ryegrass emerged. On the other hand, when root separation was carried out as in treatment (b) and each component given its own rooting area due to the polythene barriers, the clover exhibited slightly more vigorous growth. These observations were verified by measurements taken on 21st June and the mean values recorded were:-

Treatment	Grass height cm	Clover height cm
(a) Root systems combined	15.7	9.9
(b) Root systems separated	13.4	10.5

These differences, which were manifest approximately two months after brairding, are thought to have occurred due to below ground competition for root area, to differential competitive ability of the two species and, linked with both of these, the comparative root morphology of each component.

On July 9th an establishment cut was taken, firstly to aid uniformity in further growth and secondly to eradicate further problems with annual weeds. The various hand weedings carried out after brairding within and between the rows of grass and clover were effective and data recorded at this period is accurate and worthy of examination.

2.22 Summary of results - Establishment cut - July 9th, 1963

2.221 Yield of D.M. (lb/acre). [Grass D.M. + Clover D.M.]

The differences between treatments a and b recorded in June were again present when the establishment cut was taken and if anything were more pronounced.

Treatment	Yield	Contribution made by grass dry matter as % Total D.M.
a	1,894 (128%)	72%
b	1,476 (100%)	52%
S.E.	± 67	-

Total D.M. yields were 28% higher on treatment (a) and this was highly significant. At the same time the contribution made by the perennial ryegrass component was 72% as against only 52% on treatment (b). Where the perennial ryegrass is not restricted below ground it contributes much more to total D.M. production and as a result enhances total D.M. yield partly from the greater green yield present and partly through having a higher D.M. % compared with the clover companion species.

2.222 <u>Chemical analyses</u>

Variations in both D.M. % and N % in the grass and clover were extremely small and only one significant difference was recorded between treatments a and b, this being in the nitrogen content of the clover on these two treatments.

Treatment	% D.M.		% N.	
-	Grass	Clover	Grass	Clover
a	14.12	8.55	1.77	3.11
b	14.49	8.67	1.78	3.21
S.E.	± 0.36	± 0.15	±0.042	± 0.027*

2.223 Nitrogen yield (lb/acre N) [Grass N + Clover N]

The nitrogen yield expressed in lb N/acre is slightly but not significantly higher on treatment (a). This increase amounting to 10% compared with treatment b is much lower than that experienced in the comparable D.M. yield data. It is almost wholly accounted for by the change in proportional representation to yield by the two components and the relatively large differences in % N; the grass component having only half the nitrogen compared with the clover counterpart.

Treatment	N Yield in lbs./acre	Contribution made by grass N as % total N
a	40.6 (110%)	60%
b	36.8 (100%)	37%
S.E.	± 1.98	-

At this stage it is important to recognise the 28% difference in dry matter yield and 10% difference in nitrogen yield, both in favour of treatment (a) as a direct effect of the polythene barriers employed. The allocation of rooting space which has automatically followed the introduction of polythene below ground has reduced the contribution made by the perennial ryegrass and has enhanced the contribution made by the white clover. The largest contribution has been from the grass and a subsequent reduction in this has had an overall negative effect. If N-transference had taken place it is reasonable to assume that the % N in the grass which was not separated from its clover companion species would be noticeably higher than where it had been kept separate.

In order to measure the physical effect of the polythene used as underground barriers, a separate trial was established in 1964. Similar plot sizes were involved and the grass and clover grown in alternate rows as before. Here, however, the polythene of similar thickness and dimension was placed at right angles to the rows of herbage. It was incorporated in six plots with the same number used as controls.

2.23 Observations and details of treatment cuts

2.231 Post establishment cut braird check

On July 15th when the trial had recovered, assessments of the actual "take" of each component were considered. Examination of each plot indicated a satisfactorily uniform plant stand of clover. In respect of the grass component, tiller counts revealed a satisfactory braird, with both treatments having over 300 tillers per linear foot of row and no significant differences between them.

Following the establishment cut on July 9th the various treatment cuts, modified as reported earlier, were taken throughout the remainder of the 1963 season. These have been separately analysed and grouped according to main treatment and appear in Tables I, II and III.

2.232 Main treatment 2

This involved a single cut in 1963 and is reported in Table I. A significantly higher D.M. yield was obtained on subsidiary treatment (a) but no difference was recorded in respect of N yield. The analysis of the clover fraction showed no significant difference in either % D.M. or % N. The grass analysis however indicated a higher D.M. % on treatment (a) but a lower % N.

2.233 Main treatment 4

Two cuts were taken on this main treatment and these are analysed and presented in Table II. Results from August 15th indicate nothing significant in D.M. yields or N yields but minor trends appear in the chemical analyses. D.M. % is higher on treatment (a) for both grass and clover but the reverse trend occurs in respect of N %. At the later date nothing significant appears in the data.

2.234 Main treatment 6

In addition to the establishment cut, three treatment cuts were taken during 1963 on August 15th, September 16th and October 16th. Individual analyses appear in Table III and indicate a similar pattern to the one exhibited in treatment 4. Similar D.M. and N yields were experienced with each cut and the chemical analysis revealed higher D.M. % and lower N % on herbage from treatment (a) during the first cut on August 15th. The two later cuts however indicated a similar chemical composition.

2.24 Total production in 1963

2.241 Dry matter

The figures incorporated in this final analysis for 1963 are the establishment cut plus the various appropriate treatment cuts. The statistical breakdown of this data is in Table IV together with a summary of the total yield of D.M. produced. During the first year 4,528 pounds of dry matter were produced per acre on treatment (a) which represented an 18% increase over treatment (b). During the first few weeks after brairding this difference between (a) and (b) was quite marked, and in the establishment cut in July it amounted to 28%. Since then the magnitude of the difference between the two subsidiary treatments has progressively decreased and although the final figure for total D.M. yield shows 18% in favour of (a), half of this can be attributed to differential growth up to the establishment cut. A reasonable explanation for this phenomena lies in the mode of root colonisation of the area available. Without polythene barriers below the surface, grass and clover roots develop unimpeded and this will contribute towards growth above ground without any check. The introduction of impenetrable barriers will temporarily inhibit root development and until they have changed direction from a lateral to an upward/downward movement on meeting the obstacle, there is likely to be a check which could be manifest in the above ground growth. It would appear that underground barriers have had a slight differential effect on the two species concerned particularly in the early stages of growth. The outcome being a reduction in the contributing power of the grass compensated by an increased development of the clover. It is in many ways similar to, but not identical with, the effect of frequent defoliation of mixed swards. The average contribution to D.M. yield by the grass component throughout the 1963 season was 74% and 55% for treatments (a) and (b) respectively.

2.242 <u>Nitrogen</u>

As with total dry matter production, all recorded weighings in the first year were used to assess the total nitrogen yield from the trial and statistical analysis and summary of this data appears in

Table V. Main treatments 2 v 4 v 6 are significantly different at P 0.05, 2 representing the least number of cuts being significantly lower in total N yield than 4 (medium cutting frequency) and 6 (the highest cutting frequency). Where the plots were cut twice during the season sub-maximal yield resulted due to shading, overcrowding and some senescence in the grass and clover growing close to the soil and this was particularly prevalent in respect of the clover component. Subsidiary treatments (a) and (b) gave similar yields - 109 and 108.6 lb N per acre respectively and the interaction of cutting frequency x (a) v (b) comparisons were insignificant. The average contribution to N yield by the grass component throughout the first year was 63% and 43% for treatments (a) and (b) respectively. These proportional representations to total N yield by the grass are approximately 10% lower than the corresponding figure for total D.M. production and are partly responsible for similar N yields from these two treatments. Higher clover contribution with its naturally higher nitrogen analysis has compensated for lower D.M. yield on those plots where the roots are separated (b).

2.25 Summary of the 1963 results

2.251 The effect of root segregation

The introduction of polythene barriers as in treatment (b) affected the initial growth of grass and clover and although the evidence showed this to be minimal by the end of the season, it was responsible for a small but significantly lower yield (18%) in total D.M. production.

Total N yield was similar for both subsidiary treatments. This was brought about through a higher clover contribution to fresh weight on treatment (b) coupled with the fact that this material contained almost double the % protein compared with the grass. As a result, in spite of the higher D.M. yield reported above, total nitrogen yield remained the same.

2.252 Effect of main treatments 2, 4 and 6 cuts per annum

Cutting twice gave significantly lower total D.M. and N yields compared with those plots receiving more numerous defoliations throughout the season.

2.253 Interactions

The interactions between main and subsidiary treatments were insignificant both in respect of total D.M. production and total N yield.

2.254 Chemical composition

Some significant differences were recorded between treatments (a) and (b) in respect of D.M.% and N%. Grass and clover D.M.% were higher on (a) at the beginning and on occasions at the end of the season but the N% was lower. These isolated differences are not considered of great importance when viewing the chemical data as a whole.

2.255 Interim hypotheses on nitrogen economy

From the 1963 data the following tentative hypotheses may be propounded.

(a) During the establishment year of a grass and clover ley grown under the conditions described in this experiment and with a soil N status of 0.26% (a figure above average compared with Kay, 1934, but higher than those reported by Walker et al 1954) all the atmospheric nitrogen fixed by an effective symbiotic rhizobia may be utilised by the bacteria themselves and the legume host.

(b) N in excess of the bacteria and legume requirement is present in significant quantities yet unavailable to the grass companion species.

(c) N in excess of the bacteria and legume requirement is available in small quantities the uptake of which is masked by other larger agronomic features. (One possible explanation could be through the changing pattern of proportional representations to yield by the two components).

(d) On those plots where the root systems are separated, soil mineralisation has released sufficient N to maintain a high protein content of the grass in these areas, again masking a relatively small amount of transfer on the corresponding plots.

(e) Ineffective nodulation has meant little or no N fixation and therefore no release of N surplus to bacteria and legume requirements.

2.3 RESULTS FROM THE MAIN TRIAL IN ITS 2nd YEAR 1964

2.31 Preliminary details
2.31 Preliminary details

Following the final cut in 1963, the trial area received a dressing of phosphate and potash, (168 lbs. per acre P_2O_5 and 168 lbs. per acre K_2O) to replenish the soil levels of these major nutrients. In 1964 the full number of treatment cuts were made on the following dates:-

Cut Number	Treatment 2 Cuts/annum	Treatment 4 Cuts/annum	Treatment 6 Cuts/annum
1	May 1st	April 24th	April 16th
2	Oct. 1st	June 15th	May 13th
3	-	Aug. 5th	June 23rd
4	-	Oct. 7th	July 28th
5	-	-	Sept. 9th
6	-	-	Oct. 14th

During this year the clover began to grow into the grass rows and throughout the season invading stolons had to be retrained to grow within the limits of the polythene barriers employed. At the same time botanical separation of the cut herbage had to be introduced to obtain an accurate measure of the grass and clover components in the yield data.

2.32 Results from the main treatments

2.321 Main treatment 2 cuts per annum

Summaries of these data appear in tables VI, VII and VIII in the appendix and represent the 1st cut, 2nd cut and total production respectively. Taking a broad view of the results through the total production for the year, there appears no significant differences in yield between the two subsidiary treatments a and b in respect of green weight, dry matter yield or nitrogen yield.

Total production measured as:-	Treatment a	Treatment b
Green yield Tons/acre	17.17	15.76
D.M. yield (lb D.M. per acre)	7641	6617
Nitrogen yield (lb N. per acre)	138.64	139.93

However there still remained a difference between these treatments when the proportional representation of the two components for yield were assessed. Under treatment a, clover contributed 23.6%, 16.3% and 32.2% towards green yield, D.M. yield and nitrogen yield respectively. Those figures rose to 38.1%, 27.3% and 47.6% for the corresponding yield data under treatment b.

Chemical composition of the grass and clover, in the form of D.M. % and nitrogen % appeared unaffected by segregation of roots.

2.322 Main treatment 4 cuts per annum

Individual treatment cuts are summarised in tables IX, X, XI and XII and show a similar but not identical pattern to the less frequently cut plots just reported. There are no yield differences between the subsidiary treatments and neither does the chemical analysis reveal any significant changes in % D.M. and % N in the herbage. The contribution to yield by each component under this more frequent defoliation system now appears unaffected by root segregation. These observations are well corroborated when the total production for the year is examined in Table XIII, an extract from which appears below:-

Total production measured as:-	Treatment a	Treatment b
Green yield Tons/acre	22.45 (46.4%)	22.89 (50.3%)
D.M. yield (lb/acre)	7,295 (37.0%)	7,191 (40.1%)
Nitrogen yield (lb N./acre)	197.73 (52.9%)	196.66 (56.3%)

(Figures in brackets indicate the contribution made by clover as a percentage total yield)

2.323 Main treatment 6 cuts per annum

The results of the individual cuts from the most frequently defoliated plots are summarised in tables XIV, XV, XVI, XVII, XVIII and XIX in the appendix. A pattern emerged from these data which is similar to that already described in respect of main treatments 2 and 4. Yield and chemical analysis indicate no significant differences between the subsidiary treatments and as in main treatment 2 the clover contribution is notably higher where the root systems have been segregated. Again these observations are well confirmed when the total production for the year is examined (Table XX), an extract of the main features appear below:-

Total production measured as:-	Treatment a	Treatment b
Green yield Tons/acre	20.28 (53.0%)	21.93 (64.3%)
D.M. yield (lb/acre)	6,597 (43.4%)	6,956 (56.4%)
Nitrogen yield (lb N./acre)	206.76 (55.97%)	229.07 (67.49%)

(Figures in brackets indicate the contribution made by clover as a percentage total yield).

2.324 Examination of the combined data in split plot analysis form

Amalgamation of the data from each of the main treatments gives an overall picture of total green yield, total dry matter yield and total nitrogen yield in tables XXI, XXII and XXIII, and the percentage clover contribution to these yield measurements can be studied in tables XXIV, XXV and XXVI.

2.3241 Total dry matter yield in 1964

Total dry matter yields for 1964 appear to be unaffected by differing frequencies of defoliation - 2, 4 and 6 cuts per annum being responsible for 7254, 7119 and 6777 lb of dry matter per acre respectively. A small but significant difference was recorded between the subsidiary treatments a and b and this could still be legacy of root restriction which was clearly manifest in the establishment year. The interaction between main and subsidiary treatments is significant and can be partially interpreted through the higher clover contribution to yield where the root systems are segregated compared with those areas where the roots are combined (Table XXV).

2.3242 Total nitrogen yield in 1964

Increasing the number of defoliations had little effect on total dry matter yield but they were responsible for large and significant increases in the total nitrogen production, 2, 4 and 6 cuts per annum recorded 139, 197 and 218 lb of nitrogen harvested per acre respectively. This is in line with present concepts and is accounted for by the fact that young growth obtained by frequent cutting is rich in nitrogen whereas old material, although high in dry matter is not correspondingly high in protein.

There was no apparent difference between the subsidiary treatments in respect of nitrogen yield. This can be explained via a higher clover contribution to yield under treatment b, which would delete any advantage which treatment a, might have enjoyed through ahigher dry matter yield. The interaction between main and subsidiary treatments in respect of total nitrogen yield for the year was insignificant.

2.4 PERIOD BETWEEN THE LAST CUT IN 1964 AND THE FIRST CUT IN 1965

Towards the end of the 1964 growing season, small, random areas of clover on the trial exhibited dark brown or black lesions. The causal organisms were later identified as one or a combination of the following fungi:-

(a) *Sphaerulina trifolii* (Rostr) - Burn

(b) *Pseudopeziza trifolii* (Fr) Fuckel - Leaf spot

(c) *Cymadothea trifolii* Wolf

Stat. con. *Polythrincium trifolii* (Fr) - Black blotch

On October 15th the trial area and surrounds were sprayed with 2 lb of zineb (65-70% zinc dithiocarbamate) in 30 gallons of water per acre. This remedial measure was again effected the following spring on April 2nd and the clover remained healthy throughout 1965.

Following the final cut in 1964 any clover stolons invading the area allocated to the neighbouring grass rows were retrained to grow in their own space within the limits set by the polythene barriers. This laborious but necessary task was completed by November 10th. On the following day the main trial area received its winter dressing of phosphate and potash to replenish the soil supply of these major nutrients. (Rate of application:- 112 lb of P_2O_5 and 112 lb of K_2O per acre).

During the period 5th-7th April, 1965, all dicotyledonous weeds were removed by hand from the trial and on April 8th a further application of phosphate and potash took place. (Similar application rate).

2.5 RESULTS FROM THE MAIN TRIAL IN ITS 3rd YEAR 1965

2.51 Preliminary details

In 1965 the full number of treatment cuts were effected as in the previous year and they occurred on the following dates:-

Cut Number	Treatment 2 Cuts/Annum	Treatment 4 Cuts/Annum	Treatment 6 Cuts/Annum
1	May 25th	May 11th	April 27th
2	Oct. 5th	July 1st	May 26th
3	-	Aug. 31st	June 22nd
4	-	Oct. 5th	July 27th
5	-	-	Sept. 1st
6	-	-	Oct. 12th

On July 14th the trial area received a mid-season dressing of granual compound fertilizer, equivalent to 112 lb of P_2O_5 and 112 lb of K_2O per acre.

2.52 Main treatment 2

Summaries of these data appear in tables XXVII, XXVIII and XXIX in the appendix and represent the 1st Cut, 2nd Cut and total production respectively. Throughout the spring it became increasingly apparent that major differences in yield between the two subsidiary treatments were building up. By the 25th May, the date of the first cut, the grass on treatment a (root systems combined) was much taller and more vigorous than on treatment b. (Plates 7 and 8). This was largely, if not wholly responsible for the significant differences

Yield data	1st cut (25/5)		2nd cut (5/10)		Total 1965 production	
	a	b	a	b	a	b
Green yield Tons/acre	7.60 [178]	4.26 [100]	10.26 [126]	8.14 [100]	17.86 [144]	12.40 [100]
Dry matter Lb/acre	3,129 [179]	1,749 [100]	2,681 [132]	2,033 [100]	5,810 [154]	3,782 [100]
Nitrogen yield Lb N/acre	47.59 [153]	31.04 [100]	74.81 [121]	61.61 [100]	122.40 [132]	92.65 [100]

in total green weight, total dry matter and total nitrogen yield, amounting to an additional 78%, 79% and 53% respectively on the first cut (See table above). A similar position was recorded at the end of the season in the second cut on October 5th, thus presenting wide differences in yield between subsidiary treatments a and b when the total yearly production is assessed. Compared with 1964, the average yields in the third year were lower by $1\frac{1}{2}$ tons fresh weight, amounting to a reduced dry matter yield of 1 ton and 22 lb less nitrogen. The explanation for this lies partly in the fact that too low a cutting frequency on an ageing sward will restrict the production from it. Chemical composition (% D.M. and % N) of grass and clover appeared unaffected by root segregation in both cuts and also on a weighted mean basis.

2.53 Main treatment 4

Summaries of these data appear in tables XXX, XXXI, XXXII, XXXIII and XXXIV in the appendix and they represent the 1st, 2nd, 3rd and 4th Cuts and total yearly production respectively.

Plots on this medium cutting frequency (4/annum) showed a similar pattern to the less frequently cut areas just reported. Green yield, dry matter yield and nitrogen yield on each cut appear higher without root segregation, but in this instance the differences are smaller and less significant. Viewing these results as a whole through the total production for the year there appears a consistent 16-18% difference in yield between the two subsidiary treatments. The 1965 (3rd Year) yields in general are of the same magnitude as those recorded in the previous year.

Total production measured as:-	Treatment a	Treatment b
Green yield [Tons/acre]	24.07 [116]	20.78 [100]
Dry matter yield [Lb/acre]	7,294 [118]	6,162 [100]
Nitrogen yield [Lb N/acre]	198.09 [116]	170.35 [100]

No consistent difference in chemical composition of the herbage emerged and % D.M. and % N may be considered similar for grass and clover whether or not their root systems are segregated.

2.54 Main treatment 6

Summaries of the individual cuts on this treatment appear in Tables XXXV to XL in the appendix and the overall production for the year is reviewed in Table XLI. The general level of yield in 1965 from these frequently defoliated plots was higher than in the two previous years. Twenty four tons of fresh material was harvested compared with 21 tons and 16 tons in 1964 and 1963 respectively and there was a concomitant increase in the dry matter and nitrogen yields.

A consistent yield difference occurred between the subsidiary treatments a and b. Plots with their root systems combined (a) produced higher dry matter yields than those where root segregation had been employed (see Table below).

Dry matter yields (lb/acre) throughout 1965 from main treatment 6 cuts per annum

Treatment	1st Cut	2nd Cut	3rd Cut	4th Cut	5th Cut	6th Cut	Total yearly production
a	1,006 145	1,127 132	1,400 105	1,652 113	1,596 111	830 112	7,612 117
b	695 100	852 100	1,329 100	1,467 100	1,438 100	741 100	6,522 100
S.E.	± 51 *	± 62 N.S.	±106 N.S.	± 17 * *	± 34 *	± 13 *	± 230 *

This was particularly pronounced in the first and last cuts where the differences were statistically significant and also in respect of the total yearly production which varied by 1090 lb of dry matter.

The amount of nitrogen harvested from treatment a was consistently higher than from treatment b and this was particularly significant in the first and last cuts (See Table below).

Nitrogen yields (lb N per acre) throughout 1965, from main treatment 6 cuts per annum

Treatment	1st Cut	2nd Cut	3rd Cut	4th Cut	5th Cut	6th Cut	Total yearly production
a	24.51 163	36.24 147	45.82 103	57.08 111	64.08 106	35.26 110	262.99 115
b	15.01 100	24.66 100	44.64 100	51.24 100	60.43 100	32.14 100	228.12 100
S.E.	± 1.09 **	± 2.98 N.S.	± 3.90 N.S.	± 1.54 N.S.	± 1.33 N.S.	± 0.26 **	± 7.89 N.S.

2.55 Chemical composition

Chemical composition of the clover as measured by % dry matter and % nitrogen appeared unaffected by the introduction of polythene root barriers as indicated in the weighted mean for the year:-

	% D.M.	% N.
a	11.17	4.08
b	11.57	4.08

[Extract from Table XLI]

There were, however, significant differences in the chemical composition of the grass on the two subsidiary treatments. % Dry matter was consistently lower and the % nitrogen was notably higher without root segregation. These differences were more pronounced during the early cuts than later on in the season. The % dry matter for the

Main treatment 6 cuts/annum. % D.M. in the grass. 1965.

Treatment	1st Cut	2nd Cut	3rd Cut	4th Cut	5th Cut	6th Cut	Weighted mean
a	19.32	15.97	15.20	16.20	12.12	12.28	15.33
b	21.98	17.35	16.18	17.25	12.58	12.48	16.53
	***	*	**	**			**

[Extracted from Tables XXXV-XLI]

season as a whole, represented by the weighted mean, was significantly lower on treatment a and the % nitrogen (weighted mean) was significantly

Main treatment 6 cuts/annum. % N in the grass. 1965.

Treatment	1st Cut	2nd Cut	3rd Cut	4th Cut	5th Cut	6th Cut	Weighted mean
a	2.31	2.53	2.49	2.88	3.13	3.86	2.76
b	2.05	2.17	2.32	2.77	3.18	3.83	2.56
	*	*	*				*

[Extracted from Tables XXXV-XLI]

higher on this treatment. This lowering of % dry matter and corresponding increase in the % nitrogen is synonymous with the results obtained when nitrogen fertilizer is applied to grassland (Tables XVI to XXV, Appendix B) and could be attributed to the underground transference of nitrogen from the clover.

2.56 1965 Results as a whole

Examination of all the 1965 data in the form of split plot analyses may be seen in Tables XLII, XLIII and XLIV in the appendix and these represent total green yield, total dry matter produced and total amount of nitrogen harvested.

2.561 Total green yield

Significant differences were recorded in total green yield between the main treatments two, four and six defoliations per year. The infrequently cut plots yielded 15.13 tons per acre and raising the number of cuts to four and six was responsible for an increase of 48% and 61% in the fresh weight respectively.

The mean yield of all the plots with their root systems segregated (subsidiary treatment b) was 18.49 tons of green material per acre and where the root systems were combined (subsidiary treatment a) this rose significantly to 22.81 tons per acre. There was no interaction between main and subsidiary treatments.

2.562 Total dry matter yield

On converting the fresh weights to dry matter yields over the season, a pattern emerged which was similar to the one already described under total green yield.

(Lb dry matter/acre)

Main treatment	a	b	Mean
2	5,810	3,782	4,796 (100)
4	7,294	6,162	6,728 (140)
6	7,612	6,523	7,067 (147)
Mean	6,905 (126)	5,489 (100)	

[Extract from table XLIII]

Maximum dry matter production was obtained from the 6 cut plots although these were not substantially higher than those on the medium cutting frequency. Dry matter yields were 26% higher without root segregation and again no significant interaction between main and subsidiary treatments was reported.

2.563 Total nitrogen yield

In 1965 the effect of different defoliation frequencies was very marked. The mean nitrogen yield rose sharply with increased cuts - 107.53 lb, 184.22 lb and 245.56 lb of nitrogen being harvested from the 2, 4 and 6 cuts respectively. This represented an increase of 71% and 128% over two cuts per annum and was highly significant. The average nitrogen yield was 19% higher without root segregation and there was no trace of an interaction between main and subsidiary treatments.

2.564 % Clover contribution in total yield

The statistical analyses of the percentage clover contributions in each of the yield measurements can be found in tables XLV, XLVI and XLVII in the appendix, the main features of which appear below.

	Green yield		Dry matter yield		Nitrogen yield	
	a	b	a	b	a	b
2	25.89	39.92	19.41	31.83	30.88	45.57
4	48.47	58.02	36.68	46.31	50.70	59.92
6	60.14	70.00	52.36	62.01	61.89	72.18
Mean	44.83	55.93	36.81	46.72	47.82	59.22

[Extract from tables XLV-XLVII]

From this extract it will be seen that the clover component contributes 10 to 11% more towards the total yield under a system of root segregation than when the root systems are combined. This is entirely due to the fact that there was an enhanced grass yield on treatment a and since the clover yields were similar the contribution of clover expressed as a % of the total yield on treatment b was bound to be higher.

Increasing the number of cuts during the year favours the less aggressive clover component and this is reflected in the rising figures for clover contribution in total yield.

<u>% Clover contribution in total yield</u>

Cuts/annum	Green yield	Dry matter yield	Nitrogen yield
2	32.91	25.62	38.23
4	53.24	42.49	55.31
6	65.07	57.19	67.03

In round figures the clover contributes approximately $\frac{1}{3}$ towards the total yield when infrequently cut, approximately $\frac{1}{2}$ when cut four times a year and $\frac{2}{3}$ when defoliated six times a season.

3. SECTION III DISCUSSION .

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3.1 OUTPUT OF DRY MATTER 1963-65

In the year of establishment when growth was naturally restricted, only half the number of defoliations were carried out and the dry matter harvested from the "2", "4" and "6" cut plots amounted to 3836, 4512, 4216 lb per acre respectively. [Table IV]. Root segregation had reduced the competitive ability and hence the yield of the grass component and this resulted in an overall reduction of 18% in the total amount of dry matter produced. The clover had benefited concomitantly by this reduced competition from the neighbouring grass and although its contribution towards total output was enhanced, this was not sufficient to counteract the drop in contribution from the grass. During this establishment year the plots where root barriers had been introduced showed a check in growth. It is suggested that this occurred when the root systems met with the impenetrable polythene barriers and had to change from a mainly lateral growth to one which was vertical.

The full number of treatment cuts were made in 1964 and the corresponding yields from 2, 4 and 6 defoliations were 7254, 7119 and 6777 lb of dry matter per acre. These proved to be insignificant on statistical analysis but a significant difference did occur as a result of root segregation. As in 1963, the root segregated plots (subsidiary treatment b) were lower than those where the root systems were combined and this amounted to 6% on average. [Table XXII]. For the first time the interaction between main and subsidiary treatments proved to be significant. Increasing the number of cuts on those plots where the grass and clover were growing normally was responsible for substantial reductions in dry matter yield whereas on the root segregated plots the greater numbers of defoliations gave small increases in dry matter yield. This descending pattern of dry matter yield exhibited by the grass and clover when grown without root restriction is one which is widely accepted. The reverse trend in dry matter yield from the other plots will therefore need some interpretation. The significantly lower total dry matter yield from the root restricted plots may well be due to a low utilisation of fixed nitrogen since any which is surplus to the clover's requirement will not be taken up. More defoliation will therefore lead to an increased efficiency in nitrogen utilisation resulting in higher dry matter yields. Partial corroboration of this can be seen in Table XXV which analyses the percentage clover contribution in the total dry matter yield. Over the season as a whole, clover contributed 11% more to dry matter yield where root restriction had been practised and this rose to 13% at the highest defoliation rate.

In 1965 the production of dry matter was lower than in the previous year as would be expected from an ageing sward. During this year the effect of nitrogen transferred from clover to grass was pronounced, giving the grass a more vigorous appearance and at the same time it was noticeably darker green in colour (Plates 7 and 8). The first occasion on which these differences were visible was on May 25th and compared with published data, notably that of Bakhuis and Kleter 1965, this is very much later. These Dutch workers demonstrated a

PLATE 7

Photograph taken on 25th May, 1965 from Replicate 4.

Treatment 2 cuts per annum, showing major difference between subsidiary treatments

b (left centre) - a (right centre)

PLATE 8

Photograph taken on 25th May, 1965 from Replicate 2.

Main Treatment 2 cuts per annum confirming major

difference between subsidiary treatments

b (left centre) - a (right centre)

significant underground nitrogen effect after 50 days with perennial ryegrass when associated with white clover whereas the data from these experiments shows that under the conditions imposed at Auchincruive in the West of Scotland significant underground effects were not manifest until two years had elapsed after sowing. The reason for this major difference in time of benefit in a perennial ryegrass - white clover association may be due to one or more of the following discrepancies between the experiments:-

(1) Soil conditions were very dissimilar. In Holland a nitrogen deficient sand was used whereas in the experiments described, the soil was a medium loam, with a loss on ignition of over 8% and an average nitrogen level of 0.267%.

(2) The experimental conditions differed fundamentally. The Dutch workers using sand had built their experiment above ground level and the soil moisture regime must therefore have been very different from that experienced at Auchincruive where the normal soil level was maintained and except for the introduction of a very thin film of polythene natural field conditions existed.

(3) Root segregating materials had different thicknesses and would therefore have a differential effect on the reduction of "effective" plot width.

(4) From the illustration of the field layout it would appear that the Dutch experiment may have suffered more from neighbouring row competition since effective discard rows were absent. At Auchincruive however four rows out of eight were harvested for yield determinations and chemical composition.

(5) The examination of the meteorological data for both places shows some fairly large variations, one of the most notable being the discrepancy between the solar radiation figures (Makkink 1959 and Tables XI-XV - Appendix B).

Average solar radiation data

(Total Energy received in Cals per square centimetre per day)

	April	May	June	July	Aug.	Sept.	Total
Holland (1931-1957 Average)	294 (130)	379 (137)	392 (135)	371 (158)	321 (159)	233 (181)	1990 (146)
Auchincruive (1964-1965 Average)	226 (100)	276 (100)	291 (100)	234 (100)	202 (100)	129 (100)	1358 (100)

Taking the six months from April to September which virtually form the growing season as a whole, Holland on average has 46% more total solar radiation than the West of Scotland received on average during two years of the experiment.

From the various points listed above, together with the fact that in the Dutch trials the clover yield was computed from a De Wit 1960 model, rather than separated out by botanical analysis and weighed as at Auchincruive, it would be reasonable to assume that the Scottish results would be a more accurate guide to the picture in a normal field soil.

The actual dry matter yields obtained in 1965 (Table XLIII) from the 2, 4 and 6 cutting frequencies was 4,796, 6,728 and 7,067 lb per acre respectively. Statistical examination of this data showed that two cuts per annum on a three year old sward gave significantly lower yields than either four or six defoliations. At the same time substantial differences were being recorded between root segregated plots and the controls. On average this proved to be 26% higher without root restriction and this was significant at the P = 0.001 level. As in previous years the clover contributed about 10% more to dry matter yield on the root segregated plots compared with its contribution under free growing conditions and again this is accounted for by larger yields of grass on treatment a.

The average yearly production of dry matter between 1963 and 1965 can be summarised as follows:-

Main treatment	Yield/acre in lb dry matter	
2 Cuts/annum	5295	(100%)
4 Cuts/annum	6119	(116%)
6 Cuts/annum	6019	(114%)
Subsidiary treatments		
a (roots combined)	6321	(117%)
b (roots separated)	5392	(100%)

3.2 OUTPUT OF NITROGEN 1963-65

In general terms the establishment year produced about a hundredweight of elemental nitrogen per acre, and more specifically the three main treatment "2", "4" and "6" cuts per annum yielded 90.11, 117.44 and 188.82 lb of nitrogen per acre respectively, remembering that only half the treatment cuts were applied in this first year. Statistical examination of the data verified what was, at first sight apparent, that the infrequently cut plots gave significantly lower nitrogen yields than the medium and high cutting frequency and that "4" cuts and "6" cuts gave similar levels of productivity. Segregating the roots of the grass and clover components did not affect the total amount of nitrogen harvested from the plots, the yield was 108.56 lb of nitrogen per acre compared with the control plot yield of 109.04 lb (Table V in appendix). This emerged in spite of higher dry matter production from the control area, where root systems were combined. The explanation being that the higher clover contribution to total dry matter on treatment b, with its correspondingly higher nitrogen content together were sufficient to outweigh any advantage through total dry matter yield enjoyed by the controls (treatment a).

In 1964 the amount of nitrogen harvested was approximately double that of the establishment year and the main treatments in order of increasing defoliation produced 139.29, 197.20 and 217.92 lb of nitrogen per acre as a total yearly production (Table XXIII Appendix). These results were highly significant and were similar to much of the published data relating cutting frequency and yield. At these cutting frequencies the clover component was responsible for approximately 40%, 55% and 62% of the total nitrogen yield which is a familiarly significant pattern. It indicates the differential effect of cutting frequency on the two components namely that low rates will favour grass whereas high rates will favour the less aggressive clover.

The effect of root segregation on total nitrogen yield again proved to be insignificant with 188.56 lb being harvested from the root segregated plots compared with 181.04 lb of nitrogen from those without barriers below ground. A lower total dry matter yield in 1964 on these plots with separated grass and clover components was offset by a higher percentage clover contribution and thus when nitrogen yields are examined they appear similar to the control.

The examination of the third year data for nitrogen yield (Table XLIV) again reveals a familiar pattern in that 2, 4 and 6 cuts are responsible for increasing yields, 107.53, 184.22 and 245.56 lb of nitrogen being harvested per acre respectively. The clover component contributed 38%, 55% and 67% towards this total nitrogen yield and thus indicates no significant change from the previous year. All three years produced very similar trends as can be seen in the above table and the overall effect as measured by the three yearly mean was very pronounced in favour of the highest rate of defoliation. (p. 84)

Yield of nitrogen in lbs. per acre

Main Treatment	1963	1964	1965	Mean
2 cuts	90.11	139.29	107.53	112.31 (100)
4 cuts	117.44	197.20	184.22	166.27 (148)
6 cuts	188.82	217.92	245.56	217.43 (194)

In the final year there appeared a highly significant difference in total nitrogen yield between the two subsidiary treatments which amounted to a 19% increase where the root systems were combined. (Table XLIV). This verifies the field observations apparent during April and May 1965 which were recorded in plates 7 and 8 signifying that nitrogen transference from clover to associated grass species was at last demonstrable. The effects of nitrogen transference were very marked during the early part of the year with much more vigorous grass of a darker hue from those plots without root segregating barriers. Mid-season and autumn growth did not demonstrate the large differences recorded in the spring and this can be verified on examination of the individual treatment cuts for 1965 when arranged in chronological order. (See table overleaf). This phenomena may be correlated with the nitrogen released through nodule drop from the previous year. It certainly appears this way in that major significant differences in nitrogen uptake as measured through nitrogen yield were only demonstrable in the spring.

Table C.

1965 Nitrogen yields from the individual cuts in chronological order.

(Grass N + clover N - lb N/acre)

*E

Treatment	1st: 6 Cut April 27th	1st: 4 Cut May 11th	1st: 2 Cut May 25th	2nd: 6 Cut May 26th	3rd: 6 Cut June 22nd	2nd: 4 Cut July 1st	4th: 6 Cut July 27th	3rd: 4 Cut Aug. 31st	5th: 6 Cut Sept. 1st	4th: 4 Cut Oct. 5th	2nd: 2 Cut Oct. 5th	6th: 6 Cut Oct. 12th
(a) Root systems combined	24.51 (163)	33.76 (148)	47.59 (153)	36.24 (147)	45.82 (103)	54.42 (117)	57.08 (111)	79.27 (107)	64.08 (106)	30.64 (111)	74.81 (121)	35.26 (110)
(b) Root systems segregated	15.01 (100)	22.75 (100)	31.04 (100)	24.66 (100)	44.64 (100)	46.32 (100)	51.24 (100)	73.76 (100)	60.43 (100)	27.53 (100)	61.61 (100)	32.14 (100)
	Major differences between treatments easily visible in the field				Relatively small differences in respect of total nitrogen yield but treatments could be identified on visible examination through a much more vigorous grass component except where indicated by - *E							

In 1965, as in previous years, the clover on treatment b was responsible for a significantly higher contribution to total nitrogen yield than on treatment a, 59.22% as against 47.82%. This was an apparent effect rather than a real one since the actual contribution in each case was similar:-

Treatment a 47.82% of 199.50 lb = 95.01 lb directly contributed by clover in 1965.

Treatment b 59.22% of 163.71 lb = 96.95 lb directly contributed by clover in 1965.

It was fortuitously caused by a higher contribution to total nitrogen yield by the grass component on treatment a.

3.3 CHEMICAL COMPOSITION OF THE HERBAGE 1963-1965

The two measurements of chemical composition, namely % dry matter and % nitrogen were similar for each of the subsidiary treatments when the establishment cut was taken on July 9th, 1963. Early treatment cuts in the first year showed small consistent increases in the % D.M. on the clover component of these plots. A simultaneous decrease in the % nitrogen of this dry matter on treatment a in respect of both grass and clover resulted. These synergistic trends of increasing dry matter percentage and decreased nitrogen concentration were due mainly to the initial root growth which developed unimpeded compared with that on treatment b which was temporarily restricted. Confirmation of this temporary restriction emerged from the data of the cuts made later on in the season when % D.M. and % nitrogen appeared unaffected by the introduction of barriers (Tables II and III).

No significant trends and few significant differences were recorded in respect of chemical composition in 1964. Results in general from this year were reviewed as a whole by using weighted means and including all possible data for that year in table D and for 1965 in table E.

% D.M. and % N in 1964 (weighted means)

Table D

Treatment	2 Cuts				4 Cuts				6 Cuts			
	% D.M.		% N		% D.M.		% N		% D.M.		% N	
	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover
a	21.77	13.66	1.496	3.59	17.07	11.53	2.110	3.919	17.48	11.87	2.479	4.13
b	22.05	13.94	1.560	3.68	16.94	11.19*	2.076	3.865*	17.30	12.41	2.503	4.03

[Extract from Tables VIII, XIII and XX]

(* Significant at P = 0.05)

% D.M. and % N in 1965. (Weighted means) Table E

	2 Cuts				4 Cuts				6 Cuts			
	% D.M.		% N		% D.M.		% N		% D.M.		% N	
	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover	Grass	Clover
a	15.74	10.84	1.81	3.39	16.11	10.80	2.18	3.56	15.33	11.17	2.76	4.08
b	15.38	10.59	1.96	3.61	16.92	10.56	2.06	3.58	16.53	11.57	2.56	4.08
					*				**		*	

[Extract from Tables XXIX, XXXIV and XLI].

(**, * significant at P = 0.01 and 0.05 respectively).

The trend of increasing nitrogen content in the grass component, emerging from the above figures would suggest that nothing was to be gained from the clover at low defoliation rates and that it was only when at least four cuts were made during the season that a positive benefit could be demonstrated. Statistical examination of this data in the form of a split plot analysis, using all the available measurements in 1965 to produce weighted means, appears in full in the appendix Table XLVIII, an extract from which appears below:-

<u>% Nitrogen in the grass.</u> (Weighted means 1965)

	a	b	Mean	
2	1.81	1.96	1.89	
4	2.18	2.06	2.12	S.E. ± 0.04
6	2.76	2.56	2.66	
Mean	2.25	2.20		

S.E. ± 0.02

S.E. of figures within the table ± 0.03

[Extract from Table XLVIII]

The effect of infrequent defoliation, which favoured the grass, thus shading the less aggressive clover was more pronounced where the root system of the two species had not been isolated. The introduction of root barriers had helped to offset this tendency and should there be lower grass yields on treatment b this could result in a higher % nitrogen, as was experienced here. When the herbage was removed four or six times during the season the clover component was favoured. This in turn fixed more atmospheric nitrogen an increasing amount of which was surplus to the clover requirement and found its way to the associated grass. Differences in nitrogen % in the grass between treatments a and b showed the following trend:- -0.15%, +0.12% and +0.20% and was highly significant in the statistical analysis. (Interaction [2.4.6.] x [a v b] Table XLVIII).

In the final year, chemical analyses revealed nothing between the subsidiary treatments when defoliated twice, little at the medium defoliation frequency and some highly significant differences at six cuts per year.

3.4 GRASS AND CLOVER COMPARED WITH GRASS PLUS FERTILIZER N

3.41 Dry matter yield

The main trial involving grass and clover and also the grassland observation plots were sown in 1963 and comparisons of productivity are best achieved by a study of their yields in 1964 and 1965 on well established swards. Details of the individual cuts from the perennial ryegrass plots with fertilizer nitrogen appear in Tables XVI-XXV in Appendix B and are reported on at length in Section V (p.121). Here a comparison is made between these plots and those of the main trial in order to answer the following questions.

How much fertilizer N has to be applied to a pure stand of New Zealand perennial ryegrass in order to obtain

(i) the same dry matter yield

and (ii) the same nitrogen yield as that harvested from the mixed stand of grass and clover in the main trial?

The pertinent yields have been drawn up in the following table which summarises the data for both years.

Table F.

Level of total dry matter output in 1964 and 1965. (lb D.M. per acre).

A comparison of grass and clover versus grass with added fertilizer nitrogen

	1964				1965				
	Grass + clover yield		Grass yield			Grass + clover yield		Grass yield	
	a	b				a	b		
2 cuts	7,891	6,616	N_0	5,131	2 cuts	5,810	3,782	N_0	3,985
4 cuts	7,295	6,942	N_1	9,092	4 cuts	7,294	6,162	N_1	7,508
6 cuts	6,597	6,956	N_2	11,405	6 cuts	7,612	6,523	N_2	9,619
Mean	7,261	6,838	N_3	12,989	Mean	6,905	5,489	N_3	10,919

Key:- N_0 - Control plot, no fertilizer nitrogen.

N_1 - 94.08 lb of nitrogen per acre per annum.

N_2 - 188.16 lb of nitrogen per acre per annum.

N_3 - 282.24 lb of nitrogen per acre per annum.

In 1964, the maximum amount of dry matter, namely 7,891 lb per acre, was obtained from these plots with combined rooting systems which had been cut twice in the year. However, since the grass plots received four cuts throughout the season it would perhaps be more appropriate to compare them with those of main treatment 4 - 7,295 lb of dry matter per acre. This yield is lower than that obtained from grass with 94.08 lb of fertilizer nitrogen added. Assuming that the response to N_1 is linear and using the factor of 42.1 lb of dry matter per pound of nitrogen applied (Table XX Appendix B) by interpolation the gross effect of clover can be equated to approximately 51 lb of fertilizer nitrogen when yield of dry matter is considered.

The corresponding figure for dry matter yield in 1965, from the grass and clover plots was 7,294 lb per acre and this again appeared lower than that obtained from grass alone at N_1. The gross effect of clover in the third year, calculated in a manner similar to that just described but using the 1965 incremental factor of 37.4 lb of dry matter per pound of nitrogen applied (Table XXV Appendix B), could be considered equal to the effect of 89 lb of fertilizer nitrogen when applied to a pure grass stand. This increased value of clover in 1965 compared with the previous year was anticipated since it was thought that the soil contribution towards the general nitrogen economy would diminish with time. It could also be due to a more homoplectic root association between the two species.

These results indicate a lower gross effect from the clover in respect of dry matter yield than the published data which has been summarised in Section I (page 20). Holmes and MacLusky 1955, working with several grasses over a five year period showed that on average clover was equivalent to 121 lb of fertilizer nitrogen applied to grass alone. This figure is the nearest to the findings of this experiment and is perhaps not surprising since both sets of yield trials were carried out in the West of Scotland and within a mile radius of each other. The actual difference experienced between these two sets of data could be accounted for in the fact that root systems of material grown in separate rows 6" apart would be less closely knit than in broadcast swards and may result in smaller and-or later nitrogen benefits from legume to associated grass species.

3.42 Nitrogen yield

Results for comparing the total amount of nitrogen harvested from the main trial compared with that from grass with added fertilizer nitrogen are set out below. Both 1964 and 1965 have been included and the information in respect of yields from the grass only plots has been extracted from tables XVI-XXV in appendix B a longer discussion of which appears in Section V.

Table G

Level of total nitrogen yield in 1964 and 1965. (lb of nitrogen per acre)

A comparison of grass and clover versus grass with added fertilizer nitrogen

1964	Grass and clover yield a	Grass and clover yield b	Grass yield		1965	Grass and clover yield a	Grass and clover yield b	Grass yield	
2 Cuts	138.64	139.94	N_0	88.82	2 Cuts	122.40	92.65	N_0	65.03
4 Cuts	197.74	196.66	N_1	154.65	4 Cuts	198.10	170.35	N_1	128.25
6 Cuts	206.76	229.08	N_2	197.83	6 Cuts	262.99	228.12	N_2	195.02
Mean	181.04	188.56	N_3	263.75	Mean	194.50	163.71	N_3	283.14

Key:- N_0 - Control plot, no fertilizer nitrogen
N_1 - 94.08 lb of nitrogen per acre per annum
N_2 - 188.16 lb of nitrogen per acre per annum
N_3 - 282.24 lb of nitrogen per acre per annum.

In 1964, 197.74 lb of nitrogen were harvested from those plots on the main trial which were defoliated four times during the year and this was identical with that obtained from the grass only plots with 188.16 lb of fertilizer nitrogen added. A very similar yield of nitrogen, namely 198.10 lb, was obtained the next year from the corresponding treatment and by interpolation this meant that the gross effect of the clover would be equal to approximately 191 lb of fertilizer nitrogen applied to a pure grass stand.

These results fall within the range expressed in the summary table on page 21 of the introduction but are a little on the low side when compared with the findings from experiments carried out in Britain. Again the data collected by Holmes and MacLusky 1955 at the Hannah Dairy Research Institute is the nearest to that reported in these experiments. The reason for the slightly lower gross values of clover in the trials at Auchincruive may again be attributed to the sowing method practised. Establishing grass and clover in separate rows six inches apart could mean a long delay in complete root integration whereas an immediately intermingling is likely with a broadcast sward. Under such circumstances it would be reasonable to expect a lower gross value from the clover component through a less efficient underground transfer of nitrogen where the two species start off some distance apart.

3.43 Maximum value of clover

By using different defoliation frequencies it was possible to obtain higher yields of dry matter and nitrogen from the main experiment than those used in the previous comparison. The maximum gross value of clover grown in free association with grass was as follows:-

Table H

Amount of nitrogen fertilizer required (lb N/acre) by grass alone to replace the gross effect of clover in the experiment

	Equity on dry matter yield basis	Equity on N yield basis
1964	66 (2 cuts)	201 (6 cuts)
1965	99 (6 cuts)	261 (6 cuts)

3.44 Amount of nitrogen harvested as a result of underground nitrogen transference

Only in the third year of the experiment was there any evidence of underground nitrogen transference. Those areas of the experiment where clover roots and grass roots were not allowed to come in contact produced 163.71 lb of total nitrogen compared with 194.50 lb of nitrogen from the plots with unrestricted rooting systems. With similar above ground conditions this would suggest an underground transfer of nitrogen, 31 lb of which was capable of being harvested through the grass. The nitrogen yields harvested from the mixed stand ranged between those obtained from N_1 and N_2 fertilizer level applied to grass alone. Within these levels 66.77 lb of herbage nitrogen was harvested thus suggesting that for each one pound of applied nitrogen 0.709 lb could be recovered. On this basis the actual difference between treatments a and b, namely 31 lb of nitrogen would be equivalent to approximately 44 lb of fertilizer nitrogen applied to grass.

3.5 EXPERIMENTAL FINDINGS OF 1965 COMPARED WITH PREDICTED DATA USING THE THEORY PUT FORWARD BY WALKER, ORCHISTON AND ADAMS (1954) ON THE CLOVER CONTRIBUTION IN MIXED SWARDS.

Walker and his colleagues working at Canterbury, New Zealand, and incorporating many other results from England and America produced a theory which would evaluate the clover contribution to grass nitrogen (G_n) in a mixed sward.

$$G_n = a\,S_n + b\,C_n + c\,F_n$$

Where S_n = Soil nitrogen

C_n = Clover nitrogen

F_n = Fertilizer nitrogen, and a, b and c are constants.

From the various sets of data correlations were obtained and multiple regressions (method of least squares) compounded by keeping one of the factors constant and the above authors arrived at the following expression:-

The Approximate Clover Contribution = Total Nitrogen in the grass − 170 x per cent N in the Soil.

In the following table this theory has been used to predict the clover contribution to grass nitrogen, it has also been calculated using the grass observation plots and these are compared with direct measurements made in the main experiment which were reported earlier. The predicted value of clover contribution to grass nitrogen when both species are in free association, obtained from the theory of Walker and his colleagues of 52.27 lb of nitrogen is much higher than the

Table I

Clover contribution to grass nitrogen - a comparison of results and predictions

1965 Yield data from 4 Cut plots without root segregation

Total production of nitrogen (lb N/acre)	Grass nitrogen (lb/acre)	Clover nitrogen (lb/acre)	
198.10	97.66	100.44	Experimental data from the main trial

Grass nitrogen (lb/acre)		
97.66 lb	Soil contribution 45.39 lb (soil N% only) ∴ Clover contribution 52.27 lb	Predicted Value usi theory of Walker, Orchiston and Adams

Grass nitrogen (lb/acre)		
97.66 lb	Soil contribution 65.03 lb (all sources) ∴ Clover contribution 32.63 lb	Predicted value usi grass observation p without added fertilizer

observed value through the grass plots - namely 32.63 lb of nitrogen. The latter is very close to the average value of 31 lb of nitrogen which emerged from the main trial as the amount of nitrogen harvested in the grass which could be attributed to underground transference. It would appear to indicate that the use of soil N% by itself in predicting the clover contribution to associated grasses could lead to an erroneously high figure. This discrepancy has arisen since little or no credit was afforded to other sources of nitrogen i.e. from the free living soil organisms and from rainfall. These have been discussed in Section I and could well account for the difference between the predicted value (52 lb N) and the observed values of 31-32 lb nitrogen which were harvested in the grass component being the result of subterranean transfer from the legume.

3.6 METEOROLOGICAL DATA

3.61 General weather conditions

In order to obtain some general impressions of weather conditions at Auchincruive Table VII, Appendix B has been included which summarises monthly temperatures, rainfall and sunshine in the form of a twenty year average. From this table it can be seen that 1273 hours of sunshine are to be expected in this district of South-west Scotland, with approximately 204 rain days and a total precipitation of just over 37 inches for the year. In 1963, the establishment year, the overall weather pattern was one of slightly lower total rainfall and above average sunshine hours, with slightly lower temperatures recorded in the air and in the soil. A closer look at the data in respect of the growing season (March-September inclusive) reveals that during this period sunshine was a little over average (+ 5%), in line with the general pattern but that the rainfall during growth was substantially higher than normal (+ 20%). This is not evident from the data totals. (Table VIII, Appendix B).

Monthly weather data for 1964 appears in Table IX, Appendix B indicating that the second year was normal in respect of sunshine hours but rainfall was significantly lower when yearly totals are considered. Restricting observations to the growing season, sunshine hours were down by 6% and rainfall was similar to the twenty year mean. The early spring of 1964 was not as cold as the previous year and temperatures in general were a little higher throughout the season. A Siemens integrating photometer was installed at the beginning of the year in order to record total solar radiation. These measurements of solar energy expressed in calories per square centimetre have been included with the other meteorological data in Appendix B. Tables XI-XIV contain the daily records and Table XV summarises the daily means on a monthly basis for both 1964 and 1965.

Total precipitation in 1965 was very close to the average in a smaller number of rain days whereas sunshine hours were slightly above normal. Restricting the comparison to the growing season, the duration of bright sunshine closely followed the long term mean but rainfall during active growth was 15% higher than usual. Temperatures in general were slightly lower than average and total solar energy was 10% lower than the previous year.

3.62 <u>Comparisons with New Zealand and Holland</u>

The solar radiation received at Auchincruive has been compared with the average recorded in Holland and has already been discussed in Section III (p. 80). The photosynthetic rate of the clover will be affected by solar energy and this in turn will influence the amount of nitrogen fixed and the amount of nitrogen transferred in a grass-legume association through the carbohydrate status of the clover. Since there appears to be a large difference (46%) in the total amount of radiation between the West of Scotland and Holland, this factor alone could account for major variations in nitrogen fixation and nitrogen transference between these areas.

New Zealand farming and weather conditions would appear to induce very high rates of nitrogen fixation by strains of their own white clover, Sears (1960) estimated these for the North and South Islands to be 400-500 lb and 200-250 lb of nitrogen per acre per annum respectively.

Table J. New Zealand and West of Scotland weather comparisons

Country and Place	Average Annual Rainfall (inches)	Average Annual Raindays	Average Annual Sunshine (hours)	Average Annual Temperature °F
New Zealand				
North Island (Auckland	50	184	2033	59
(Napier	32	114	2416	56
(Wellington	43	162	2050	54
South Island (Nelson	38	119	2510	54
(Christchurch	26	126	1967	52
(Dunedin	40	161	1711	50
(Invercargill	45	199	1626	50
West of Scotland				
Auchincruive	37	204	1273	48

(New Zealand weather data taken from "Farming in New Zealand" Published by Department of Agriculture, Wellington, New Zealand).

The above weather summary helps to indicate the major differences in climate between New Zealand and the West of Scotland explaining in part why the white clover in the North Island in particular is able to fix more atmospheric nitrogen. The two main features of difference are hours of sunshine and temperatures. In the case of the former, approaching twice the amount of bright sunshine is recorded in the North Island compared with Auchincruive and at the same time temperatures would appear to be substantially higher. Both these factors would favour more active clover growth over a longer period and could account for the large nitrogen fixation reported in this part of the world.

3.63 Microclimatic temperatures 1964

Readings of maximum and minimum temperatures were taken daily from mid-April to the end of July at ground level using screened thermometers. These were placed between the grass and clover rows and observations were made daily at 9.00 a.m. B.S.T. Details of individual records appear in Tables XXXVI-XL, Appendix B and mean monthly temperatures are summarised in Table XLI. From this summary it appears that the introduction of the polythene barriers did not materially affect the temperatures at ground level.

3.64 <u>Microclimatic temperatures 1965</u>

In 1965 it was decided to look into the ground level temperatures between the three major treatments 2, 4 and 6 defoliation frequencies. At the same time a comparison between the grass and clover sward temperatures and those experienced by grass alone was considered of interest since Johnstone-Wallace (1937) reported significant differences in diurnal fluctuations between these two regimes. Daily records appear in Tables XLII-XLV in Appendix B and mean monthly data is summarised in Table XLVI.

Minimum temperatures on the grass and clover sward appeared to be unaffected by varying the cutting frequency but the maximum temperature showed a general rise with the higher cutting rates. This was due to more sunlight and therefore more heat reaching the ground where the plots were frequently defoliated.

Similar minimum temperatures were recorded on the grass plots but when maximum temperatures of the grass only plots are compared with those of the mixed sward at the same degree of defoliation they are substantially higher, amounting to between 4 and 7°F. The main reason for these discrepancies may result from the differing growth habits of grass and clover. The former may be considered mainly panphotometric whereas the clover with its leaves parallel to the ground tends to be euphotometric.

Working at Cornell and recording diurnal fluctuations of temperature during May at 1" below ground level, Johnstone Wallace (1937) reported a range of 40-70°F on a grass only sward and 47-68°F in the mixed sward. These higher maximum temperatures recorded on the grass sward were similar to those experienced at Auchincruive and the dissimilarity in minimum temperature fluctuations is largely connected with the differential heat-losses exhibited by soil and air. The limited observations of temperature on the trials would therefore appear to corroborate in principle the findings of Johnstone Wallace that the inclusion of clover to a grassland sward would be instrumental in reducing the range of diurnal fluctuations of temperature.

3.7 SUPPLEMENTARY EXPERIMENTS

3.7 SUPPLEMENTARY EXPERIMENTSots sown with inoculated New Zealand white clover. (Certified mother seed as used in the main trial).

Since the general theme in this thesis revolves around nitrogen, the majority of which is likely to result from the symbiotic association of white clover and rhizobial bacteria, it was thought essential to have some overall assessment of the native rhizobia. Manil and Bonnier (1950) showed that improved growth was possible with clover and lucerne after the introduction of a strain of good competitive ability in the presence of the native strain. Under these circumstances they were able to demonstrate that up to 60% of the nodules on the legume could be attributable to the introduced strain of rhizobium.

Inoculated white clover seed was sown on April 19th, 1963 and untreated seed from the same stock was put in simultaneously as a control. An effective strain of rhizobium was used, namely R.157 from Professor Vincent, Sydney, Australia and throughout the growing season assessments of plant height, colour and vigour were made. No visible differences were recorded in the inoculated plants compared with the controls and when nodule numbers, nodule weights and a measurement of nitrogen percentage were taken no substantial variation could be detected. From this very limited evidence it would appear that the indigenous strain of rhizobium at Auchincruive was an effective one and it may be comparable with the introduced strain R.157.

3.72 Grass observation plots

These were established in 1963 and observations were made on them during the next two years. They received various levels of nitrogenous fertilizer in the form of nitro-chalk and were cut four times during the growing season coinciding with the defoliation of plots on main treatment 4 from the mixed sward. Details of individual cuts appear in Tables XVI-XIX and XXI-XXIV, with yearly summaries in Tables XX and XXV in Appendix B. They are reported on in detail in Section V, p. 121, and due to their proximity to the main trial the results have been used for comparisons with the grass and clover trial.

Green and Cowling (1960) have suggested that on average the soil would not be capable of supplying more than about 50 lb of nitrogen per acre per annum for the growth of herbage. The results from these observation plots suggest that some soils of the West of Scotland may be capable of larger nitrogen releases since the total amount of nitrogen harvested in the year was 89 and 65 lb per acre in 1964 and 1965 respectively. This was to be expected because the organic matter levels are notably higher in the north and west of Britain thus enhancing the soil nitrogen status. Once the soil nitrogen contribution has been estimated the amount fixed by clover in a mixed sward may be deduced. On this basis it may be assumed that about 109 and 133 lb of nitrogen were fixed per acre during 1964 and 1965 respectively. These assessments are approximate since it is assumed that the uptake of combined nitrogen by grass and clover is the same as that by grass alone. It may very well be that the clover of a grass-clover sward is not as demanding on combined soil nitrogen since it is not totally dependent on this source for its nutrition. From this hypothesis it would be reasonable to assume that at least 109 and 133 lb of nitrogen were fixed per acre in the two years 1964 and 1965.

3.73 Physical effects of a double layer of 500 gauge black polythene

Although the reduction of plot width by the introduction of eight barriers of double layer 500 gauge black polythene is very small (0.17%) it was deemed necessary to investigate the physical effects of this treatment on the yield of a grass and clover sward. A special trial was sown for this purpose, containing grass and clover of the same cultivars and at similar seeding rates to those used in the main trial. By introducing the underground barriers at right angles to the line of sowing an assessment could be made of any physical effects of the material without interfering with underground nitrogen transference between the species. Results from this trial have been reported in Section V (p. 125) and they contain details of three cuts taken in 1964 and four cuts the following year. There were no significant differences in yield between the polythene plots and the controls, neither were there any significant variations in chemical composition of the herbage. Trace element analyses on the herbage in July 1963 and May 1965 (Tables III-VI Appendix B) indicate that the uptake of minor elements was not affected by introducing the polythene root barriers. Conclusive evidence is therefore obtained to demonstrate that the physical effects below ground are minimal by the introduction of these thin barriers.

3.74 Permeability tests on the polythene

Two tests on the permeability of the polythene barrier material were made, one in the laboratory and the other in the field and these have been reported in detail in Section V (p. 127). These were initiated in order to examine the effectiveness of polythene in its ability to restrain the movement of nitrogenous substances. Under controlled conditions two layers of polythene were able to prohibit the passage of L-aspartic acid to its surrounding jacket of distilled water. In the field this barrier material was tested by applying a large quantity of nitrogenous fertilizer (752 lb of nitrogen) in four applications to grass grown in rows. Grass growing alongside this but separated by polythene was compared with control plots to see whether any of the unused ferilizer nitrogen had percolated through. No significant differences were recorded in dry matter yield, nitrogen yield or chemical composition of the herbage from the four cuts taken in 1965. The overall average percentage nitrogen in the dry matter was 2.185 and 2.105% for grass alongside the fertilizer compared with the control plots respectively. The closeness of these means and the fact that significant variations in the individual cuts were absent suggest that the barriers have proved effective.

Total nitrogen uptake from the soil was 83.47 lb of nitrogen throughout the year from the control plots (Treatment C in Tables XXXIII-XXXVI) and 88.31 lb from treatment B - i.e. those plots in close proximity to fertilizer application but separated by polythene. These are sufficiently close to each other and to the figure obtained from the grass observation plots in 1964 (88.82 lb of nitrogen harvested) to confirm that the fertilizer nitrogen had not migrated through the barriers.

One interesting and unexpected feature from these permeability test plots was the high recovery of fertilizer nitrogen applied. From the grass observation area with the differential nitrogen levels, an overall recovery of 51% was recorded at the maximum fertilizer application of 564 lb of nitrogen. It was decided to apply a much higher rate to the permeability test area in order that a reasonable amount of nitrogen would be in excess and available for percolation should the barriers prove permeable. The polythene has acted as a concentrating barrier as indicated by the percentage recovery figures in the following table, where individual and overall results are compared with data from the grass observation plots. (Figures extracted from Tables XVI-XXV Appendix B).

Table K

A comparison of the percentage recovery of added fertilizer nitrogen, grass plots versus permeability test plots

% Recovery of fertilizer nitrogen

	1st Cut	2nd Cut	3rd Cut	4th Cut	Overall % recovery
1964 Data	April 24th	June 15th	August 5th	October 7th	
Grass plots receiving 564 lb N/acre/annum	34.3	69.3	54.5	47.6	51.4
1965 Data	May 4th	June 29th	July 29th	September 28th	
Permeability test plot receiving 752 lb N/acre/annum.	41.8	89.4	74.5	100.7	76.6

From these results it could be inferred that the polythene barriers have proved successful in restricting the migration of the fertilizer nitrogen through the significantly higher percentage recovery figures obtained.

4. <u>SECTION IV SUMMARY AND CONCLUSIONS</u> .

4. SECTION IV SUMMARY AND CONCLUSIONS

1. An experiment was conducted over the period 1963-65 with New Zealand cultivars of *Lolium perenne* (L) and *Trifolium repens* (L). They were sown in alternate rows, 6" apart, and half the plots in the trial area were established to maintain root segregation between the species. The method adopted is described and illustrated by photographic plates, and revolves around the introduction of double layers of 500 gauge black polythene between the grass and clover components. The statistical layout involved was a split-plot design, with four replicates, three main treatments of 2, 4 and 6 defoliations per annum and two subsidiary treatments corresponding to root segregation on the one hand compared with normal root integration on the other. Throughout the period of these investigations the mixed sward received liberal quantities of phosphate and potash fertilizer but relied upon soil mineralisation, fixation by free-living organisms, rainfall and symbiotic fixation for its nitrogen nutrition.

2. The yearly output of dry matter from this perennial ryegrass-white clover sward was approximately 4,000, 7,000 and 6,000 pounds per acre for 1963, 1964 and 1965 respectively.

3. Variations in defoliation frequency during 1963 and 1964 had little effect on dry matter yield, but in the final year a drop of 2,000 pounds was recorded at the lowest rate.

4. The overall mean yield of dry matter from 2, 4 and 6 cuts per annum amounted to 5,300, 6,100 and 6,000 pounds per acre per year.

5. In 1963 and 1964, the output of dry matter from the medium cutting frequency (4) was 300 pounds more than that recorded at the

highest rate (6) but the following year this tendency was reversed.

6. Root segregation was responsible for an 18% reduction in dry matter yield during the establishment year and it is suggested that the check to root growth which was experienced on encountering the polythene barriers may be the main cause. In 1964 a similar pattern was recorded, with a 6% lower yield from those plots where the grass and clover were separated below ground compared with plots with integrated rooting systems. As a result of this lower reduction in dry matter yield and from other data namely nitrogen yield and chemical composition it is inferred that the reductions in yield in these two years are not due to the arrest of underground nitrogen transference from clover to associated grass.

7. In 1965 large, visible differences in yield between root segregated and root integrated plots were recorded. In terms of dry matter yield over the season as a whole this amounted to 26% and was significant at P = 0.001.

8. The percentage clover contribution to dry matter yield (mean of 1964 and 1965 results) was affected both by cutting frequency and the introduction of root barriers. When cut twice during the year clover supplied 24% of the dry matter yield and this rose to 41% and 54% as defoliation frequency increased. Where the two species had integrated rooting systems the clover contributed 34% towards the dry matter yield. Underground barriers, by confining the root systems of each species to their own territory, reduced the competitive ability of the grass and enhanced the value of the clover whose contribution to dry matter yield rose to 44%.

9. The mean yield of nitrogen from this grass-legume association during the three years 1963-1965 was 132, 184 and 179 pounds of elemental N per acre/annum. The low output in 1963 is a combination of slow growth in the maiden year compared with established swards and also the fact that half the number of defoliations were employed by comparison with succeeding years.

10. The average yearly production of nitrogen during the experimental period from 2, 4 and 6 cuts was 112, 166 and 217 pounds of nitrogen per acre respectively which meant a 48% increase by doubling the cutting frequency and a 94% increase when three times the number of defoliations were employed.

11. Segregating the roots of perennial ryegrass and white clover when grown in close association had no effect on the total amount of nitrogen harvested in the first two years.

	lb of nitrogen harvested/acre/annum	
	Segregated	Integrated
1963	108.56	109.04
1964	188.56	181.56

These results can be directly attributed to a higher proportion of clover in the dry matter yield where barriers had been introduced.

	% clover contribution in D.M. Yield	
	Segregated	Integrated
1963	41.75	26.32
1964	41.31	32.20

12. In 1965, however, there was a significantly higher nitrogen yield from those plots with combined root systems compared with those where barriers had been introduced. The difference was of the order

of 19% and amounted to 30.79 lb of elemental N. These results confirm the visual observations of April and May 1965 which have been recorded in plates 7 and 8. They indicate that under the conditions of this experiment the effects of underground transference of nitrogen between white clover and perennial ryegrass are first demonstrable in the spring of the third year.

13. On the whole the interaction between main and subsidiary treatments in respect of green weight, dry matter 'production' and nitrogen yield proved insignificant, the only exception to this general trend was in the dry matter yield in 1964.

14. The nitrogen economy of a perennial ryegrass - white clover association has been studied and the 1965 clover contribution to grass nitrogen is compared with a predicted value using the theory of Walker, Orchiston and Adams. From the main trial 30.79 pounds of nitrogen can be ascribed to clover via underground transference whereas a value of 52.27 pounds is obtained from Walker's theory. Using the data from the grassland observation plots to estimate the total nitrogen contribution from the soil, clover contribution to grass nitrogen in a mixed stand in theory should amount to 32.63 pounds of nitrogen during the year.

15. It is suggested that Walker's theory may lead to an erroneously high figure when predicting the clover contribution to grass nitrogen in mixed swards since it does not recognise free-living organisms and rainfall as significant sources of nitrogen.

16. From an established sward of perennial ryegrass and white clover, drilled in alternate rows and cut four times during the season,

the total amount of dry matter harvested in 1964 and 1965 was the same as that obtained from perennial ryegrass only with approximately 50 and 90 pounds of added fertiliser nitrogen respectively.

17. Similarly, the amount of nitrogen harvested in 1964 and 1965 from the mixed stand was equivalent to that from a grass monoculture receiving 188 and 191 pounds of fertiliser nitrogen respectively.

18. By varying the cutting frequency it was possible to obtain slightly higher gross values from clover in the experiment.

Amount of fertiliser nitrogen required, in pounds of Nitrogen per acre, by grass alone to replace the gross effect of white clover

	Equity on Dry Matter yield basis	Equity on Nitrogen yield basis
1964	66	201
1965	99	261

19. Chemical composition of the clover as measured by % D.M. and % N in 1964 and 1965 appeared unaffected by introducing below ground barriers. The % D.M. (weighted means) was not influenced by cutting rates but higher defoliation frequencies lead to small consistent increases in the % N in the legume.

20. Quality determinations on the perennial ryegrass component indicated a significantly higher average % D.M. at low cutting rates and the effect of root segregation showed no consistent trend in the first two years. During the last year, however, the below ground

barriers produced lower dry matter percentages and higher nitrogen percentages in the grass compared with the herbage from the control plots with integrated rooting systems. As defoliation frequency increased the level of N% in the grass rose, 1.89, 2.12 and 2.66 %N were the weighted means obtained from 2, 4 and 6 cuts per annum respectively.

There was also a significant interaction between main and subsidiary treatments in respect of the %N in the grass component in 1965. Infrequent cutting favoured root segregation, whereas frequent defoliation produced higher nitrogen contents where the grass and clover were grown normally.

21. From the limited evidence of the additional trials and observations on inoculated clover seed it would be reasonable to assume that the indigenous strain of rhizobia at Auchincruive was an effective one.

22. Observations on micro-climatic temperatures at ground level partially corroborate the findings of Johnstone-Wallace who showed lower diurnal fluctuations of temperature with a mixed sward compared with grass alone.

23. A general account of the meteorological conditions during the trial period is given and comparisons with long term averages are studied. Comparisons with New Zealand and Holland are also made, the most noteable features of climatic difference being in solar radiation. It would appear that the North Island of New Zealand will receive twice as much sunshine, and Holland records show 46% more total solar energy compared with Auchincruive.

24. The physical effects of introducing a double layer of 500 gauge black polythene have been measured and are reported and laboratory and field tests on the permeability of this material are discussed.

5. SECTION V SUPPLEMENTARY EXPERIMENTS AND TESTS.

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5.1 GRASS OBSERVATION PLOTS

5.11 Preliminary details

Seven grassland observation plots were established next to the main trial on April 8th, 1963. Lime, phosphate and potash had previously been applied and worked into the soil and New Zealand Certified Mother Strain perennial ryegrass at 20 lb per acre was sown in rows 6" apart. Brairding was complete by April 24th and throughout the remainder of the year the area was hand weeded and topped when necessary. In 1964 and 1965 these grassland observation plots had a basic management treatment similar to that of the main trial. They were cut four times each year on the same day as the 4 Cut plots, the only difference being that they received differential levels of inorganic nitrogenous fertilizer. The object of these plots was to indicate the approximate level of fertilizer nitrogen required by grass alone to equal the yield of dry matter and nitrogen simultaneously obtained by a mixed sward as in the main study.

5.12 Yield data 1964

During 1964 the calendar of events was as follows:-

	N Fertilizer application	Cutting date
1st	March 3rd	April 24th
2nd	April 27th	June 15th
3rd	June 16th	August 5th
4th	August 6th	October 7th

The nitrogen fertilizer was applied to the appropriate plots

6-8 weeks in advance of the date of cutting and on that date the plots were weighed and sampled for D.M. and nitrogen analyses as in the main study. The yield data from the individual cuts, namely green weight, dry matter yield and nitrogen yield appears in the appendix together with estimates of percentage recovery of fertilizer nitrogen in the herbage and the increment of dry matter yield per pound of fertilizer nitrogen applied. An overall picture is obtained from the summary table which indicates the level of total production for the year.

A yield of 12.576 tons fresh material, containing 5,131 lb of dry matter with 88.82 lb of nitrogen was obtained from the control plot without added fertilizer nitrogen. This is a high yield from grass relying solely on soil nitrogen for its nutrition and it indicates perhaps a slightly higher nitrogen contribution from the soil than was anticipated in Section 3 (p. 99) The application of increasing quantities of fertilizer nitrogen was responsible for corresponding increments in yield and at the highest level of combined nitrogen (564.48 lb of nitrogen per acre throughout the season - 24 cwts. of 21% N Nitro-chalk) 47.302 tons of fresh material resulted, containing 15,724 lb of dry matter with 379.21 lb of nitrogen.

The overall percentage recovery of the nitrogen applied ranged from 70% at the low dosage rate to 51% at the highest level and the concomitant increment of dry matter yield per pound of nitrogen applied varied between 42 lb and 17 lb.

5.13 Yield data obtained in 1965

The pattern of events during 1965 was similar to that of the previous year except that the penultimate nitrogen level was omitted due to rodent activity on the plot.

	N Fertilizer application	Cutting date
1st	April 9th	May 11th
2nd	May 11th	July 1st
3rd	July 2nd	August 31st
4th	August 31st	October 5th

The yield data from individual cuts appears in the appendix together with a summary of total productivity for the year and estimates of nitrogen recovery. Compared with the previous year the general level of yield was considerably lower.

From the control plot (no fertilizer nitrogen) a yield of 8.138 tons of fresh material was harvested containing 3,985 lb of dry matter with 65.03 lb of nitrogen. In 1965 the application of increasing quantities of nitrogenous fertilizer again resulted in corresponding increments in yield. These were, however, smaller than in 1964 and in spite of some interference through field mice, there was evidence to suggest that application of nitrogen in excess of 376.32 lb per acre would be unfruitful.

The recovery of nitrogen was similar to 1964, ranging from 67% to 46% as the fertilizer level increased, and a similar scale of dry matter yield increments per unit of fertilizer nitrogen applied was recorded. When the total amount of nitrogen fertilizer in the year amounted to 94.08 lb per acre, each pound of this was responsible for a yield increase of 37.4 lb of dry matter whilst at the highest nitrogen level 564.48 lb, each pound of nitrogen gave 14.5 lb of dry matter.

5.2 FIELD TRIALS TO MEASURE THE PHYSICAL EFFECT, IF ANY, OF THE BLACK POLYTHENE USED AS BARRIERS IN THE MAIN TRIAL

5.21 Preliminary details

Although the double layer of black polythene used as underground root segregating barriers was only 0.01" thick, thus reducing the effective plot width by 0.08", (equivalent to 0.17%) it was considered essential to have an estimate of its physical effect. A field trial was established during March 1964 for this purpose. The land prior to sowing had an application of lime to correct the pH to 6.25 and 112 lb of P_2O_5 and 112 lb of K_2O were worked in as a basal fertilizer dressing. The plots were established in a manner similar to that used for the main trial and followed the pattern illustrated by Plates 1 to 6. Sowing was done in rows, this time at right angles to the polythene barriers in order that both sets of plots had grass and clover with integrated rooting systems. New Zealand cultivars of perennial rye-grass (20 lb per acre) and white clover (2 lb per acre) were sown in alternate rows 6" apart on March 23rd and 24th.

5.22 Details of the 1964 cut

Three cuts were taken from these plots in 1964, an establishment cut on July 15th and two further ones on September 2nd and October 15th.

No significant differences in production between the two sets of plots were recorded either as dry matter or nitrogen yield and no consistent difference appeared in the chemical composition of the herbage (Tables XXVI-XXVIII Appendix B).

It is concluded that in the establishment year physical effects from introducing the polythene were insignificant.

5.23 Details of the 1965 cuts

Full details of the four cuts taken on May 18th, July 6th, September 7th and October 19th are found in Tables XXIX-XXXII in appendix B, an extract from which appears below:-

Treatment	May 18th		July 6th		Sept. 7th		Oct. 19th	
	Total D.M. yield lb/ acre	Total nitrogen yield lb N/ acre	Total D.M. yield lb/ acre	Total nitrogen yield lb N/ acre	Total D.M. yield lb/ acre	Total nitrogen yield lb N/ acre	Total D.M. yield lb/ acre	Total nitrogen yield lb N/ acre
Polythene	3,286	49.25	2,000	47.25	2,316	75.39	892	34.41
No polythene control	3,084	44.58	2,080	49.55	2,257	73.15	868	33.13
S.E.	± 59 N.S.	±1.58 N.S.	± 52 N.S.	±2.05 N.S.	± 47 N.S.	±1.12 N.S.	±19 N.S.	±0.96 N.S.

As in 1964, there were no significant differences in dry matter yield or nitrogen yield between the polythene plots and the control and the grass : clover ratios in both these yield parameters were almost identical for each set of plots. Similar chemical composition of the herbage was also encountered.

These results are similar in every respect to those previously obtained and thus confirm the hypothesis that the physical effect of the polythene introduced in the main trial for underground root segregating barriers is insignificant.

5.3 PERMEABILITY TESTS ON THE POLYTHENE

Thin polythene sheeting is not completely impervious and it was therefore deemed necessary to test its permeability with nitrogenous substances both in the laboratory and under field conditions.

5.31 Laboratory tests

5.31 Laboratory tests

It was decided to use L-aspartic acid in the laboratory tests as Virtanen and his co-workers had shown this to be the major nitrogenous excretion product from nodules. A near 0.5% solution of this amino acid was enclosed in a double layer of 500 gauge black polythene and then placed in a beaker surrounded by distilled water. This water jacket was sampled 24 hours, 36 hours, 1 week, 1 month and 6 months later and tested for the presence of L-aspartic acid. The amino acid paper chromatogram technique using nin-hydrin in ethanol was employed. (Consden, Gordon and Martin 1944, as modified by Patton and Chism 1951). Each of the tests proved to be negative indicating that a double layer of 500 gauge black should be a sufficient barrier for this amino acid.

5.32 Field Tests

During the first week of July 1964 a field experiment was established to test the permeability of the black polythene to an inorganic nitrogenous fertilizer. As in the main trial these plots were excavated and the polythene barriers were introduced at 6" intervals. The same New Zealand cultivar of perennial ryegrass was sown at 20 lb per acre between the lines of polythene and nitrogen fertilizer was applied to one out of every three rows. [Plate 9]

PLATE 9

Illustrating the method of root segregation using double layer 500 gauge polythene and also the permeability tests of polythene in the field. (3 white pegs showing line of N fertilizer application to grass rows).

This fertilizer nitrogen was applied in advance of the four defoliations in 1965 at 8 cwt per acre nitrochalk (21% N) per application. The total amount of nitrochalk applied during the season namely 32 cwt per acre, equal to a dressing of 752 lb of elemental nitrogen, was considered in excess of the grass requirement and some would therefore be available for migration. In this experiment there were six replications of the following three treatments:-

A. Grass receiving 188 lb of N per acre per cut.

B. Grass in close proximity to fertilizer nitrogen applied in A but separated by polythene.

C. Control grass without fertilizer nitrogen and distant from that applied to A.

Details of the yields obtained from the four cuts taken on May 4th, June 29th, July 29th and September 28th in 1965 appear in Appendix B (Tables XXXIII-XXXVI). The statistical analysis of each cut indicated no significant differences between treatments B and C in respect of dry matter yield, nitrogen yield, dry matter % or nitrogen percentage.

Treatment	Total D.M. yield lb/acre	Total nitrogen yield N/acre	Mean % D.M. in grass	Mean % N in grass
B	4,300	88.79	14.98	2.185
C	4,098	83.47	15.50	2.105

The total production for the year and mean values for chemical composition are shown in the above table and reiterate the statistical conclusions of the separate analyses.

As a result of both of these tests and bearing in mind the fact that the polythene at the end of the experiment was in good order it would be reasonable to assume that the double layer of 500 gauge black polythene had been a reliable barrier to any nitrogenous excrement from the clover nodules.

5.4 OBSERVATIONS FROM PLOTS SOWN WITH INOCULATED CLOVER SEED

5.41 1963 Data

A small trial to ascertain the effects, if any, of using inoculated clover seed against a non-inoculated control was established in April 1963. This was undertaken to give some indication of the effectivity of the native strain of rhizobia at Auchincruive.

Observations up to the time of sampling, September 23rd, on the height, vigour and colour of the clover indicated no visible or recordable differences between inoculated and non-inoculated plants.

Sowing of these plots had been done in a manner similar to that used on the main trial and to facilitate plant and nodule counts to be done one linear foot of row was extracted per plot. The entire plants were removed together with one foot of soil and this was transferred gently to the laboratory. The soil was carefully removed from the roots by a slow washing process so that nodules remained intact. They were removed from the plants at the time of counting and dried to a constant weight. Details of the results obtained appear in table L overleaf.

Table L

Nodule counts, dry weight and chemical composition

Treatment	Mean number of plants per linear foot of row	Nodule * numbers per linear foot of row	Nodules per plant	Dry wt. of nodules in grams per linear foot of row	Dry wt. of individual nodule micro grams	% N in nodule (on D.M.)
Inoculated	112	609	5.44	0.2299	377	6.56
Control (Not inoculated)	110	568	5.16	0.1657	292	7.55
S.E.	-	± 33	-	±0.0182	-	-

* (The nodules removed from the plant and included in the totals were those deemed to be effective ones. Vestigial, ineffective, immature and decomposed ones were not considered).

The introduced strain of rhizobium was R.157 originating from Sydney, Australia and it is considered to be an effective one. From the limited evidence of 1963, it would appear that the indigenous strain at Auchincruive is fairly comparable with this one. No significant differences could be detected in nodule numbers or in nodule dry weight.

The % nitrogen in the nodules, on D.M. basis, was approximately 7% agreeing well with published data (Wilson, 1942 and Butler and Bathurst 1956).

5.42 <u>1964 Data</u> (Sampled July 6th)

A procedure similar to that in 1963 was adopted, taking one linear foot of row, counting and weighing the nodules. A summary of this data appears in table M, but does not contain the % N in the nodules as mice ate them before analysis took place.

Table M

1964 Nodule counts and dry weight

Treatment	Mean number of plants per linear foot of row	Nodule * numbers per linear foot of row	Nodules per plant	Dry wt. of nodules in grams per linear foot of row	Dry wt. of individual nodule micro grams
Inoculated	23	190	8.26	0.1196	629
Control (Not inoculated)	25	250	10.00	0.1226	490
S.E.	-	±87	-	±0.0251	-

* (Nodules removed and included as in 1963)

The results in 1964 serve to corroborate the previous year's findings and tend to indicate that the indigenous strain of rhizobium is an effective one and again no visible or recordable differences existed above ground between inoculated and control plots.

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7. SECTION VII APPENDICES

7.1 APPENDIX A

7.11 1963 Yield data

7.11 1963 Yield data from the main trial

TABLE I. Statistical Analysis of Individual treatment cut in 1963, involving (a) versus (b) comparisons only on Main Treatment 2. (September 30th)

	Total D.M. Yield lbs./acre (% Due to Grass D.M.)		Total N. Yield lbs./acre (% Due to Grass N.)		D.M.%		N. %	
					Grass	Clover	Grass	Clover
(a)	2,517	(87)	53.83	(78)	18.55	13.08	1.93	3.57
(b)	1,897	(73)	50.15	(61)	16.75	12.57	2.24	3.80
S.E.	± 105		±1.31		±0.18	±0.29	± 0.05	±0.06
Sign. Levels	*		N.S.		**	N.S.	*	N.S.

TABLE II. Statistical Analysis of Individual treatment cuts in 1963 involving (a) versus (b) comparisons only on Main Treatment 4. (August 15th and September 30th)

		Total D.M. Yield lbs./acre (% Due to Grass D.M.)		Total N. Yield lbs./acre (% Due to Grass N.)		D.M. %		N. %	
						Grass	Clover	Grass	Clover
August 15th	(a)	1,662	(63)	40.12	(50)	14.28	12.05	1.91	3.25
	(b)	1,502	(45)	44.33	(33)	13.48	10.22	2.17	3.58
	S.E.	± 60		±1.87		±0.16	±0.26	±0.024	±0.022
	Sign. Levels	N.S.		N.S.		*	*	**	**
Sept. 30th	(a)	1,221	(76)	32.57	(64)	16.72	13.43	2.24	4.05
	(b)	1,188	(61)	35.41	(47)	16.05	12.90	2.32	4.03
	S.E.	± 73		±1.78		±0.24	±0.20	±0.027	±0.076
	Sign. Levels	N.S.		N.S.		N.S.	N.S.	N.S.	N.S.

TABLE III. Statistical Analysis of Individual treatment cuts in 1963 involving (a) versus (b) comparisons only on Main Treatment 6. (August 15th, September 16th and October 16th)

		Total D.M. Yield lbs./acre (% Due to Grass D.M.)		Total N. Yield lbs./acre (% Due to Grass N.)		D.M. %		N. %	
						Grass	Clover	Grass	Clover
Aug. 15th	(a)	1,587	(64)	38.22	(50)	14.25	12.20	1.91	3.27
	(b)	1,427	(40)	43.18	(30)	13.37	10.58	2.27	3.56
	S.E.	± 86		±3.63		±0.20	±0.32	±0.08	±0.05
	Sign. Levels	N.S.		N.S.		N.S.	*	*	*
Sept. 16th	(a)	871	(77)	25.35	(65)	14.33	11.62	2.46	4.44
	(b)	829	(52)	28.66	(38)	14.00	11.60	2.54	4.47
	S.E.	± 51		±2.34		±0.14	±0.09	±0.07	±0.03
	Sign. Levels	N.S.		N.S.		N.S.	N.S.	N.S.	N.S.
Oct. 16th	(a)	443	(81)	15.52	(76)	17.63	16.35	3.31	4.27
	(b)	365	(61)	13.60	(55)	18.25	16.18	3.35	4.30
	S.E.	±32		±1.12		±0.20	±0.24	±0.04	±0.04
	Sign. Levels	N.S.		N.S.		N.S.	N.S.	N.S.	N.S.

Table IIIa. Total Green Yield in Establishment Year (Establishment cut plus appropriate treatment cuts)

Split Plot Analysis

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots					
Blocks	3	2,194,393	731,464	8.931	*
2 v 4 v 6	2	1,342,695	671,347	7.305	*
Error	6	551,382	81,897		
Within Main Plots					
a v b	1	153,120	153,120	2.106	N.S.
[2.4.6.] x [a v b]	2	115,425	57,712	0.7937	N.S.
Error	9	654,373	72,708		

Total Yield of Green Material in Establishment Year (Tons/Acre)

	a	b	Mean
2	13.598	11.618	12.612 (100)
4	16.148	16.506	16.327 (129)
6	16.949	15.141	16.048 (127)
Mean	15.563 (108)	14.419 (100)	

S.E. of means (2, 4, 6): S.E. ± 0.765

S.E. ± 0.557

S.E. of figures within the table ± 0.957

TABLE IV. Total D.M. Yield in Establishment Year (Established cut plus appropriate treatment cuts)

(Analysis done on total yield of D.M. in grams/plot)

Variant	D.F.	S.O.S.	M.S.	V.R.	Sign. Level
Between Main Plots.					
Blocks	3	13,166	4388.7	7.758	*
2 v 4 v 6	2	8,401	4200.5	7.425	*
Error	6	3,394	565.7		
Total	11	24,961			
Within Main Plots					
a v b	1	15,504	15,504	26.897	***
(2.4.6) x (a v b)	2	3,049	1,525	2.645	N.S.
Error	9	5,188	576.4		

D.M. Yields lbs./acre

	a	b	Mean
2	4432	3240	3836 (100)
4	4672	4352	4512 (118)
6	4480	3952	4216 (110)
Mean	4528 (118)	3848 (100)	S.E. $\pm$ 135

S.E. $\pm$ 111

TABLE V. Total 'N' Yield in Establishment Year (Established cut plus appropriate treatment cuts)

(Analysis done on total 'N' yield (i.e. grass N + clover N) in grams/plot

Variant	D.F.	S.O.S.	M.S.	V.R.	Sign. Level
Between Main Plots					
Blocks	3	17.4644	5.8214	5.6977	*
2 v 4 v 6	2	16.3856	8.1928	8.018	*
Error	6	6.1307	1.0217		
Total	11	39.9807			
Within Main Plots					
a v b	1	0.0060	0.0060	0.001	N.S.
(2.4.6) x (a v b)	2	1.1656	0.5828	1.099	N.S.
Error	9	4.7706	0.5301		

N. YIELDS lbs./acre

	a	b	Mean
2	94.83	85.39	90.11 (100)
4	113.56	121.24	117.44 (130)
6	118.76	118.88	118.82 (132)
Mean	109.04 (100.4)	108.56 (100)	S.E. ± 5.76

S.E. ±3.52

7.12 1964 Yield data

7.12 1964 Yield data from the main trial

TABLE VI. 1964 Data From Main Trial. Treatment 2 Cuts/Annum

1st Cut. May 1st

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	10.02	8.18	4,874	4.85	61.99	12.85
b	8.39	20.87	3,856	12.26	54.10	30.85
S.E.	± 0.41 N.S.	± 2.67 *	± 220 *	± 1.58 *	± 2.04 N.S.	± 3.63 *

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	22.50	12.93	1.165	3.34
b	22.80	12.10	1.105	3.55
S.E.	± 0.13 N.S.	± 0.17 *	± 0.028 N.S.	± 0.04 *

TABLE VII. 1964 Data from Split Plot Trial. Main Treatment 2 Cuts/Annum

2nd Cut. October 1st

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield Lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	7.16	45.31	2,767	36.36	76.65	47.94
b	7.38	57.61	2,761	48.40	85.83	58.09
S.E.	± 0.22 N.S.	± 2.26 *	± 101 N.S.	± 1.85 *	± 3.03 N.S.	± 1.97 *

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	20.30	13.85	2.268	3.66
b	20.48	14.13	2.525	3.74
S.E.	± 0.17 N.S.	± 0.48 N.S.	± 0.029 **	± 0.09 N.S.

TABLE VIII. 1964 Data From Main Trial. Treatment 2 Cuts/Annum

Total Production

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield Lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	17.17	23.64	7,641	16.27	138.64	32.18
b	15.76	38.10	6,617	27.37	139.93	47.55
S.E.	± 0.61 N.S.	± 2.59 *	± 298 N.S.	± 1.86 *	± 4.87 N.S.	± 2.73 *

	D.M. % (Weighted mean over both cuts)		N. % (Weighted mean over both cuts)	
	Grass	Clover	Grass	Clover
a	21.77	13.66	1.496	3.59
b	22.05	13.54	1.560	3.68
S.E.	± 0.14 N.S.	± 0.38 N.S.	± 0.027 N.S.	± 0.08 N.S.

TABLE IX. 1964 Data From Main Trial. Treatment 4 Cuts/Annum

1st Cut. 24th April

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield lbs. N./acre	Contribution Made By Clover as % Total Nitrogen Yield
a	3.65	5.6	1,603	4.1	36.00	9.03
b	3.33	8.3	1,426	6.0	33.95	11.71
S.E.	± 0.24 N.S.	± 0.7 N.S.	± 77 N.S.	± 0.5 N.S.	± 2.25 N.S.	± 1.0 N.S.

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	19.97	14.50	2.13	4.92
b	19.57	13.80	2.24	4.70
S.E.	± 0.38 N.S.	-	± 0.05 N.S.	-

TABLE X. 1964 Data From Main Trial. Treatment 4 Cuts/Annum

2nd Cut. 15th June

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield Lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N./ Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	6.54	37.7	2,298	27.8	42.82	47.07
b	7.09	40.7	2,399	29.7	45.16	50.19
S.E.	± 0.34 N.S.	± 1.7 N.S.	± 84 N.S.	± 1.5 N.S.	± 1.98 N.S.	± 1.69 N.S.

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	18.27	11.48	1.35	3.18
b	18.22	11.13	1.32	3.18
S.E.	± 0.26 N.S.	± 0.18 N.S.	± 0.03 N.S.	± 0.06 N.S.

TABLE XI. 1964 Data From The Main Trial. Treatment 4 Cuts/Annum

3rd Cut. 5th August

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield Lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	5.59	68.2	1,808	61.6	58.60	72.61
b	5.53	69.5	1,767	62.6	56.10	73.54
S.E.	± 0.11 N.S.	± 3.2 N.S.	± 38 N.S.	± 3.8 N.S.	± 0.87 N.S.	± 2.77 N.S.

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	17.45	13.03	2.32	3.83
b	17.55	12.90	2.25	3.73
S.E.	± 0.20 N.S.	± 0.16 N.S.	± 0.05 N.S.	± 0.01 **

TABLE XII. 1964 Data From The Main Trial. Treatment 4 Cuts/Annum

4th Cut. 7th October

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield Lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	6.67	58.4	1,586	54.7	60.32	63.51
b	7.02	64.9	1,600	61.4	61.45	69.42
S.E.	± 0.14 N.S.	± 1.7 N.S.	± 39 N.S.	± 1.7 N.S.	± 0.80 N.S.	± 2.06 N.S.

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	11.55	9.95	3.07	4.42
b	11.20	9.63	3.04	4.35
S.E.	± 0.13 N.S.	± 0.05 *	± 0.03 N.S.	± 0.07 N.S.

TABLE XIII. 1964 Data From Main Trial. Treatment 4 Cuts/Annum

Total Production For The Year

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield Lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	22.454	46.4	7,295	37.0	197.73	52.87
b	22.885	50.3	7,191	40.1	196.66	56.31
S.E.	± 0.606 N.S.	± 2.2 N.S.	± 193 N.S.	± 1.9 N.S.	± 3.88 N.S.	± 2.43 N.S.

	D.M. % (weighted mean)		N. % (weighted mean)	
	Grass	Clover	Grass	Clover
a	17.07	11.53	2.110	3.919
b	16.94	11.19	2.076	3.865
S.E.	± 0.15 N.S.	± 0.07 *	± 0.025 N.S.	± 0.009 *

TABLE XIV. 1964 Results From The Main Trial. Treatment 6 Cuts/Annum

1st Cut. 16th April

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	1.97	5.7	918	4.6	24.84	8.02
b	1.53	13.8	704	10.3	20.01	17.08
S.E.	± 0.17 N.S.	± 1.0 *	± 74 N.S.	± 0.6 **	± 2.06 N.S.	± 1.11 *

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	21.03	16.70	2.610	4.70
b	21.48	16.60	2.610	4.66
S.E.	± 0.31 N.S.	-	± 0.021 N.S.	-

TABLE XV. 1964 Results From the Main Trial. Treatment 6 Cuts/Annum

2nd Cut. 13th May

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield Lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	2.37	20.8	877	17.0	21.33	30.16
b	2.69	38.9	956	31.4	26.66	50.88
S.E.	± 0.06 *	± 1.6 **	± 29 N.S.	± 1.4 **	± 0.65 *	± 1.31 **

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	17.35	13.55	2.025	4.35
b	17.90	12.80	1.965	4.61
S.E.	± 0.21 N.S.	± 0.09 **	± 0.061 N.S.	± 0.09 N.S.

TABLE XVI. 1964 Results From The Main Trial. Treatment 6 Cuts/Annum

3rd Cut. 23rd June

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield Lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	3.99	60.5	1,583	52.4	41.87	66.95
b	5.09	72.6	1,986	66.6	56.80	77.42
S.E.	± 0.34 N.S.	± 2.5 *	± 102 N.S.	± 2.5 *	± 3.62 N.S.	± 2.41 N.S.

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	21.53	15.28	1.798	3.38
b	21.28	15.98	1.928	3.33
S.E.	± 0.22 N.S.	± 0.25 N.S.	± 0.040 N.S.	± 0.09 N.S.

TABLE XVII. 1964 Results From The Main Trial. Treatment 6 Cuts/Annum

4th Cut. 28th July

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	3.87	66.6	1,254	59.3	42.44	70.29
b	4.23	74.5	1,339	69.7	47.49	77.61
S.E.	± 0.13 N.S.	± 0.9 **	± 32 N.S.	± 1.4 *	± 1.63 N.S.	± 0.70 **

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	17.65	12.90	2.470	4.01
b	16.83	13.23	2.625	3.95
S.E.	± 0.31 N.S.	± 0.23 N.S.	± 0.073 N.S.	± 0.04 N.S.

TABLE XVIII. 1964 Results From The Main Trial. Treatment 6 Cuts/Annum

5th Cut. 9th September

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield Lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	6.49	66.6	1,363	59.2	51.97	69.54
b	7.08	73.9	1,467	68.7	57.58	76.69
S.E.	± 0.14 N.S.	± 1.1 *	± 32 N.S.	± 0.7 **	± 1.81 N.S.	± 0.82 **

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	11.48	8.35	2.848	4.48
b	11.10	8.63	2.918	4.38
S.E.	± 0.20 N.S.	± 0.30 N.S.	± 0.077 N.S.	± 0.08 N.S.

TABLE XIX. 1964 Results From The Main Trial. Treatment 6 Cuts/Annum

6th Cut. 14th October

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield Lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	1.59	49.4	603	46.9	24.30	52.91
b	1.31	58.8	503	56.0	20.54	61.87
S.E.	± 0.05 *	± 1.3 *	± 20 *	± 1.6 *	± 0.66 *	± 1.47 *

	D.M. %		N. %	
	Grass	Clover	Grass	Clover
a	17.78	16.05	3.568	4.55
b	18.40	16.40	3.513	4.51
S.E.	± 0.21 N.S.	± 0.11 N.S.	± 0.049 N.S.	± 0.04 N.S.

TABLE XX. 1964 Results From The Main Trial. Treatment 6 Cuts/Annum

Total Production For The Year

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution Made By Clover as % Total Green Yield	Total D.M. Yield lbs./Acre	Contribution Made By Clover as % Total D.M. Yield	Total Nitrogen Yield lbs. N./Acre	Contribution Made By Clover as % Total Nitrogen Yield
a	20.28	53.0	6,597	43.4	206.76	55.97
b	21.93	64.3	6,956	56.4	229.07	67.49
S.E.	± 0.64 N.S.	± 0.9 **	± 225 N.S.	± 1.3 **	± 7.35 N.S.	± 1.09 **

	D.M. % (as weighted mean)		N. % (as weighted mean)	
	Grass	Clover	Grass	Clover
a	17.48	11.87	2.479	4.13
b	17.30	12.41	2.503	4.03
S.E.	± 0.20 N.S.	± 0.18 N.S.	± 0.042 N.S.	± 0.05 N.S.

TABLE XXI. 1964 Results. Total Green Yield

Split Plot Analysis:-

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots:-					
Blocks	3	6.5311093	2.1770364	2.755	N.S.
2 v 4 v 6	2	166.38940	83.194702	105.269	***
Error	6	4.7418399	0.79030665		
Within Main Plots:-					
a v b	1	0.29260254	0.29260254	0.191	N.S.
[2. 4. 6.] x [a v b]	2	9.4697876	4.7348938	3.095	N.S.
Error	9	13.769312	1.5299235		

Total Yield Of Green Material In 1964 (Tons/Acre)

	a	b	Mean	
2	17.1745	15.7625	16.4685	(100)
4	22.4540	22.8847	22.6694	(138) S.E. ± 0.3143
6	20.2832	21.9270	21.1051	(128)
Mean	19.9706 (99)	20.1914 (100)		

S.E. ± 0.3571.

S.E. of figures within the table ± 0.6185

TABLE XXII. 1964 Results. Total D.M. Yield

Split-Plot Analysis

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots:-					
Blocks	3	292508.11	97502.704	0.406	N.S.
2 v 4 v 6	2	966888.00	483444.00	2.014	N.S.
Error	6	1440587.9	240097.98		
Within Main Plots:-					
a v b	1	1075116.0	1075116.0	6.495	*
[2. 4. 6.] x [a v b]	2	2681324.0	1340662.0	8.099	**
Error	9	1489771.3	165530.15		

Total Yield Of Dry Matter In 1964 (Lbs./Acre)

	a	b	Mean	
2	7890.97	6616.45	7253.71	(100)
4	7295.47	6941.55	7118.51	(98) S.E. ± 175.24
6	6597.47	6956.00	6776.74	(93)
Mean	7261.31 (106)	6838.00 (100)		

S.E. ± 117.45

S.E. of the figures within the table ± 166.10

TABLE XXIII. 1964 Results. Total Nitrogen Yield

Split Plot Analysis:-

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots:-					
Blocks	3	1032.4093	344.13645	4.237	N.S.
2 v 4 v 6	2	26575.565	13287.782	163.588	***
Error	6	487.36212	81.227020		
Within Main Plots:-					
a v b	1	338.91406	338.91406	2.737	N.S.
[2. 4. 6.] x [a v b]	2	663.13086	331.56543	2.677	N.S.
Error	9	1114.5718	123.84131		

Total Yield of Nitrogen (Lbs./Acre)

	a	b	Mean	
2	138.64	139.94	139.29	(100)
4	197.74	196.66	197.20	(142) S.E. ± 3.19
6	206.76	229.08	217.92	(156)
Mean	181.04 (96)	188.56 (100)		

S.E. ± 3.21

S.E. of the figures within the table ± 5.56

TABLE XXIV. 1964 Results. % Clover Contribution In Total Green Weight

Split Plot Analysis:-

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots:-					
Blocks	3	173.19526	57.731752	3.807	N.S.
2 v 4 v 6	2	3149.3503	1574.6752	103.844	***
Error	6	90.982964	15.163827		
Within Main Plots					
a v b	1	590.22022	590.22022	36.271	***
[2. 4. 6.] x [a v b]	2	115.62183	57.810913	3.553	N.S.
Error	9	146.45145	16.272383		

% Clover Contribution In Total Green Weight

	a	b	Mean
2	23.64	38.09	30.87
4	46.36	50.34	48.35
6	52.96	64.27	58.62
Mean	40.99	50.90	

S.E. $\pm$ 1.38

S.E. $\pm$ 1.16

S.E. of the figures within the table $\pm$ 202

TABLE XXV. 1964 Results. % Clover Contribution In Total Dry Matter Yield

Split Plot Analysis:-

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots:-					
Blocks	3	155.02858	51.676192	2.568	N.S.
2 v 4 v 6	2	3194.0485	1597.0243	79.362	***
Error	6	120.73901	20.123168		
Within Main Plots:-					
a v b	1	497.79755	497.79755	42.408	***
[2. 4. 6.] x [a v b]	2	109.13116	54.565582	4.648	*
Error	9	105.64536	11.738373		

% Clover Contribution In Total Dry Matter Yield

	a	b	Mean
2	16.27	27.36	21.82
4	36.96	40.14	38.55
6	43.38	56.43	49.91
Mean	32.20	41.31	

S.E. $\pm$ 1.59

S.E. $\pm$ 0.99

S.E. of the figures within the table $\pm$ 1.71.

TABLE XXVI. 1964 Results. % Clover Contribution In Total Nitrogen Yield

Split Plot Analysis:-

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots:-					
Blocks	3	171.11714	57.039047	3.915	N.S.
2 v 4 v 6	2	1989.5101	994.75507	68.285	***
Error	6	87.406792	14.567799		
Within Main Plots:-					
a v b	1	613.57654	613.57654	31.712	***
[2. 4. 6.] x [a v b]	2	148.21545	74.107727	3.83	N.S.
Error	9	174.13750	19.348611		

% Clover Contribution In Total Nitrogen Yield

	a	b	Mean
2	32.18	47.55	39.864
4	52.87	56.31	54.588
6	55.97	67.49	61.732
Mean	47.005	57.118	

S.E. $\pm$ 1.349 (Mean column)

S.E. $\pm$ 1.270 (Mean row)

S.E. of figures within the table $\pm$ 2.199

7.13 1965 Yield data

7.13 1965 Yield data from the main trial

Table XXVII. 1965 Data from the Main Trial. Treatment 2 Cuts/Annum.

1st Cut May 25th

Subsidiary Treatment	Total Green Yield Tons/acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N/acre	Contribution made by clover as % Total Nitrogen Yield
a	7.60	10.39	3,129	6.86	47.59	16.17
b	4.26	26.77	1,749	17.01	31.04	35.23
S.E.	± 0.45*	± 3.26*	± 155**	± 2.10*	± 2.57*	± 3.74*

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	19.12	12.20	1.37	3.63
b	20.72	11.50	1.39	3.84
S.E.	± 0.19**	± 0.39 N.S.	± 0.03 N.S.	± 0.07 N.S

Table XXVIII. 1965 Data from the Main Trial. Treatment 2 Cuts/Annum.

2nd Cut October 5th

Subsidiary Treatment	Total Green Yield Tons/acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N/acre	Contribution made by clover as % Total Nitrogen Yield
a	10.26	36.89	2,681	34.09	74.81	40.05
b	8.14	46.75	2,033	44.20	61.61	50.79
S.E.	± 0.29*	± 4.48N.S.	± 144*	± 4.92 N.S.	± 3.69N.S	± 3.99 N.S.

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	12.15	10.63	2.57	3.36
b	11.63	10.35	2.69	3.54
S.E.	± 0.37 N.S.	± 0.29 N.S.	± 0.06 N.S.	± 0.14 N.S.

Table XXIX. 1965 Data from the Main Trial. Treatment 2 Cuts/Annum.

Total Production for the Year

Subsidiary Treatment	Total Green Yield Tons/acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield lbs.N/acre	Contribution made by clover as % Total Nitrogen Yield
a	17.86	25.89	5,810	19.40	122.40	30.88
b	12.40	39.92	3,782	31.83	92.65	45.56
S.E.	± 0.72*	± 3.43 N.S.	± 285*	± 3.33 N.S.	±6.01*	± 3.21*

	D.M. % (weighted mean)		N % (weighted mean)	
	Grass	Clover	Grass	Clover
a	15.74	10.84	1.81	3.39
b	15.38	10.59	1.96	3.61
S.E.	± 0.17 N.S.	±0.22 N.S.	± 0.03 N.S.	± 0.10 N.S.

Table XXX. 1965 Data from the Main Trial. Treatment 4 Cuts/Annum.

1st Cut May 11th

Subsidiary Treatment	Total Green Yield Tons/acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs.N/acre	Contribution made by clover as % Total Nitrogen Yield
a	4.25	11.80	1,753	8.03	33.76	17.45
b	2.90	10.25	1,241	6.57	22.75	15.81
S.E.	± 0.35 N.S.	± 1.50 N.S.	± 113 *	± 0.88 N.S.	± 2.51 N.S.	± 2.17 N.S.

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	19.25	12.53	1.72	4.11
b	20.00	12.60	1.65	4.40
S.E.	± 0.60 N.S.	± 0.33 N.S.	± 0.03 N.S.	± 0.15 N.S.

TABLE XXXI. 1965 Data from the Main Trial. Treatment 4 Cuts/Annum.

2nd Cut July 1st

Subsidiary Treatment	Total Green Yield Tons/acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N/acre	Contribution made by clover as % Total Nitrogen Yield
a	6.13	55.46	2,284	45.02	54.42	61.79
b	5.32	56.96	2,012	45.44	46.32	63.94
S.E.	± 0.34 N.S.	± 2.75 N.S.	± 101 N.S.	± 3.39 N.S.	± 3.19 N.S.	±2.24 N.S.

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	20.70	13.58	1.65	3.27
b	21.85	13.53	1.49	3.23
S.E.	± 0.50 N.S.	± 0.23 N.S.	± 0.05 N.S.	± 0.02 N.S.

Table XXXII. 1965 Data from the Main Trial. Treatment 4 Cuts/Annum.

3rd Cut August 31st

Subsidiary Treatment	Total Green Yield Tons/acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs.N/acre	Contribution made by clover as % Total Nitrogen Yield
a	10.79	59.50	2,526	52.72	79.27	58.91
b	9.91	71.11	2,275	64.44	73.76	69.65
S.E.	± 0.22 N.S.	± 0.89 **	± 50 *	± 1.78 *	± 2.15 N.S.	± 1.78 *

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	12.22	9.30	2.73	3.52
b	12.65	9.33	2.77	3.50
S.E.	± 0.30 N.S.	± 0.28 N.S.	± 0.04 N.S.	± 0.04 N.S.

Table XXXIII. 1965 Data from the Main Trial. Treatment 4 Cuts/Annum

4th Cut October 5th

Subsidiary Treatment	Total Green Yield Tons/acre	Contribution made by Clover as % Total Green Yield	Total D.M. Yield lbs./acre	Contribution made by Clover as % Total D.M. Yield	Total Nitrogen Yield Lbs.N/acre	Contribution made by Clover as % Total Nitrogen Yield
a	2.90	44.01	730	41.20	30.64	44.12
b	2.65	58.83	634	57.06	27.53	60.49
S.E.	± 0.23 N.S.	± 1.25 **	± 65 N.S.	± 1.95 *	± 2.61 N.S.	± 1.74 **

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	11.85	10.58	3.99	4.50
b	11.23	10.35	3.99	4.60
S.E.	± 0.75 N.S.	± 0.18 N.S.	± 0.02 N.S.	± 0.05 N.S.

Table XXXIV. 1965 Data from the Main Trial. Treatment 4 Cuts/Annum.

Total Production for the Year

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield Lbs./Acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs. N/Acre	Contribution made by clover as % Total Nitrogen Yield
a	24.07	48.46	7,294	38.68	198.09	50.70
b	20.78	58.02	6,162	46.31	170.35	59.92
S.E.	± 0.77 N.S.	± 1.57 *	± 232 *	± 1.68 *	± 6.07 *	± 1.44 *

	D.M. % (Weighted mean)		N % (Weighted mean)	
	Grass	Clover	Grass	Clover
a	16.11	10.80	2.18	3.56
b	16.92	10.56	2.06	3.58
S.E.	± 0.15 *	± 0.12 N.S.	± 0.03 N.S.	± 0.03 N.S.

Table XXXV. 1965 Data from the Main Trial. Treatment 6 Cuts/Annum.

1st Cut April 27th

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield Lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs.N/acre	Contribution made by clover as % Total Nitrogen Yield
a	2.38	7.34	1,006	5.28	24.51	9.79
b	1.45	6.86	695	4.72	15.01	9.76
S.E.	± 0.11 **	± 1.14 N.S.	± 51 *	± 0.83 N.S.	± 1.09 **	± 1.56 N.S.

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	19.32	13.60	2.31	4.53
b	21.98	15.00	2.05	4.53
S.E.	± 0.12 ***	-	± 0.05 *	-

Table XXXVI. 1965 Data from the Main Trial. Treatment 6 Cuts/Annum.

2nd Cut May 26th

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield Lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs.N/acre	Contribution made by clover as % Total Nitrogen Yield
a	3.69	49.11	1,127	41.38	36.24	53.22
b	2.62	51.36	852	42.17	24.66	56.45
S.E.	± 0.28 N.S.	± 3.38 N.S.	± 62 N.S.	± 3.17 N.S.	± 2.98 N.S.	± 3.75 N.S.

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	15.97	11.60	2.53	4.12
b	17.35	11.95	2.17	3.87
S.E.	± 0.23 *	± 0.22 N.S.	± 0.08 *	± 0.13 N.S.

Table XXXVII. 1965 Data from the Main Trial. Treatment 6 Cuts/Annum.

3rd Cut June 22nd

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield Lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs.N/acre	Contribution made by clover as % Total Nitrogen Yield
a	4.98	69.87	1,400	63.58	45.82	72.20
b	4.56	78.17	1,329	72.65	44.64	80.83
S.E.	± 0.23 N.S.	± 2.26 N.S.	± 106 N.S.	± 3.56 N.S.	± 3.90 N.S.	± 3.08 N.S.

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	15.20	11.45	2.49	3.71
b	16.18	12.25	2.32	3.73
S.E.	± 0.10 **	± 0.79 N.S.	± 0.04 *	± 0.05 N.S.

Table XXXVIII. 1965 Data from the Main Trial. Treatment 6 Cuts/Annum

4th Cut July 27th

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield Lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs.N/acre	Contribution made by clover as % Total Nitrogen Yield
a	5.51	72.34	1,652	66.56	57.08	72.10
b	4.69	79.43	1,467	74.58	51.24	79.96
S.E.	± 0.14 *	± 1.64 N.S.	± 17 **	± 1.99 N.S.	± 1.54 N.S.	± 1.51 *

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	16.20	12.30	2.88	3.74
b	17.25	13.15	2.77	3.76
S.E.	± 0.12 **	± 0.39 N.S.	± 0.08 N.S.	± 0.08 N.S.

Table XXXIX. 1965 Data from the Main Trial. Treatment 6 Cuts/Annum.

5th Cut September 1st

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield Lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs.N/acre	Contribution made by clover as % Total Nitrogen Yield
a	6.73	70.57	1,596	66.41	64.08	73.88
b	6.10	80.78	1,438	77.05	60.43	82.66
S.E.	± 0.14 *	± 1.62 *	± 34 *	± 1.75 *	± 1.33 N.S.	± 1.18 *

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	12.12	9.97	3.13	4.47
b	12.58	10.03	3.18	4.51
S.E.	± 0.11 N.S.	± 0.10 N.S.	± 0.08 N.S.	± 0.03 N.S.

Table XL. 1965 Data from the Main Trial. Treatment 6 Cuts/Annum.

6th Cut October 12th

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield Lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs.N/acre	Contribution made by clover as % Total Nitrogen Yield
a	3.19	50.24	830	47.71	35.26	52.49
b	2.88	67.16	741	64.35	32.14	68.51
S.E.	± 0.05 *	± 3.47 *	± 13 *	± 3.36 *	± 0.26 **	± 2.92 *

	D.M. %		N %	
	Grass	Clover	Grass	Clover
a	12.28	11.00	3.86	4.70
b	12.48	10.97	3.83	4.63
S.E.	± 0.12 N.S.	± 0.06 N.S.	± 0.05 N.S.	± 0.04 N.S.

Table XLI. 1965 Data from the Main Trial. Treatment 6 Cuts/Annum.

Total Production for the year

Subsidiary Treatment	Total Green Yield Tons/Acre	Contribution made by clover as % Total Green Yield	Total D.M. Yield Lbs./acre	Contribution made by clover as % Total D.M. Yield	Total Nitrogen Yield Lbs.N/acre	Contribution made by clover as % Total Nitrogen Yield
a	26.48	60.14	7,612	52.36	262.99	61.88
b	22.31	70.01	6,522	62.01	228.12	72.18
S.E.	± 0.60 *	± 1.17 **	± 230 *	± 1.63	± 7.89 N.S.	± 1.26 *

	D.M. % (weighted mean)		N % (weighted mean)	
	Grass	Clover	Grass	Clover
a	15.33	11.17	2.76	4.08
b	16.53	11.57	2.56	4.08
S.E.	± 0.11 **	± 0.28 N.S.	± 0.04 *	± 0.03 N.S.

Table XLII. 1965 Results. TOTAL GREEN YIELD.

Split Plot Analysis:-

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots					
Blocks	3	2.6721500	0.89071665	0.171	N.S.
2 v 4 v 6	2	380.94754	190.47377	36.469	***
Error	6	31.337340	5.2228901		
Within Main Plots					
a v b	1	111.62897	111.62897	57.201	***
[2.4.6.] x [a v b]	2	4.7447205	2.3723602	1.216	N.S.
Error	9	17.563560	1.9515067		

Total Yield of green material in 1965 (Tons/acre)

	a	b	Mean
2	17.86	12.40	15.13 (100)
4	24.07	20.77	22.43 (148)
6	26.48	22.31	24.40 (161)
Mean	22.81 (123)	18.49 (100)	

S.E. ± 0.81

S.E. ± 0.40

S.E. of figures within the table ± 0.70

Table XLIII. 1965 Results. TOTAL DRY MATTER YIELD.

Split Plot Analysis:-

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots					
Blocks	3	767405.43	255801.81	0.386	N.S.
2 v 4 v 6	2	24020644	12010322	18.105	**
Error	6	3980302.6	663383.76		
Within Main Plots					
a v b	1	12034584	12034584	47.967	***
[2.4.6.] x [a v b]	2	1124496.0	562248.00	2.241	N.S.
Error	9	2258036.7	250892.97		

Total Yield of Dry Matter in 1965 (lbs./acre)

	a	b	Mean
2	5,810	3,782	4,796 (100)
4	7,294	6,162	6,728 (140)
6	7,612	6,523	7,067 (147)
Mean	6,905 (126)	5,489 (100)	

S.E. ± 288 (Mean column)

S.E. ± 235 (Mean row)

S.E. of figures within the table ± 250

Table XLIV. 1965 Results. TOTAL NITROGEN YIELD.

Split Plot Analysis

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots					
Blocks	3	267.73521	89.245069	0.2611	N.S.
2 v 4 v 6	2	76525.127	38262.564	111.965	***
Error	6	2050.4192	341.73653		
Within Main Plots					
a v b	1	5687.8359	5687.8359	31.555	***
[2.4.6.] x [a v b]	2	53.990234	26.995117	0.150	N.S.
Error	9	1622.2759	180.25287		

Total Nitrogen Yield in 1965

	a	b	Mean
2	122.40	92.65	107.53 (100)
4	198.10	170.35	184.22 (171)
6	262.99	228.12	245.56 (228)
Mean	194.50 (119)	163.71 (100)	

S.E. $\pm$ 6.54

S.E. $\pm$ 3.88

S.E. of figures within the table $\pm$ 6.71

Table XLV. 1965 Results. % CLOVER CONTRIBUTION IN TOTAL GREEN YIELD.

Split Plot Analysis

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots					
Blocks	3	104.42884	34.809615	1.2019	N.S.
2 v 4 v 6	2	4235.2581	2117.6290	73.117	***
Error	6	173.77379	28.962298		
Within Main Plots					
a v b	1	745.93433	745.93433	35.899	***
[2.4.6.] x [a v b]	2	24.893921	12.446960	0.599	N.S.
Error	9	187.00868	20.778742		

% Clover Contribution in Total Green Yield

	a	b	Mean
2	25.89	39.92	32.91
4	48.47	58.02	53.24
6	60.14	70.00	65.07
Mean	44.83	55.98	

S.E. $\pm$ 1.90

S.E. $\pm$ 1.32

S.E. of figures within the table $\pm$2.28

Table XLVI. 1965 Results. % CLOVER CONTRIBUTION IN TOTAL DRY MATTER YIELD.

Split Plot Analysis

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots					
Blocks	3	114.66884	38.222945	1.5682	N.S.
2 v 4 v 6	2	3993.0109	1996.5054	81.911	***
Error	6	146.24388	24.373980		
Within Main Plots					
a v b	1	588.45630	588.45630	26.649	***
[2.4.6.] x [a v b]	2	23.108765	11.554382	0.523	N.S.
Error	9	198.73483	22.081648		

% Clover Contribution in Total Dry Matter Yield

	a	b	Mean
2	19.41	31.83	25.62
4	38.68	46.31	42.49
6	52.36	62.01	57.19
Mean	36.81	46.72	

S.E. ± 1.75

S.E. ± 1.36

S.E. of figures within the table ± 2.35

Table XLVII. 1965 Results. % CLOVER CONTRIBUTION IN TOTAL NITROGEN YIELD.

Split Plot Analysis

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between Main Plots					
Blocks	3	147.30848	49.102826	1.7631	N.S.
2 v 4 v 6	2	3357.4888	1678.7444	60.276	***
Error	6	167.10461	27.850768		
Within Main Plots					
a v b	1	779.41846	779.41846	41.716	***
[2.4.6.] x [a v b]	2	33.474121	16.737061	0.896	N.S.
Error	9	168.15364	18.683738		

% Clover Contribution in Total Nitrogen Yield

	a	b	Mean
2	30.88	45.57	38.23
4	50.70	59.92	55.31
6	61.89	72.18	67.03
Mean	47.82	59.22	

S.E. ± 1.87

S.E. ± 1.25

S.E. of figures within the table ± 2.16

Table XLVIII. Nitrogen % in the grass (Weighted means using all 1965 Data)

Split-plot Analysis of Variance

Variant	D.F.	S.O.S.	M.S.	V.R.	Significance Level
Between main plots:-					
Blocks	3	0.00790854	0.00263618	0.2683	N.S.
2 v 4 v 6	2	2.5111809	1.2555904	127.785	***
Error	6	0.05895499	0.00982583		
Within main plots					
a v b	1	0.02070093	0.02070093 4.535 →		N.S.
[2.4.6] x [a v b]	2	0.13548088	0.06774044	14.838	**
Error	9	0.04108681	0.00456520		

Nitrogen % in the grass - Year 1965. (Weighted means)

	a	b	Mean
2	1.81	1.96	1.89
4	2.18	2.06	2.12
6	2.76	2.56	2.66
Mean	2.25	2.20	

S.E. ± 0.04

S.E. of figures within the table ± 0.03

S.E. ± 0.02

7.2 APPENDIX B

7.21 Brief descriptions of the methods employed in chemical analyses

7.21 Brief descriptions of the methods employed in chemical analysis

7.211 Soil analyses

7.2111 Moisture determination

The sample, as taken from the field, was dried overnight at 40°C and was then milled to pass through a 2 mm sieve. 10 grams of this air dry soil were placed in a Towson and Mercer Evenheat oven (S.222 x 223F) at 100°C for 24 hours and the moisture content was obtained by difference.

7.2112 Loss on ignition

A sample of dried soil was ignited at 650-700°C and ignition losses obtained by difference to give a guide to the organic matter of the soil.

7.2113 Measurements of pH

The soil pH was measured in a soil:water suspension (1 soil: $2\frac{1}{2}$ water). The mixture was shaken end-over-end for $\frac{1}{2}$ hour and then left for $1\frac{1}{2}$ hours. The pH determinations were made using a spear point glass electrode coupled to a MARCONI laboratory pH meter (T.F. 1093).

7.2114 Soil suspension conductivity (pC)

The same soil-water suspension is used here as for pH and the MULLARD apparatus involved in this determination gives the reading as resistance in ohms. [pC = Log. R - Log (cell constant)].

7.2115 Lime requirement

This was calculated using the pL concept (Schofield-Palmer 1956)

[i.e. pL = 2pH - pC].

7.2116 Available phosphate and potash

7.2116 Available phosphate and potash

For both determinations the extraction is done using 0.5 N acetic acid. 40 parts of this with one part of soil was shaken up for 2 hours and then filtered. The phosphate determination was carried out using the method described by Williams and Stewart (1941). Briefly this involves the use of ammonium molybdate and stannous chloride, the latter being used in an acid solution to reduce the phosphomolybdic acid to the blue complex. Available phosphate is then a colorimetric determination using a SPEKKER ABSORPTIOMETER (Type H.760).

In the case of potash, the extract is passed into an EEL flame photometer giving a direct reading.

7.2117 N determinations

The soil sample having been dried was then milled to pass through a 2mm sieve. A sub-sample was taken and re-milled in a mortar. Duplicate samples of 10 grams were taken, 30 ml conc. H_2SO_4 were added, digested for 2½ hours and then diluted to 500 ml. To 50 ml of this solution was added 20 ml of NaOH and two anti foam tablets. Distillation was into boric acid and titration was against 0.1 N-HCl.

7.2118 Trace elements

Spectrographic analysis was used to determine several trace elements and the soil extraction methods used are indicated in Table II of this appendix.

7.212 Herbage analyses

7.2121 Dry-matter

Dry-matter determinations were done in a CRAIG and DERRIOTT oven, overnight at 100°C.

7.2122 Nitrogen analyses

Nitrogen determinations were done taking 1 gram of dried material, adding 30 ml conc. H_2SO_4 and a selenium catalyst allowing $2\frac{1}{2}$ hours for the digestion period. Distillation and titration which followed were similar to those described under soil N.

7.2123 Trace elements

Spectrographic analysis was employed to determine the total trace elements from the ashed dry matter of grass and clover.

7.22 Data from soil and herbage analyses

7.22 <u>Data from soil and herbage analyses</u>

TABLE I. Soil analyses done on individual plot basis on the main trial. March 1963.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Moisture %	1.9	1.9	1.8	1.9	2.0	1.9	1.9	1.8	1.9	1.8	1.9	1.7	2.3	2.1	1.8	2.0	2.2	2.3	2.0	1.9	2.1	2.3	2.3	2.3
Loss on ignition %	8.8	8.9	8.8	8.5	8.6	8.4	8.4	8.4	8.5	8.4	8.3	8.6	8.5	8.9	8.6	8.2	8.3	8.4	8.2	8.8	8.3	8.2	7.8	8.6
pH (water)	5.34	5.44	5.47	5.55	5.36	5.40	5.31	5.36	5.28	5.29	5.31	5.32	5.38	5.50	5.42	5.39	5.30	5.29	5.30	5.28	5.34	5.28	5.20	5.22
Lime requirement (Tons/acre) (to pH 6.25)	3.25	2.75	2.75	2.50	3.00	3.00	3.25	3.00	3.25	3.25	3.25	3.25	3.00	2.50	3.00	3.00	3.25	3.25	3.25	3.25	3.00	3.25	3.75	3.50
Available P_2O_5	5	5	5	3	3	3	3	3	4	3	4	4	4	4	4	3	3	3	3	3	3	4	3	3
Available K_2O	16	22	16	9	10	9	10	11	11	10	12	13	16	25	21	12	10	11	15	18	12	13	9	9
pC	4.14	4.17	4.16	4.17	4.10	4.16	4.17	4.14	4.11	4.11	4.13	4.10	4.18	4.14	4.17	4.17	4.14	4.14	4.14	4.08	4.13	4.09	4.09	4.10
% N. (on air dry)	0.29	0.28	0.28	0.27	0.28	0.28	0.26	0.27	0.27	0.26	0.25	0.28	0.28	0.26	0.26	0.26	0.26	0.26	0.27	0.25	0.26	0.26	0.27	0.27

TABLE II. Spectrographic analyses of trace elements in a composite soil sample taken from the main trial area. March 1963.

Element	Extraction Method	Soil Level
Sodium	0.5 N. Acetic Acid	17.5 mgs. per 100 grams air dry soil
Magnesium	0.5 N. Acetic Acid	5.8 mgs. per 100 grams air dry soil
Manganese	Water soluble	0.44 p.p.m.
Copper	E.D.T.A.	4.08 p.p.m.
Cobalt	0.5 N. Acetic Acid	0.45 p.p.m.

TABLE III. Trace Element Analysis on herbage from Establishment Cut 9.7.63. (All p.p.m. of D.M. except Magnesium which is % D.M.)

Element	GRASS (ROOTS SEPARATED FROM CLOVER)						GRASS (ROOTS COMBINED WITH CLOVER)					
	I	II	III	IV	TOTAL	MEAN	I	II	III	IV	TOTAL	MEAN
Magnesium	0.150	0.143	0.136	0.128	0.557	0.139	0.165	0.113	0.125	0.130	0.533	0.133
Iron	806	1,085	810	832	3,533	883	506	575	559	476	2,116	529
Cobalt	0.54	0.71	0.49	0.55	2.29	0.57	0.38	0.45	0.39	0.34	1.56	0.39
Nickel	2.77	3.43	2.98	2.97	12.15	3.04	3.46	3.83	2.60	2.81	12.70	3.17
Molybdenum	2.37	1.61	1.87	1.68	7.53	1.88	0.65	0.62	0.70	0.71	2.68	0.67
Vanadium	4.85	5.22	4.85	5.20	20.12	5.03	1.58	2.01	16.9	1.75	7.03	1.76
Lead	2.08	3.23	1.87	1.86	9.04	2.26	3.10	3.09	3.05	3.17	12.41	3.10
Copper	6.6	7.3	7.4	7.0	28.3	7.1	7.4	6.4	6.9	7.5	28.2	7.1
Manganese	130	141	112	125	508	127	128	116	127	118	489	122
Aluminium	720	1,055	590	698	3,063	765	528	490	520	488	2,026	506

TABLE IV. Trace Element Analysis on herbage from Establishment Cut 9.7.63
(All p.p.m. of D.M. except Magnesium which is % of D.M.)

Element	CLOVER (Roots Separated From Grass)						CLOVER (Roots Combined With Grass)					
	I	II	III	IV	Total	Mean	I	II	III	IV	Total	Mean
Magnesium	0.228	0.238	0.238	0.213	0.917	0.229	0.235	0.238	0.235	0.204	0.912	0.228
Iron	484	954	847	804	3,089	772	556	1,196	737	583	3,072	768
Cobalt	0.43	0.76	0.64	0.57	2.40	0.60	0.49	0.80	0.52	0.43	2.24	0.56
Nickel	3.36	3.65	3.36	3.99	14.36	3.59	3.41	5.47	3.71	3.29	15.88	3.97
Molybdenum	0.87	1.11	1.59	0.82	4.39	1.10	1.20	1.27	1.15	0.99	4.61	1.15
Vanadium	1.19	2.52	2.50	2.15	8.36	2.09	1.37	3.07	1.96	1.62	8.02	2.01
Zinc	87	93	49	60	289	72	56	88	61	69	274	69
Tin	-	0.67	0.45	0.35	1.47	0.49	0.95	0.88	0.29	0.27	2.39	0.60
Lead	1.72	4.44	2.84	2.14	11.14	2.78	2.86	5.42	2.05	1.38	11.71	2.93
Copper	10.7	9.5	10.6	10.6	41.4	10.3	9.1	11.5	10.6	10.5	41.7	10.4
Manganese	70	105	82	85	342	86	90	106	92	95	383	96
Aluminium	382	980	755	480	2,597	649	562	1,055	725	360	2,702	676

TABLE V. Trace Element Analysis on herbage from Cut taken on 25th May, 1965. (p.p.m./D.M.)

Element	GRASS (Roots Separated From Clover By Polythene)					GRASS (Roots Combined With Clover)				
	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean
Iron	105	138	105	136	121	79	105	101	92	94
Cobalt	0.07	0.09	0.09	0.12	0.09	0.06	0.08	0.07	0.08	0.07
Nickel	2.05	2.17	1.87	2.87	2.24	1.89	2.1	2.0	2.44	2.11
Molybdenum	0.40	0.38	0.46	0.32	0.39	0.28	0.43	0.38	0.44	0.38
Titanium	8.9	12.7	11.6	15.9	12.3	6.7	11.0	8.7	7.3	8.4
Vanadium	0.29	0.41	0.40	0.42	0.38	0.19	0.34	0.28	0.29	0.28
Zinc	23	27	27	34	28	28	27	29	27	28
Lead	1.14	1.58	1.62	1.08	1.36	0.99	1.34	1.32	1.89	1.39
Copper	4.6	4.9	4.6	4.6	4.7	4.3	5.0	4.5	4.9	4.7
Manganese	96	130	100	149	119	100	119	87	121	107

TABLE VI. Trace Element Analysis on herbage from Cut taken on 25th May. 1965.
(p.p.m./D.M.)

Element	CLOVER (Roots Separated From Grass By Polythene)					CLOVER (Roots Combined With Grass)				
	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean
Iron	140	128	109	117	123	157	116	129	118	130
Cobalt	0.25	0.22	0.22	0.30	0.25	0.25	0.21	0.26	0.23	0.24
Nickel	2.77	2.41	2.50	2.78	2.62	2.22	2.41	3.14	2.70	2.62
Molybdenum	0.74	0.96	1.72	0.68	1.03	1.36	1.43	1.61	1.08	1.37
Titanium	11.6	10.1	12.2	8.4	10.6	13.0	10.7	10.8	10.9	11.4
Vanadium	0.15	0.19	0.14	0.27	0.19	0.21	0.11	0.16	0.14	0.16
Zinc	47	53	32	54	46.5	59	39	30	33	40.2
Lead	2.44	2.17	4.37	1.64	2.66	1.97	3.12	3.66	2.90	2.91
Copper	10.4	10.9	10.7	9.9	10.5	10.9	11.0	9.1	10.3	10.3
Manganese	65	64	54	70	63	63	64	66	56	62

7.23 Meteorological data

Table VII. METEOROLOGICAL DATA. Twenty year averages. Auchincruive 1935-1954

Month:-	Mean Soil Temp. °F. at 1 ft.	Air Temperature °F.			Rainfall (inches)	Rain days	Sunshine (hours)
		Mean Maximum	Monthly Mean	Mean Minimum			
January	38.85	43.04	37.91	32.79	3.61	18.9	40.99
February	38.99	44.86	39.47	34.09	2.68	17.2	63.78
March	41.32	48.94	42.51	36.11	2.14	15.1	103.44
April	45.69	53.22	46.01	38.82	2.06	14.2	143.04
May	51.28	59.22	50.88	42.54	1.99	12.55	197.24
June	56.48	63.41	55.55	47.69	2.52	14.95	172.14
July	59.23	65.11	58.41	51.74	3.38	18.0	145.2
August	59.39	65.46	58.18	50.91	3.27	16.4	141.0
September	56.02	61.45	54.79	48.13	3.82	18.3	103.98
October	50.77	55.17	49.28	43.40	4.12	18.2	79.01
November	45.32	49.17	43.51	37.87	3.88	19.2	48.91
December	41.37	44.67	39.74	34.82	3.72	21.0	34.78
TOTAL	-	-	-	-	37.19	204	1273.51

Table VIII. METEOROLOGICAL DATA. AUCHINCRUIVE. 1963

Month:-	Soil Temperatures °F.				Air Temperatures °F.				Grass Minimum °F.		Monthly Rainfall (inches)	Rain days	Sunshine (hours)
	4 ft.	1 ft.	8"	4"	Mean Maximum	Highest Maximum	Mean Minimum	Lowest Minimum	Mean	Lowest			
January	41.0	34.5	33.0	30.4	36.9	46	27.2	15	19.7	5	0.49	5	85.8
February	38.3	33.3	32.5	30.2	39.0	47	26.4	20	18.6	8	0.14	3	126.2
March	39.4	38.3	38.4	37.9	48.1	55	36.7	29	29.2	12	3.20	19	110.6
April	42.3	43.8	43.5	43.6	51.9	61	40.1	31	34.1	20	4.55	22	136.6
May	46.2	48.9	48.4	49.4	55.3	74	42.1	34	37.6	28	3.29	20	206.4
June	50.9	55.9	56.0	57.9	64.2	76	49.6	43	45.6	32	2.54	16	190.9
July	53.1	56.9	56.6	57.9	63.1	75	50.0	44	44.7	32	2.81	18	164.0
August	54.4	56.7	56.3	56.6	60.9	71	49.4	38	44.0	32	3.55	20	109.3
September	53.6	54.2	53.5	53.1	59.6	68	47.3	39	41.5	32	3.07	18	137.6
October	51.9	50.9	50.0	49.3	55.3	61	45.4	37	39.8	28	4.43	22	77.7
November	49.1	45.8	44.4	42.6	48.8	56	37.7	26	31.9	18	6.45	20	51.7
December	44.5	39.4	37.9	37.8	42.9	51	32.7	18	25.8	10	0.85	8	44.5
TOTAL	-	-	-	-	-	-	-	-	-	-	35.37	191	1441.3

Table IX. METEOROLOGICAL DATA. AUCHINCRUIVE. 1964

Month:-	Soil Temperatures °F.				Air Temperatures °F.				Grass Minimum °F.		Monthly Rainfall (inches)	Rain days	Sunshine (hours)
	4 ft.	1 ft.	8"	4"	Mean Maximum	Highest Maximum	Mean Minimum	Lowest Minimum	Mean	Lowest			
January	42.8	40.4	39.6	38.4	45.0	50	35.9	27	30.0	15	2.50	12	42.9
February	42.5	40.0	39.5	37.6	44.9	54	35.3	23	29.4	14	0.70	13	78.0
March	42.1	40.4	39.6	37.9	45.7	50	34.6	24	29.2	14	0.72	7	78.0
April	43.8	45.0	44.7	45.0	52.7	63	40.7	28	34.9	19	1.60	20	128.6
May	48.1	52.0	51.9	53.8	60.5	75	46.3	41	40.6	27	2.60	18	178.3
June	51.6	55.0	54.5	57.0	60.7	71	47.9	34	44.1	28	2.29	14	148.5
July	53.7	57.1	57.3	59.2	62.3	68	51.6	44	47.2	35	2.41	16	122.0
August	55.1	57.4	57.1	58.4	62.7	72	50.1	35	47.5	34	4.38	20	156.5
September	54.6	55.0	54.1	54.1	60.9	67	48.1	32	44.0	27	5.57	18	131.1
October	52.0	49.9	48.6	46.9	53.6	62	41.6	32	36.4	23	3.61	14	73.8
November	48.9	46.1	44.9	42.8	49.6	55	39.3	28	34.5	22	3.33	18	58.7
December	45.9	41.2	40.0	37.6	43.7	53	33.8	22	28.5	17	4.26	21	45.7
TOTAL	-	-	-	-	-	-	-	-	-		33.97	191	1242.1

Table X. Meteorological Data. Auchincruive. 1965

Month:-	Soil Temperatures °F				Air Temperatures °F				Grass Minimum °F		Monthly Rainfall (inches)	Rain-days	Sunshine (hours)
	4 ft.	1 ft.	8"	4"	Mean Maximum	Highest Maximum	Mean Minimum	Lowest Minimum	Mean	Lowest			
January	42.8	38.7	37.7	35.3	42.1	52	32.7	22	28.5	16	4.79	17	75.3
February	40.7	37.1	36.4	34.3	42.9	48	31.6	24	27.0	18	0.74	6	71.4
March	41.2	39.5	38.8	38.0	47.4	64	34.2	12	30.2	14	1.38	12	133.8
April	44.0	44.8	44.2	45.5	51.8	61	38.0	30	33.3	22	2.76	16	160.8
May	47.1	50.2	49.8	52.2	56.7	71	44.3	31	40.6	25	2.05	15	143.8
June	51.4	55.8	55.9	58.5	62.2	69	48.7	37	46.5	32	3.51	16	176.2
July	53.5	56.6	56.3	58.3	60.6	69	47.9	36	44.5	32	3.35	14	153.1
August	54.5	56.5	56.2	57.4	62.2	75	49.3	37	46.6	34	4.17	17	163.0
September	53.7	53.9	53.1	52.7	58.6	66	47.0	38	43.2	34	4.76	20	76.4
October	52.1	50.7	49.3	48.0	56.3	66	44.0	34	39.8	28	3.55	13	105.2
November	48.4	43.7	41.6	38.6	44.3	57	32.5	24	29.9	18	1.76	13	93.1
December	44.5	40.7	39.5	37.6	40.5	53	33.9	20	29.9	19	4.54	26	46.2
Total:-	-	-	-	-	-	-	-	-	-	-	37.34	185	1398.3

TABLE XI Daily readings of radiant energy (cals/sq. cm.) January - June 1964

Date	January	February	March	April	May	June
1	20.7	48.3	138.0	213.9	358.8	117.3
2	6.9	62.1	103.5	227.7	151.8	379.5
3	6.9	82.8	75.9	172.5	248.4	393.3
4	6.9	48.3	96.6	186.3	227.7	248.4
5	27.6	117.3	172.5	400.2	193.2	227.7
6	13.8	34.5	186.3	138.0	255.3	82.8
7	20.7	48.3	186.3	324.3	248.4	124.2
8	6.9	55.2	172.5	82.8	345.0	441.6
9	20.7	34.5	131.1	158.7	220.8	117.3
10	41.4	13.8	96.6	213.9	331.2	317.4
11	41.4	48.3	227.7	213.9	241.5	269.1
12	20.7	62.1	220.8	220.8	220.8	227.7
13	34.5	82.8	69.0	269.1	351.9	269.1
14	13.8	69.0	27.6	234.6	420.9	96.6
15	34.5	75.9	138.0	138.0	455.4	110.4
16	69.0	82.8	165.6	138.0	372.6	117.3
17	69.0	75.9	62.1	138.0	303.6	386.4
18	69.0	144.9	117.3	379.5	69.0	310.5
19	62.1	96.6	48.3	41.4	338.1	331.2
20	75.9	158.7	75.9	296.7	420.9	531.3
21	27.6	158.7	55.2	207.0	124.2	351.9
22	20.7	41.4	55.2	220.8	338.1	393.3
23	55.2	13.8	158.7	289.8	434.7	345.0
24	20.7	62.1	41.4	289.8	427.8	434.7
25	20.7	96.6	234.6	131.1	489.9	372.6
26	41.4	172.5	276.0	186.3	483.0	289.8
27	27.6	62.1	200.1	193.2	476.1	434.7
28	48.3	124.2	96.6	351.9	427.8	372.6
29	20.7	213.9	151.8	331.2	282.9	414.0
30	48.3	-	138.0	351.9	144.9	276.0
31	48.3	-	103.5	-	117.3	-

TABLE XII Daily readings of radiant energy

(cals/sq. cm.) July - December 1964

Date	July	August	September	October	November	December
1	386.4	276.0	310.5	69.0	41.4	27.6
2	248.4	96.6	193.2	138.0	75.9	13.8
3	434.7	172.5	227.7	138.0	69.0	27.6
4	372.6	255.3	276.0	207.0	20.7	41.4
5	455.4	124.2	276.0	20.7	6.9	13.8
6	234.6	262.2	103.5	41.4	69.0	6.9
7	69.0	151.8	69.0	41.4	62.1	6.9
8	303.6	331.2	55.2	69.0	75.9	13.8
9	103.5	220.8	62.1	48.3	75.9	27.6
10	269.1	358.8	55.2	138.0	69.0	34.5
11	303.6	365.7	255.3	158.7	13.8	20.7
12	317.4	317.4	138.0	82.8	41.4	13.8
13	303.6	351.9	131.1	62.1	20.7	13.8
14	158.7	241.5	131.1	75.9	20.7	20.7
15	407.1	131.1	138.0	89.7	41.4	27.6
16	317.4	110.4	131.1	69.0	34.5	27.6
17	96.6	48.3	131.1	96.6	20.7	13.8
18	165.6	241.5	131.1	41.4	20.7	20.7
19	124.2	303.6	103.5	48.3	6.9	20.7
20	241.5	379.5	248.4	48.3	6.9	20.7
21	220.8	310.5	110.4	131.1	13.8	27.6
22	276.0	158.7	62.1	34.5	20.7	13.8
23	296.7	103.5	172.5	69.0	20.7	13.8
24	117.3	82.8	96.6	69.0	20.7	13.8
25	262.2	151.8	89.7	82.8	34.5	20.7
26	200.1	62.1	89.7	69.0	13.8	20.7
27	131.1	220.8	158.7	34.5	20.7	13.8
28	276.0	241.5	131.1	62.1	41.4	20.7
29	144.9	331.2	186.3	75.9	27.6	13.8
30	151.8	310.5	200.1	20.7	6.9	20.7
31	213.9	324.3	-	34.5	-	13.8

TABLE XIII Daily readings of radiant energy (cals/sq. cm.) January-June 1965

Date	January	February	March	April	May	June
1	20.7	69.0	158.7	276.0	317.4	386.4
2	20.7	34.5	179.4	207.0	124.2	469.2
3	48.3	75.9	55.2	310.5	82.8	414.0
4	27.6	69.0	158.7	296.7	372.6	220.8
5	13.8	69.0	207.0	310.5	103.5	269.1
6	6.9	41.4	172.5	158.7	131.1	138.0
7	6.9	27.6	89.7	220.8	144.9	234.6
8	6.9	75.9	158.7	276.0	213.9	365.7
9	13.8	34.5	117.3	124.2	172.5	441.6
10	13.8	34.5	186.3	262.2	372.6	420.9
11	27.6	82.8	207.0	200.1	172.5	207.0
12	41.4	34.5	89.7	179.4	241.5	103.5
13	13.8	69.0	96.6	234.6	351.9	345.0
14	13.8	96.6	48.3	144.9	282.9	186.3
15	20.7	96.6	89.7	103.5	317.4	186.3
16	13.8	75.9	165.6	151.8	158.7	448.5
17	13.8	75.9	241.5	158.7	75.9	331.2
18	55.2	34.5	124.2	227.7	414.0	358.8
19	55.2	75.9	172.5	276.0	345.0	345.0
20	48.3	69.0	151.8	310.5	317.4	103.5
21	27.6	62.1	34.5	372.6	89.7	345.0
22	13.8	96.6	41.4	262.2	179.4	414.0
23	13.8	103.5	96.6	303.6	255.3	241.5
24	62.1	110.4	55.2	269.1	151.8	179.4
25	27.6	82.8	165.6	151.8	414.0	234.6
26	34.5	96.6	179.4	220.8	262.2	276.0
27	62.1	89.7	151.8	186.3	227.7	207.0
28	55.2	34.5	282.9	131.1	345.0	207.0
29	55.2	-	303.6	124.2	420.9	276.0
30	82.8	-	310.5	400.2	227.7	345.0
31	75.9	-	324.3	-	324.3	-

TABLE XIV Daily readings of radiant energy (cals/sq. cm.) July - December 1965

Date	July	August	September	October	November	December
1	158.7	172.5	144.9	27.6	20.7	34.5
2	420.9	103.5	358.8	27.6	27.6	13.8
3	483.0	400.2	69.0	34.5	55.2	20.7
4	172.5	151.8	75.9	41.4	110.4	13.8
5	227.7	248.4	103.5	48.3	34.5	6.9
6	310.5	172.5	55.2	110.4	48.3	62.1
7	207.0	241.5	96.6	69.0	34.5	6.9
8	276.0	269.1	96.6	138.0	41.4	6.9
9	296.7	179.4	69.0	158.7	55.2	6.9
10	241.5	234.6	110.4	151.8	27.6	20.7
11	207.0	220.8	138.0	103.5	27.6	20.7
12	172.5	213.9	186.3	117.3	34.5	13.8
13	103.5	138.0	151.8	34.5	55.2	13.8
14	138.0	69.0	96.6	48.3	48.3	20.7
15	345.0	103.5	69.0	55.2	55.2	13.8
16	483.0	138.0	207.0	96.6	20.7	13.8
17	607.2	103.5	69.0	41.4	6.9	6.9
18	276.0	172.5	186.3	179.4	13.8	13.8
19	138.0	138.0	186.3	69.0	20.7	13.8
20	138.0	345.0	69.0	62.1	13.8	13.8
21	262.2	117.3	48.3	75.9	48.3	6.9
22	103.5	124.2	103.5	103.5	41.4	13.8
23	103.5	124.2	41.4	69.0	20.7	20.7
24	103.5	82.8	62.1	75.9	27.6	20.7
25	234.6	144.9	41.4	62.1	27.6	13.8
26	282.9	248.4	110.4	13.8	27.6	20.7
27	103.5	172.5	131.1	6.9	34.5	13.8
28	69.0	124.2	75.9	48.3	20.7	20.7
29	69.0	172.5	27.6	62.1	27.6	27.6
30	103.5	96.6	89.7	20.7	41.4	27.6
31	103.5	276.0	-	20.7	-	6.9

Radiant Energy Data. Auchincruive. 1964 and 1965.

Mean daily radiant energy (cal. cm^{2}) *

Month:-	1964	1965
January	33.6	32.1
February	82.3	68.5
March	129.8	155.4
April	224.7	228.4
May	307.2	245.5
June	292.8	290.0
July	245.3	223.9
August	227.0	177.4
September	148.8	109.0
October	76.3	70.1
November	33.8	35.7
December	19.6	17.1
Yearly Total	1,827.8	1,653.1

* Measurements recorded on Siemens Integrating Photometer.

7.24. <u>Data from supplementary trials, observation plots and temperature recordings</u>

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Grass Observation Plots:- Details of 1st Cut on April 24th, 1964. **Table XVI.**

N. TREATMENT Cuts./acre 21% Nitro-Chalk	Lbs./Acre N.	Green Yield Tons/Acre	% D.M.	% N.	D.M. Yield Lbs./Acre	N. Yield Lbs./Acre	% Recovery of Fertiliser N.
Nil	Nil	3.073	18.8	2.48	1,294	32.09	-
1	23.52	3.473	17.1	2.94	1,330	39.10	29.8
2	47.04	3.430	16.8	2.96	1,291	38.21	13.0
3	70.56	5.145	15.2	3.42	1,752	59.92	39.4
4	94.08	6.045	14.3	3.97	1,936	76.86	47.6
5	117.60	6.288	13.6	3.98	1,916	76.27	37.6
6	141.12	6.145	14.2	4.12	1,955	80.54	34.3

Table XVII

Grass Observation Plots:- Details of the 2nd Cut on June 15th, 1964.

N. TREATMENT Cwts./Acre 21% Nitro-Chalk	Lbs./Acre N.	Green Yield Tons/Acre	% D.M.	% N.	D.M. Yield Lbs./Acre	N. Yield Lbs./Acre	% Recovery of Fertiliser N.
Nil	Nil	5.716	18.1	1.15	2,317	26.65	-
1	23.52	9.075	18.1	1.19	3,679	43.78	72.8
2	47.04	12.804	16.9	1.31	4,847	63.50	78.3
3	70.56	15.505	14.5	1.57	5,036	79.07	74.3
4	94.08	15.791	14.4	1.79	5,094	91.18	68.6
5	117.60	17.606	14.7	1.89	5,797	109.56	70.5
6	141.12	20.293	15.3	1.79	6,955	124.49	69.3

Table XVIII

Grass Observation Plots:- Details of 3rd Cut on August 5th, 1964.

N. TREATMENT Cwts./Acre 21% Nitro-Chalk	N. TREATMENT Lbs./Acre N.	Green Yield Tons/Acre	% D.M.	% N.	D.M. Yield Lbs./Acre	N. Yield Lbs./Acre	% Recovery of Fertiliser N.
Nil	Nil	1.529	23.9	1.78	817	14.54	-
1	23.52	4.458	22.7	1.52	2,285	34.73	85.8
2	47.04	5.544	22.3	1.64	2,769	45.41	65.6
3	70.56	6.502	21.5	1.71	3,132	53.56	55.3
4	94.08	8.002	18.3	2.25	3,279	73.78	62.9
5	117.60	7.745	17.6	2.25	3,056	68.29	45.7
6	141.12	10.217	18.0	2.22	4,119	91.44	54.5

Table XIX

Grass Observation Plots:- Details of 4th Cut on October 7th, 1964.

N. TREATMENT Cuts./Acre 21% Nitro-Chalk	Lbs./Acre N.	Green Yield Tons/Acre	% D.M.	% N.	D.M. Yield Lbs./Acre	N. Yield Lbs./Acre	% Recovery of Fertiliser N.
Nil	Nil	2.258	13.9	2.21	703	15.54	-
1	23.52	5.816	13.8	2.06	1,798	37.04	91.4
2	47.04	8.646	12.9	2.03	2,498	50.71	74.7
3	70.56	10.961	12.5	2.32	3,069	71.20	78.9
4	94.08	12.319	10.9	2.75	3,008	82.72	71.4
5	117.60	10.575	10.2	3.04	2,416	73.45	50.8
6	141.12	10.647	11.3	3.07	2,695	82.74	47.6

Table XX

SUMMARY OF 1964 DATA FROM GRASS OBSERVATION PLOTS

Total Amount of N. Fertiliser Applied (Lbs./N./Acre)	Total Yield of Green Material Tons/Acre	Total D.M. Yield Lbs./Acre	Total N. Yield Lbs./Acre	Overall % Recovery of Fertiliser N.	Lbs. D.M. Yield per Lb. of N. Applied
Nil	12.576	5,131	88.82	-	-
94.08	22.822	9,092	154.65	70	42.1
188.16	30.424	11,405	197.83	58	33.3
282.24	38.113	12,989	263.75	62	27.8
376.32	42.157	13,317	324.54	63	21.7
470.40	42.214	13,182	327.57	51	17.1
564.48	47.302	15,724	379.21	51	18.8

Table XXI

Grass Observation Plots:- Details of the 1st Cut on May 11th, 1965.

N Treatment Cwt./acre 21% Nitro-chalk	N Treatment Lbs./acre N.	Green Yield Tons/acre	% D.M.	% N.	D.M. Yield Lbs./acre	Nitrogen Yield Lbs. nitrogen/acre	% Recovery of Fertilizer N.
Control	Nil	3.540	20.9	1.62	1,658	26.85	-
1	23.52	6.082	18.6	1.76	2,534	44.60	75.5
2	47.04	7.474	14.9	2.88	2,495	71.85	95.7
3	70.56	8.873	14.9	2.86	2,962	84.71	82.0
4	94.08	9.265	14.5	3.07	3,010	92.40	69.7
6	141.12	9.722	15.1	3.29	3,289	108.20	57.6

Table XXII

Grass Observation Plots:- Details of the 2nd Cut on July 1st, 1965.

N Treatment Cwt./acre 21% Nitro-chalk	Lbs./acre N.	Green Yield Tons/acre	% D.M.	% N.	D.M. Yield Lbs./acre	Nitrogen Yield Lbs. nitrogen/acre	% Recovery of Fertilizer N.
Control	Nil	2.463	26.7	1.31	1,473	19.30	-
1	23.52	5.404	20.5	1.22	2,482	30.27	46.6
2	47.04	8.353	23.2	1.27	4,341	55.12	76.1
3	70.56	9.902	21.4	1.39	4,746	65.98	66.2
4	94.08	10.708	20.0	1.68	4,797	80.60	65.1
6	141.12	10.423	19.7	1.83	4,599	84.16	46.0

Table XXIII

Grass Observation Plots:- Details of the 3rd Cut on August 31st, 1965.

N Treatment Cut./acre 21% Nitro-chalk	N Treatment Lbs./acre N.	Green Yield Tons/acre	% D.M.	% N.	D.M. Yield Lbs./acre	Nitrogen Yield Lbs. nitrogen/acre	% Recovery of Fertilizer N.
Control	Nil	1.014	20.7	1.54	470	7.24	-
1	23.52	3.755	18.8	1.36	1,581	21.51	60.7
2	47.04	4.498	16.2	1.46	1,632	23.83	35.3
3	70.56	5.975	14.2	1.64	1,901	31.17	33.9
4	94.08	7.482	13.3	2.17	2,229	48.37	43.7
6	141.12	6.511	12.8	3.08	1,867	57.49	35.6

Table XXIV

Grass Observation Plots:- Details of the 4th Cut on October 5th, 1965.

N Treatment Cwt./acre 21% Nitro-chalk	N Treatment Lbs./acre N.	Green Yield Tons/acre	% D.M.	% N.	D.M. Yield Lbs./acre	Nitrogen Yield Lbs. nitrogen/acre	% Recovery of Fertilizer N.
Control	Nil	1.121	15.3	3.03	384	11.64	-
1	23.52	3.227	12.6	3.50	911	31.87	86.0
2	47.04	4.283	12.0	3.84	1,151	44.22	69.3
3	70.56	5.176	11.3	4.18	1,310	54.75	61.1
4	94.08	5.533	11.7	4.26	1,450	61.77	53.3
6	141.12	* 4.176	11.8	4.37	1,104	48.25	-

* [Low yield due partly to invasion by field mice].

Table XXV

Summary of 1965 Data from Grass Observation Plots

Total Amount of N. fertilizer applied (lbs. nitrogen per acre)	Total Yield of Green Material Tons/acre	Total Dry Matter Yield lbs./acre	Total Nitrogen Yield lbs. N./acre	Overall % Recovery of Fertilizer N.	lbs. Dry Matter per lb. of nitrogen applied
Nil	8.138	3,985	65.03	-	-
94.08	18.468	7,508	128.25	67.2	37.4
188.16	24.608	9,619	195.02	69.1	29.9
282.24	29.926	10,919	236.61	60.8	24.6
376.32	32.988	11,486	283.14	57.9	19.9
564.48	30.832	10,859	298.10	*46.4	*14.5

* [Data calculated omitting Cut 4]

7.242 **Effect of the Polythene used as below-ground barriers in segregating the root systems of the Ryegrass and clover components. 1964 Results.**

Establishment Cut - taken on July 15th, 1964. **Table XXVI**

Treatment	Total D.M. Yield lbs./acre	Total N. Yield lbs./acre	% D.M.		% N.	
			Grass	Clover	Grass	Clover
Polythene (at right angles to rows) [GRASS : CLOVER RATIO]	574 (100%) [59 : 41]	15.36 (100%) [55 : 45]	17.4	14.6	2.42	3.04
No Polythene [GRASS : CLOVER RATIO]	655 (114%) [46 : 54]	18.89 (123%) [35 : 65]	18.7	13.5	2.25	3.41
S.E.	$\pm$ 35 N.S.	$\pm$ 1.04 N.S.	$\pm$0.32 °	$\pm$0.29 #	$\pm$0.08 N.S.	$\pm$0.12 N.S.

Table XXVII — Effect of the Polythene used as below-ground barriers in segregating the root systems of the Ryegrass and clover components — 1964 Results.

Details of 1st Cut taken on September 2nd, 1964.

Treatment	Total D.M. Yield lbs./acre	Total N. Yield lbs./acre	% D.M.		% N.	
			Grass	Clover	Grass	Clover
Polythene (at right angles to rows) [GRASS : CLOVER RATIO]	1513 (100%) [62:38]	42.09 (100%) [47 : 53]	17.8	14.6	2.14	3.89
No Polythene [GRASS : CLOVER RATIO]	1557 (103%) [62 : 38]	42.73 (101%) [47 : 53]	17.6	14.8	2.11	3.79
S.E.	± 83 N.S.	± 2.56 N.S.	± 0.2 N.S.	± 0.3 N.S.	± 0.02 N.S.	± 0.07 N.S.

Table XXVIII   Effect of the Polythene used as below-ground barriers in segregating the root systems of the Ryegrass and clover components.   1964 Results.

Details of the 2nd Cut taken on October 15th, 1964.

Treatment	Total D.M. Yield lbs./acre	Total N. Yield lbs./acre	% D.M.		% N.	
			Grass	Clover	Grass	Clover
Polythene (at right angles to rows) [GRASS : CLOVER RATIO]	708 (100%) [73 : 27]	22.52 (100%) [64 : 36]	14.9	13.1	2.75	4.28
No Polythene [GRASS : CLOVER RATIO]	703 (99%) [74 : 26]	21.58 (96%) [62 : 38]	15.0	12.9	2.62	4.30
S.E.	± 45 N.S.	± 1.47 N.S.	±0.2 N.S.	±0.3 N.S.	±0.04 N.S.	±0.02 N.S.

Table XXIX

Effect of the Polythene used as below-ground barriers in segregating the root systemsof the Ryegrass and Clover components

Details of the 1st Cut taken on May 18th, 1965

Treatment:-	Total D.M. Yield lbs./acre	Total N. Yield lbs. Nitrogen/acre	% D.M.		% N.	
			Grass	Clover	Grass	Clover
Polythene (at right angles to rows) [Grass : Clover Ratio]	3,286 [94:6]	49.25 [85:15]	15.7	8.8	1.36	3.50
No Polythene [Grass : Clover Ratio]	3,084 [93:7]	44.58 [84:16]	16.2	8.8	1.31	3.39
S.E.	$\pm$ 59 N.S.	$\pm$ 1.58 N.S.	$\pm$0.2 N.S.	$\pm$ 0.3 N.S.	$\pm$0.011 N.S.	$\pm$0.194 N.S.

Table XXX

Effect of the Polythene used as below-ground barriers in segregating the root systems of the Ryegrass and Clover components

Details of the 2nd Cut taken on July 6th, 1965

Treatment:-	Total D.M. Yield lbs./acre	Total N Yield lbs. nitrogen/acre	% D.M.		% N.	
			Grass	Clover	Grass	Clover
Polythene (at right angles to rows) [Grass : Clover Ratio]	2,000 [66:34]	47.25 [49:51]	20.2	14.2	1.76	3.49
No Polythene [Grass : Clover Ratio]	2,080 [65:35]	49.55 [50:50]	20.0	14.4	1.82	3.42
S.E.	± 52 N.S.	±2.05 N.S.	±0.2 N.S.	± 0.3 N.S.	±0.05 N.S.	±0.03 N.S.

Table XXXI

Effect of the Polythene used as below-ground barriers in segregating the root systems of the Ryegrass and Clover components

Details of the 3rd Cut taken on September 7th, 1965

Treatment:-	Total D.M. Yield lbs./acre	Total N Yield lbs. nitrogen/acre	% D.M.		% N.	
			Grass	Clover	Grass	Clover
Polythene (at right angles to rows) [Grass : Clover Ratio]	2,316 [64:36]	75.39 [59:41]	11.40	8.42	3.005	3.703
No Polythene [Grass : Clover Ratio]	2,257 [65:35]	73.15 [59:41]	11.43	8.40	2.965	3.768
S.E.	± 47 N.S.	±1.12 N.S.	± 0.25 N.S.	± 0.10 N.S.	±0.063 N.S.	± 0.051 N.S.

Table XXXII

Effect of the Polythene used as below-ground barriers in segregating the root systems of the Ryegrass and Clover components

Details of the 4th Cut taken on October 19th, 1965

Treatment:-	Total D.M. Yield lbs./acre	Total N Yield lbs. nitrogen/acre	% D.M.		% N.	
			Grass	Clover	Grass	Clover
Polythene (at right angles to rows) [Grass : Clover Ratio]	892 [73:27]	34.41 [69:31]	12.4	11.4	3.66	4.36
No Polythene [Grass : Clover Ratio]	868 [72:28]	33.13 [69:31]	12.5	11.4	3.62	4.37
S.E.	± 19 N.S.	±0.96 N.S.	±0.2 N.S.	±0.09 N.S.	±0.04 N.S.	±0.06 N.S.

7.243 Permeability tests on black polythene in the field

Details of 1st Cut, taken on 4th May, 1965 Table XXXIII

Treatment:-	D.M. Yields (lbs./acre)	N Yields (lbs./acre)	% D.M.	% N.
A. Grass receiving 188 lbs. of N. per acre per cut	1834	96.58	12.67	5.29
B. Grass in close proximity to fertiliser nitrogen applied in A. but separated by polythene	917	21.75	17.10	2.39
C. Control grass receiving no nitrogen and distant from fertilizer applied to A.	853	17.91	17.30	2.14
S.E. of mean	± 56 ***	± 1.92 ***	± 0.27 ***	± 0.38 ***

Permeability tests on black polythene in the field.

Details of 2nd Cut, taken on June 29th, 1965.

Table XXXIV

Treatment	D.M. Yields (lbs./acre)	N Yields (lbs./acre)	% D.M.	% N
A. Grass receiving 188 lbs. of N. per acre per cut	9,723	196.50	18.65	2.20
B. Grass in close proximity to fertilizer nitrogen applied in A. but separated by polythene	1,772	29.94	16.77	1.71
C. Control grass receiving no fertilizer nitrogen and distant from fertilizer applied to A.	1,682	28.46	17.75	1.63
S.E. of mean	$\pm$1,394 **	$\pm$23.22 ***	$\pm$ 0.35 *	$\pm$0.06 ***

Permeability tests on black polythene in the field. **Table XXXV**

Details of the 3rd Cut taken on July 29th, 1965.

Treatment	D.M. Yields (lbs./acre)	N Yields (lbs./acre)	% D.M.	% N
A. Grass receiving 188 lbs. of N. per acre per cut	4,426	156.71	14.10	3.56
B. Grass in close proximity to fertiliser nitrogen applied in A. but separated by polythene	790	15.99	12.95	2.05
C. Control grass receiving no fertilizer nitrogen and distant from fertilizer applied to A.	785	16.63	13.87	2.13
S.E. of mean	±131 ***	±4.48 ***	± 0.39 N.S.	±0.06 ***

Permeability tests on black polythene in the field. **Table XXXVI**

Details of the 4th Cut taken on September 28th, 1965.

Treatment	D.M. Yields (lbs./acre)	N Yields (lbs./acre)	% D.M.	% N.
A. Grass receiving 188 lbs. of N. per acre per cut	7,878	209.80	14.20	2.67
B. Grass in close proximity to fertilizer nitrogen applied in A. but separated by polythene	821	21.11	13.11	2.59
C. Control grass receiving no fertilizer nitrogen and distant from fertilizer applied to A.	778	20.47	13.07	2.52
S.E. of mean	±207 ***	±4.48 ***	± 0.53 N.S.	±0.81 N.S.

TABLE XXXVII. 1964 Microclimatic Temperatures (^{o}F) April 18th-30th incl. Taken at ground level by screened thermometers.

APRIL DATE	Grass and Clover (a) Max.	Grass and Clover (a) Min.	Grass and Clover (b) Max.	Grass and Clover (b) Min.
18	66	37	65	38
19	53	41	53	41
20	60	43	56	43
21	58	44	58	44
22	58	42	58	42
23	59	40	58	40
24	56	38	58	37
25	54	41	54	41
26	60	45	61	45
27	63	48	63	48
28	67	49	66	49
29	61	46	60	46
30	63	44	62	43

TABLE XXXVIII. 1964 Microclimatic Temperatures (^{o}F) taken at ground level by screened thermometers.

MAY DATE	Grass and Clover (a) Max.	Grass and Clover (a) Min.	Grass and Clover (b) Max.	Grass and Clover (b) Min.
1	62	44	62	43
2	64	43	64	42
3	60	45	60	45
4	60	46	60	46
5	64	46	63	40
6	62	44	60	43
7	62	48	61	48
8	61	43	61	42
9	61	46	61	46
10	62	46	61	46
11	61	43	61	43
12	62	48	61	48
13	60	46	61	43
14	62	44	62	44
15	63	44	66	44
16	63	46	64	45
17	68	49	68	49
18	62	52	62	52
19	61	47	60	46
20	63	45	62	44
21	64	47	63	47
22	66	46	63	47
23	65	44	64	43
24	67	47	68	46
25	72	45	70	45
26	72	43	79	43
27	85	49	84	48
28	88	49	85	48
29	81	53	80	53
30	65	55	66	55
31	54	49	52	48

TABLE XXXIX. 1964 Microclimatic Temperatures (°F) taken at ground level by screened thermometers.

JUNE DATE	Grass and Clover (a) Max.	Grass and Clover (a) Min.	Grass and Clover (b) Max.	Grass and Clover (b) Min.
1	60	42	57	42
2	66	39	63	38
3	66	44	63	44
4	64	49	62	49
5	64	50	64	48
6	58	48	58	49
7	57	52	56	51
8	68	49	65	49
9	60	52	59	53
10	65	53	64	53
11	65	50	64	50
12	66	52	65	53
13	67	55	66	55
14	56	51	56	51
15	77	49	78	48
16	Temperatures not recorded since all plots were defoliated.			
17				
18				
19				
20	73	36	73	36
21	79	44	78	43
22	81	42	80	43
23	70	48	70	46
24	83	49	85	49
25	87	54	87	54
26	82	56	83	56
27	75	56	75	56
28	79	49	80	48
29	82	50	82	49
30	74	54	75	53

TABLE XL. 1964 Microclimatic temperatures (°F) taken at ground level by screened thermometers.

JULY DATE	Grass and Clover (a) Max.	Grass and Clover (a) Min.	Grass and Clover (b) Max.	Grass and Clover (b) Min.
1	85	51	86	51
2	82	53	80	53
3	83	48	85	48
4	77	48	77	47
5	82	52	83	51
6	79	45	79	45
7	59	52	58	52
8	65	49	65	50
9	61	48	61	48
10	73	49	73	50
11	72	51	74	51
12	73	49	75	49
13	74	46	75	45
14	67	55	67	55
15	77	54	76	54
16	75	50	75	50
17	67	56	67	56
18	67	56	68	56
19	66	56	65	56
20	77	55	76	55
21	71	57	71	57
22	77	58	78	58
23	76	57	76	57
24	66	59	64	59
25	71	57	70	57
26	68	52	66	52
27	64	55	63	55
28	69	53	68	53
29	65	49	65	49
30	67	54	67	55
31	65	55	64	55

TABLE XLI. Maximum and Minimum Temperatures recorded at ground level by screened thermometers.

1964 Results. Mean monthly temperatures - $^{\circ}$F.

Treatment	April 18-30th incl.		MAY		JUNE		JULY	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Grass and clover	59.8	42.9	65.2	46.5	70.2	48.9	71.7	52.5
Grass and clover (Polythene)	59.2	42.8	65.0	45.9	69.5	48.7	71.5	52.7

TABLE XLII. 1965 Microclimatic Temperatures (°F) taken at ground level by screened thermometers.

APRIL DATE	Grass and clover 2 Cuts/ Annum		Grass and clover 4 Cuts/ Annum		Grass and clover 6 Cuts/ Annum		Grass NO		Grass N1	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
24	63	38	65	38	67	38	70	37	65	37
25	61	36	60	36	61	36	67	36	67	36
26	56	40	56	41	57	41	58	41	63	41
27	61	33	60	34	61	35	62	34	64	34
28	51	36	50	36	54	33	52	33	52	34
29	48	37	47	37	49	35	52	36	51	36
30	54	37	54	37	57	36	58	37	58	37

TABLE XLIII. 1965 Microclimatic Temperatures (°F) taken at ground level by screened thermometers.

MAY DATE	Grass and clover 2 Cuts/Annum		Grass and clover 4 Cuts/Annum		Grass and clover 6 Cuts/Annum		Grass N0		Grass N1	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1	65	40	65	40	70	39	72	42	72	42
2	67	43	67	44	71	42	71	43	76	43
3	58	46	57	45	59	45	61	45	60	46
4	53	40	52	40	55	38	55	39	55	40
5	66	47	67	46	72	46	67	47	69	47
6	54	40	53	40	55	38	54	40	54	41
7	53	46	54	46	56	46	56	47	54	47
8	55	46	55	46	58	45	57	46	56	47
9	58	45	59	46	72	45	58	46	57	46
10	59	44	57	44	65	41	62	43	60	43
11	63	44	63	42	64	43	65	41	60	41
12	60	46	64	44	65	45	75	42	73	42
13	66	48	69	46	70	46	79	42	77	43
14	69	47	78	45	76	46	90	41	87	42
15	73	52	81	51	79	52	90	51	90	51
16	62	49	67	48	64	48	75	47	75	47
17	68	45	77	45	74	45	92	48	90	47
18	46	40	48	36	48	37	50	35	49	36
19	56	32	62	30	62	32	80	31	73	32
20	61	34	70	31	70	32	90	31	81	31
21	61	46	75	45	71	46	85	45	82	45
22	-	-	-	-	-	-	-	-	-	-
23	57	47	62	47	62	48	84	47	82	48
24	62	48	67	46	66	47	82	46	77	47
25	62	46	61	46	61	47	68	45	66	46
26	71	49	69	49	70	49	81	48	77	48
27	71	44	70	45	70	44	80	44	79	45
28	77	45	75	45	76	45	85	47	83	47
29	63	44	62	44	64	44	77	44	78	42
30	80	42	75	42	78	42	84	42	82	42
31	68	42	66	44	67	42	68	43	66	44

TABLE XLIV. 1965 Microclimatic temperatures (°F) taken at ground level by screened thermometers.

JUNE DATE	Grass and clover 2 Cuts/ Annum Max.	Grass and clover 2 Cuts/ Annum Min.	Grass and clover 4 Cuts/ Annum Max.	Grass and clover 4 Cuts/ Annum Min.	Grass and clover 6 Cuts/ Annum Max.	Grass and clover 6 Cuts/ Annum Min.	Grass NO Max.	Grass NO Min.	Grass N1 Max.	Grass N1 Min.
1	71	37	69	39	73	39	77	41	71	37
2	78	41	75	41	77	41	82	40	74	41
3	85	45	78	45	82	45	84	44	79	44
4	86	49	75	50	81	49	86	48	86	49
5	78	52	70	52	78	52	72	52	70	52
6	-	-	-	-	-	-	-	-	-	-
7	74	49	68	49	73	49	75	49	71	50
8	-	-	-	-	-	-	-	-	-	-
9	73	46	67	48	73	47	75	46	72	47
10	90	46	78	47	85	48	80	46	83	46
11	87	49	77	49	81	49	80	50	79	51
12	79	54	70	54	76	54	74	54	73	55
13	-	-	-	-	-	-	-	-	-	-
14	78	53	68	53	74	54	74	52	73	53
15	74	52	67	54	72	54	74	53	73	53
16	67	51	65	51	69	52	64	51	64	52
17	76	51	63	51	69	52	68	52	68	52
18	75	54	65	55	74	55	72	55	72	55
19	75	53	65	53	68	53	67	53	66	54
20	76	53	64	55	70	55	72	53	72	54
21	68	53	62	53	67	54	62	52	62	53
22	79	48	66	48	70	48	72	48	72	48
23	77	52	64	53	76	52	72	48	72	47
24	70	51	63	51	72	49	64	48	64	50
25	62	53	60	53	62	53	60	52	61	53
26	70	49	65	50	72	48	65	49	65	49
27	71	53	65	52	75	52	68	53	-	-
28	66	52	63	53	71	51	65	52	-	-
29	61	50	61	50	70	48	64	50	-	-
30	77	56	68	55	86	56	72	55	-	-

TABLE XIV. 1965 Microclimatic temperatures (°F) taken at ground level by screened thermometers.

JULY DATE	Grass and clover 2 Cuts/Annum		Grass and clover 4 Cuts/Annum		Grass and clover 6 Cuts/Annum		Grass No	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1	73	51	65	51	78	51	70	53
2	64	53	65	52	67	53	70	53
3	75	47	79	44	80	45	90	45
4	73	43	80	40	82	41	92	41
5	65	47	67	44	69	45	74	45
6	66	48	70	46	70	47	75	48
7	70	46	76	43	74	44	84	44
8	66	46	70	44	71	45	84	44
9	68	49	71	48	74	50	86	45
10	-	-	-	-	-	-	-	-
11	70	44	79	42	78	44	92	44
12	62	49	64	46	66	46	67	48
13	71	50	75	49	75	50	83	50
14	57	49	57	49	57	49	74	51
15	56	46	56	46	57	46	58	48
16	69	43	76	42	75	44	78	44
17	75	45	84	45	79	45	80	48
18	78	48	85	47	82	47	85	48
19	72	54	78	54	79	54	81	56
20	72	52	79	51	77	51	78	53
21	72	57	75	57	76	58	76	59
22	62	55	63	55	64	55	63	56
23	67	48	70	47	69	48	70	54
24	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-
26	65	51	70	50	70	51	69	53
27	68	52	70	52	67	52	68	53
28	59	54	63	55	62	55	60	56
29	60	56	61	56	61	55	60	56
30	62	39	66	39	63	37	63	40
31	62	43	69	43	76	43	68	45

TABLE XLVI. Maximum and minimum temperatures recorded at ground level by screened thermometers. 1965 Results. Mean monthly temperatures. ^{o}F.

Treatment	April 24th-30th incl.		MAY		JUNE		JULY	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Grass and Clover 2 cuts/annum	56.3	36.7	62.8	44.2	74.9	50.1	67.1	48.7
Grass and Clover 4 cuts/annum	56.0	37.0	64.9	43.6	67.4	50.1	70.8	47.8
Grass and Clover 6 Cuts/annum	58.0	36.3	66.3	43.5	73.9	50.0	71.4	48.3
Grass only N0	59.9	36.3	73.1	43.3	71.9	49.9	74.9	49.3
Grass only N1	60.0	36.4	71.3	43.6	71.4	49.8	-	-

8. THESIS SUMMARY

8. SECTION VIII

THESIS SUMMARY

An experiment was conducted over the period 1963-65 with New Zealand cultivars of Lolium perenne (L) and Trifolium repens (L). They were sown in alternate rows, six inches apart and half the plots in the trial area were established to maintain root segregation between the species using a double layer of 500 gauge black polythene. Liberal quantities of phosphate and potash fertilizers were applied each year but the grass-legume association relied upon soil mineralisation, fixation by free-living organisms, rainfall and symbiotic fixation for its nitrogen supply.

Variations in defoliation frequency of two, four and six cuts per annum had little effect on the overall dry matter yield which amounted to 5,300, 6,100 and 6,000 pounds per acre per annum respectively. However, the average yearly production of nitrogen during the experimental period was 112, 166 and 217 pounds of nitrogen per acre which suggested a 48% increase by doubling the cutting frequency and a 98% increase when three times the number of defoliations were employed.

Root segregation of perennial ryegrass and white clover when grown in close association reduced the dry matter yield by 18% in the establishment year and by 6% in 1964 and it is suggested that root check and root restriction, particularly in respect of the grass component, were mainly responsible. In 1965 the dry matter yield was 26% lower where root barriers had been introduced and from above ground appearance of the grass and from the yield data this was clearly

the direct effect of eliminating underground nitrogen transference from the clover.

The nitrogen economy of this grass-legume association has been studied over a three year period and only in the final year was it possible to demonstrate above ground the results of underground nitrogen transfer. Clover contributed 30.79 pounds of nitrogen to its grass partner in 1965 and this figure is compared with predicted values using the theory of Walker, Orchiston and Adams and also data computed from supplementary grassland observation plots.

Micro-climatic temperatures recorded at ground level partially corroborate the findings of Johnstone-Wallace who showed lower diurnal fluctuations of temperature with a grass and clover sward compared with grass alone.

Seed of the same New Zealand cultivar of Trifolium repens (L) was inoculated with an effective strain of rhizobium (R.157 originating from Sydney, Australia) and compared with a non-inoculated control. From the limited results of this trial and from field observations it would appear that the indigenous strain of rhizobium at Auchincruive was an effective one.

The physical effects of the black polythene used for root segregation were examined through yield data in a special trial and laboratory and field tests were carried out on the permeability of this material as used in the experiments.

Dry matter production, nitrogen yields and herbage quality from the perennial ryegrass-white clover association are reported, discussed and compared with data from New Zealand and Holland.