# NUTRIENT EFFICIENCY OF SOME GRASSES AND LEGUMES IN RELATION TO ENVIRONMENTAL STRESS

A thesis presented in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy in Agronomy at
Massey University

Robin Stewart Scott
1975

# ABSTRACT

This thesis provides a definition of relative nutrient efficiency (RNE) by which species and strains of pasture plants can be differentiated in a quantifiable manner according to the shape of their response surface to applied nutrients. Attention is drawn to the fact that RNE is under genetic control and to the probability that no single casual mechanism controls RNE because of the interdependence of plant physiological processes.

Experiments are reported investigating the RNE of strains of Yorkshire fog (*Holcus lanatus* L.), perennial ryegrass (*Lolium perenne* L.), *Lotus penduncculatus* Cav. (Syn *L. uliginosis* Schkuhr., *L. major* Sm.) and white clover (*Trifolium repens* L.). Initial experiments examined a number of strains of each species for their RNE in response to N for the grasses and P for the legumes. Subsequent experiments compared strains of both grass species in response to N and legume species in response to P together with the effects of climate. Measurements were made of dry matter yields, shoot:root ratios, N absorption and utilization, and in addition for legumes their N fixing activity and P absorption and utilization.

A final experiment examined climatic paramaters affecting RNE of two strains of white clover in response to P and their relative abilities to remobolize and re-utilize N and P in younger compared to older tissue.

Results of all experiments showed differences in RNE among strains of each species. They also showed that RNE is dependent upon climate, with high RNE generally being expressed under conditions most favourable for maximum growth.

A bred strain of Yorkshire fog - Massey Basyn, was found to provide high yields relative to others at low levels of N. This was attributed to its greater ability to absorb and utilize N. Among perennial ryegrasses Mangere strain possessed low RNE under a 15/10°C day/night temperature and 200 $Wm^2$ light intensity due to its inability to absorb N through restricted root development. Comparison of the most efficient strains of Yorkshire fog and perennial ryegrass showed that they did not differ in RNE and it is concluded that the 'low fertility' classification of Yorkshire fog is unwarranted.

The initial experiment with Lotus identified strains differing in RNE but this finding was not confirmed in the subsequent experiment. A strain of white clover from the Netherlands (Tamar) was found in two experiments to have high RNE through providing high yields at medium to high levels of P. In the final experiment where Tamar was compared with Huia white clover, its greater RNE was due to a greater ability to absorb and utilize N and P, to remobolize N and P from older to younger tissue and to mobolize soluble sugars from shoots to roots.

Comparison of the most efficient strains of white clover and Lotus showed that the former responded better to high levels of P.

It is concluded from this series of experiments that genetic variability exists among the species studied and that their differing RNE could be exploited to bring about economies in the use of fertilizers.

# ACKNOWLEDGEMENTS

The author wishes to acknowledge the Ministry of Agriculture and Fisheries for the provision of a study award which enabled this study to be undertaken.

Grateful thanks is given to Professor B.R. Watkin, Agronomy Department, Massey University and Dr R.H. Jackman, Grasslands Division for the very helpful advice and support given throughout this study.

The assistance of Mr A. Hardacre and his staff of Plant Physiology Division and that of various members of the staff of the Agronomy Department, Massey University in the conduct of experiments is acknowledged. So also is the assistance of Dr R. Hazlewood of Plant Physiology Division for completion of the sugar analyses, Mr P. Nes of Grasslands Division and Mr J.L. Grigg of Invermay Agricultural Research Centre for completion of some of the N and P analyses.

The author wishes to record his appreciation to Dr G.J. Jowett and Mr P. Johnstone of Invermay Agricultural Research Centre for the advice and assistance with the statistical analyses.

# CONTENTS

List of Figures Page

# SECTION 1

# INTRODUCTION AND OBJECTIVES

New Zealand has a temperate climate well suited to grassland farming. The most important single factor limiting the attainment of levels of pasture production set by climate is the availability of soil nitrogen. Because of the high cost of artificial nitrogen in relation to the returns realised for animals products, New Zealand has evolved a grassland farming system dependent upon the fixation of nitrogen by the symbiosis between clover plants and Rhizobia bacteria and its utilization by associated grasses with a higher yield potential. The philosophy behind this system has been described by Sears in a series of articles (Sears, 1953) and by Walker (1954, 1956) and Jackman (1971a, 1971b). It needs no re-iteration here.

The attainment and maintenance of high grass/clover pasture production is dependent upon four main factors:-

1. The use of suitable species and strains of grasses and clovers.
2. The correction of mineral nutrient deficiencies.
3. Suitable grazing management.
4. Control of insect diseases and pests.

In most parts of New Zealand white clover (Trifolium repens L.) cv Grasslands Huia has been found to be the most successful clover species and cultivar. Under favourable conditions of Palmerston North, for example, Sears et al (1965) recorded levels of 670kg/ha/year of nitrogen (N) fixed by white clover grown alone or with grass where herbage was cut and removed. If clippings were returned the rate of fixation was reduced to 350kg/ha/year but with an increase in the rate of accumulation of soil nitrogen. White clover has been found to be a productive and persistent legume under close and continuous grazing.

Perennial ryegrass (Lolium perenne L.) cv Grasslands Ruanui is the standard grass species and cultivar[1] grown in New Zealand. It is not only high yielding but has the attributes of perenniality, ability to persist under relatively heavy treading and continuous grazing, and is capable of responding well to high levels of available soil nitrogen. It is compatable with white clover under suitable grazing management and adequate topdressing.

[1] In this study a number of cultivars and natural ecotypes are examined and to aid presentation they will be collectively referred to as 'strains'.

Given the most suitable species and strains of grasses and clovers, the remaining means available to farmers to increase pasture production are topdressing and grazing management. The control of pests and diseases is important in the maintenance of pasture production rather than its increase, and will not be dealt with here.

Grazing management is dependent upon the individual skill of the farmer in utilising existing knowledge to best optimize the requirements of pastures on the one hand and stock feed requirements on the other. Apart from sub-division and expenditure on extra stock, managerial requirements for capital inputs are low. Management is the means whereby a farmer can realise the potential for high production set by climate, species and strains of grasses and clovers and by the level of soil fertility - whether natural or brought about by topdressing.

Topdressing is recognised as the most powerful tool in the hands of the farmer for increasing pasture production. In general terms Elliot (1968) has drawn attention to the strong relationship between the level of livestock production in New Zealand and the level of topdressing with superphosphate - phosphorus (P) being New Zealand's most deficient major plant nutrient apart from N. In more specific terms this is well illustrated in experiments with superphosphate by Karlovsky (1966) under the mowing and clippings return technique and in terms of pasture and animal production by Scott (1968). Where potassium (K) is an additional limiting factor increases in butterfat production following its application have been demonstrated by Smith (1964). Where sulphur (S), (the remaining major nutrient deficiency of significance) is limiting, increases in pasture yields following its application have been reported by Ludecke (1965).

Increases in pasture production following topdressing are largely through the stimulation of clover growth and N fixation and the release of N to associated grasses. The demands for the major nutrients required in New Zealand agriculture are high because, in a competitive situation, grasses are more efficient in extracting available P (Jackman and Mouat, 1972), potassium (During, 1972) and sulphur (Walker, 1956) than are clovers. Thus the key to the success of New Zealand's grass/clover economy and the chief means available to increase its production is to fit the soil to the requirements of clover plants by topdressing.

In a critical review of the grass/clover pasture and symbiotic N fixation Jackman (1971a, 1971b) reached the conclusion that '.. biological research aimed at improving the potential of grass clover pasture should start with work on mineral nutrition of clovers'. He formed this conclusion (using P requirements as an illustration) on the basis that grasses are more efficient in absorbing available P than are clovers. This weakens the ability of clovers to compete for light energy in the mixed sward. Reduced light interception by clovers reduces their production of photosynthates; thereby compounding the problem as they cannot then make adequate root growth and compete with grasses for available nutrients and moisture.

This conclusion of Jackman's is in effect a suggestion that the nutrient requirement of pasture plants (and clovers in particular) should be fitted more closely to the present levels of soil fertility rather than adjusting soil fertility to the requirements of presently available plant material. In economic terms this makes sense. Sown pastures in New Zealand are generally regarded as being permanent when adequately topdressed and managed, and what little experimental evidence is available (Scott, 1971) supports this opinion. Topdressing is a recurring annual expenditure if high levels of production are to be maintained (Saunders et al, 1963; Scott & Cullen, 1965; Karlovsky, 1966). Seed costs of more efficient strains of grasses and clovers (should they be available) would be unlikely to be more than currently available strains. Thus any greater degree of efficiency in mineral nutrition that could be obtained would result in a substantial saving in money spent on fertiliser in New Zealand if current levels of pasture production are to be maintained. Alternatively much greater agricultural production could be obtained through the use of more efficient strains if current levels of fertiliser applications are continued.

Further justification for research into improved mineral nutrition of pasture plants lies in the current prospects of rapidly rising fertiliser prices. New Zealand's resources of high grade phosphate rock in Nauru and Christmas Islands are rapidly dwindling. Already plans are in train to utilise resources of B grade rock on Christmas Island - a material requiring beneficiation by heat treatment before it is suitable for manufacture into superphosphate. This will result in higher costs of production. So too will the increasing need to purchase phosphate rock on the higher priced world market. Finally, the increasing price of oil is likely, in the forseeable future, to continue to rise and bring about increased transportation costs of rock phosphate to New Zealand.

It is becoming increasingly apparent that phosphate applied to New Zealand soils is either directly or indirectly contributing to the eutrophication of waterways (Syers, 1974). Any reduction that can be brought about in phosphate topdressing through the use of plants which are more efficient in extracting and utilizing soil and applied P would be of considerable value in alleviating this problem.

It was on the basis of the economic and environmental needs and Jackman's justification in a technical sense that the objectives of this study were selected. They were as follows:-

1. To identify grasses and legumes efficient in taking up available soil nutrients (N and P) and in utilizing them for the production of dry matter.
2. To examine possible mechanisms controlling nutrient efficiency to provide a direct basis for plant breeding.

From the foregoing discussion it can be seen that a study of the nutrient efficiency of legume species was an obvious choice. In addition it was decided to study some grass species for the following reasons:- (a) As will be discussed in a later section there are presently no strains of legumes species recognised for their superior nutrient efficiency. Should this study fail to meet its objectives with legume species the possibility of progress with grass species appears greater in view of the findings of workers at Aberystwyth (see Experiment 2). Identification of mechanisms controlling efficiency in grasses could well be useful in subsequent studies of legume species. (b) The identification of species and strains of grasses with a greater ability to absorb available soil nitrogen would not only result in a direct increase in production but also result in an indirect increase through the more complete removal of available nitrogen and a resultant stimulation of nitrogen fixing activity by legumes (Walker, 1954).

White clover and perennial ryegrass were selected for study because they form the basis of New Zealand's pastoral production. Greater birdsfoot trefoil, <u>Lotus penduncul</u><u>atus</u> Cav. (Syn. <u>L.</u> <u>uliginosis</u> Schkuhr., <u>L. major</u> Sm.) was selected because (a) it shows persistency under conditions of low fertility (Levy, 1925) and has the ability to compete for low levels of available nutrients (Mouat, 1957). During the course of this programme of research Brock (1973) has produced evidence to show this is in fact true. Under low phosphorus inputs he found Lotus pendunculatus (Grasslands 4705, tetraploid) to yield 30% more dry matter than white clover (Grasslands Huia). Under high P input white clover yielded 30% more than L. pendunculatus. A comparison of Lotus with white clover, a species recognized to have a high fertility requirement, could provide useful information once it is established that strains exhibiting high nutrient efficiency are being compared. (b) Lotus has been shown to possess a high yield potential (Suckling, 1960), particularly under wet conditions and on shady faces (Levy, loc.cit.). However even under dry autumn conditions, Brock (loc.cit.) has obtained better growth of Lotus than of white clover.

Yorkshire fog (Holcus lanatus L.) was selected as a second grass for study because (a) it is recognised as a species persisting under low fertility conditions (see Experiment 1), and as in the case of legumes stated above, a comparison of a so-called 'low fertility' tolerating species with a 'high fertility' demanding species such as perennial ryegrass would be of value provided it is known that strains exhibiting high nutrient efficiency were being compared for each species. (b) It has already been demonstrated that Yorkshire fog has a good growth potential compared to perennial ryegrass, results of which have recently been reported by Jacques (1974) and by Watkin and Robinson (1974).

Superphosphate is the main fertiliser applied to New Zealand pastures. For this reason efficiency in absorption and utilization of P was selected for study with both grasses and clovers. Because of the dependence of grasses on available soil N the experiments with grasses included N as a treatment.

Initial 'screening' experiments were conducted in the glasshouse with a number of strains of each species to provide a basis for selection of strains for study in later experiments. This was to enable the conduct of more precise experiments through a reduction in number of strains examined and to ensure that the most efficient of each strain was to be compared in the interspecific comparisons.

Legume and grass species have different climatic requirements for optimum growth (Langer, 1972). Thus the production of photosynthesate is dependent upon climate and as it varies so too does the ability of plants to absorb and translocate nutrients and utilize them for the production of dry matter. For this reason the interaction of species and strains in their nutrient efficiency according to climate was studied under controlled environmental conditions. This was done after completion of screening experiments conducted in the glasshouse.

As the programme of research developed it was found desirable in the interests of experimental precision to restrict the final experiment to a study of two strains. White clover was selected because of the differences in nutrient efficiency found in one experiment and because of its importance to New Zealand agriculture. It was the aim of this experiment to investigate possible mechanisms controlling efficiency. The possibilities were wide and the 'source-sink' concept was selected for examination. Justification for this choice will be given in the introduction to Experiment 7.

## Relative nutrient efficiency (RNE)

Until now it has been convenient to refer to 'nutrient efficiency' without any clarification of its meaning. The term as used up to the present time can be misleading. It suggests that an absolute value exists which is applicable to all cultural conditions under which a plant is grown. This is unlikely to be true. Let us consider for example the nutrient efficiency of a plant grown in soil compared to one grown in solution culture. The absorption of nutrients by the plant grown in soil will be very dependent upon its root proliferation through the soil matrix whereas that of a plant grown in solution culture will not. As a further example consider two species grown either as a monoculture of as a biculture. Under the former situation there will only be intraspecific competition for nutrients or for light. Under the latter there will be both intraspecific and interspecific competition, and one species is likely to absorb less nutrients and produce less dry matter than it would in the absence of competition from an associated species. Thus to consider nutrient efficiency as an absolute and universally applicable concept is wrong. For this reason it is considered appropriate to use the term '_relative_ nutrient efficiency' in this study. It has the merit of referring to one species or strain relative to another - whether they be grown under similar cultural conditions as monocultures or as multicultures.

Basically RNE can be measured by three paramaters - nutrients absorbed, nutrients utilized and the product of these two - dry matter yields. Idealistically plants with perfect efficiency would conform to the types of response to applied nutrients shown in Fig. 1(a). Increasing the level of applied nutrient would give a linear increase in dry matter yield and nutrient

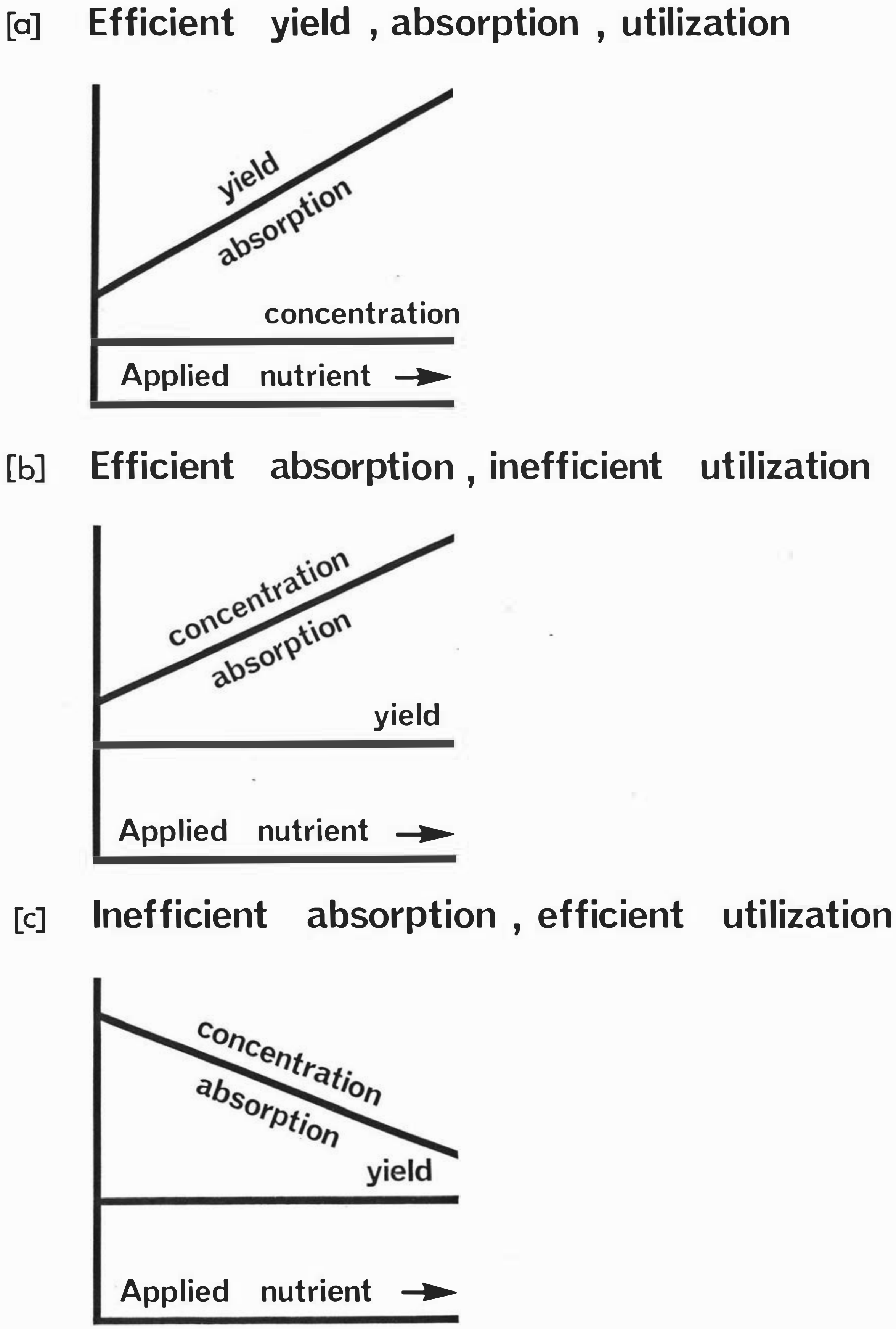

Fig. 1. Typified efficiency in absorption and utilization of applied nutrient and effect on yield.

absorbed. For near perfect utilization of absorbed nutrient it would have a low concentration (i.e. it would produce a large amount of dry matter per unit nutrient absorbed) and this would not change as the level of applied nutrient was increased. In other words, it would be equally efficient over the entire range of absorption.

Inefficiency in dry matter yield and nutrient absorption is bought about by a downward shift in the response curve or by a decrease in its slope. Inefficiency in utilization is brought about by an overall upward shift in the concentration curve or by an increase in its slope.

Absorption of a nutrient is a function of yield and nutrient concentration (and hence utilization). Because of this, identification of the cause of efficiency in yield is difficult to apportion among absorption and utilization.

The only means by which the relative contributions of absorption and utilization to yield can be gauged is by subjective comparison of their response curves. For example, an efficient absorber but inefficient user of a nutrient is typified in Fig. 1(b). Here there is increased absorption and increased concentration (decreased utilization) with the result that dry matter yields do not alter. An inefficient absorber but efficient user of a nutrient (Fig. 1(c)) would have decreasing absorption and decreasing concentration (increasing utilization), and could very well produce the same dry matter yields as the efficient absorber and inefficient user.

Previously it was pointed out that research into plant nutrition should be aimed at fitting the plant to the soil. This implies that a species or strain is required which provides high dry matter yields relative to another at low levels of available nutrient. It was also pointed out that present fertiliser practice is an attempt to fit the soil to the plant. This implies that present 'improved' species or strains require high levels of available nutrients to provide high yields. Thus there are the two extreme types of nutrient efficiency and an infinite variation between these two.

To enable differentiation of the RNE of a large number of species or strains a definition of types of efficiency is required. Such a definition must be quantifiable to enable distinctions to be made. The following are the types or RNE into which species or strains will be classified and the definitions of each types. They apply equally to

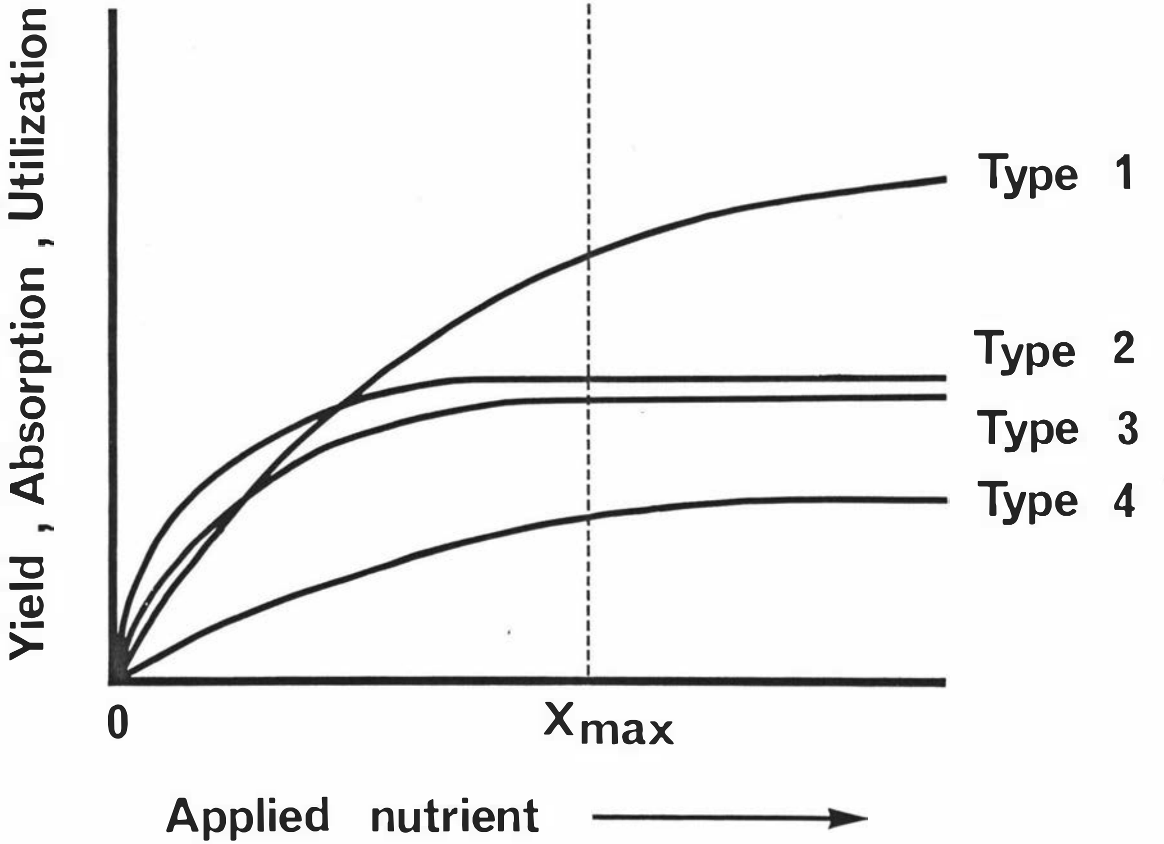

Fig. 2. Typified types of RNE in yield, absorption and utilization.

yield, absorption and utilization.

Classification of RNE

Type 1. Increasing at the maximum level of nutrient applied ($X_{max}$), where $X_{max} < 95\%$ of the asymptote value.

Type 2. Not increasing at $X_{max}$ but the best of the strains at some point in the range.

Type 3. Not increasing at $X_{max}$, not the best at any point in the range but not dominated by others.

Type 4. Not increasing at $X_{max}$, not the best at any point in the range and dominated by others.

$X_{max}$ is necessarily an arbitary level of applied nutrient but is equally applicable to all strains compared within an experiment where relative nutrient efficiency is being measured.

Examples of the above are given in Fig. 2. Type 1 illustrates a strain responding to high levels of a nutrient. It shows Type 2 as one responding at low levels of applied nutrient. Ideally a plant combining both Types 1 and 2 RNE is required. Where no such plant exists then the identificaation of strains possessing these types of RNE would open up the possibility of breeding the desirable type. It is one of the objectives of this study to so identify species and strains of some grasses and clovers.

RNE Type 3 is one which is similar to Type 2 and which should not be regarded as holding no promise for plant breeding purposes. It may be better at some point than a Type 1 strain but slightly inferior to a Type 2 at that point. A strain classified as Type 4 is one that is either of the Type 1 or Type 2 RNE but which, because of low overall yields or because of the shape of its response surface, has little to offer for plant breeding purposes.

# SECTION 2

# LITERATURE REVIEW

### (i) Nutrient efficiency.

There is adequate evidence provided in literature reviewed by Vose (1963), Gerloff (1963) and Epstein (1963, 1972) to show that RNE is under genetic control. Further, it has been shown by Bernard and Howell (1964), on crossing a P tolerant and a P sensitive variety of soybean (Glycine max.), that a pair of genes at a single loci governed the response. There are obvious opportunities to make use of mutants exhibiting greater nutrient efficiency in plant breeding, but the problem which confronts the plant breeder is the identification of mechanisms that control efficiency. Plant physiological studies have not, according to Epstein (1972, *loc.cit.*) provided much information on the genetic basis of mineral nutrition and he attributes this to physiologists working with pure lines of a relatively few species. This has precluded the opportunity of working with a large gene pool in which variation in mineral nutrition can express itself and the control mechanisms be studied.

While there are instances of the control of mineral nutrition being exerted by single loci, this does not necessarily mean that it controls one mechanism in the physiological and biochemical processes that constitute plant life. Mineral nutrition is a complex process dependent upon root morphology, root absorption and the translocation and re-distribution of absorbed ions. It is also dependent upon photosynthesis and the production of metabolites to provide energy for the absorption and translocation of ions and the elaboration of new and enlarged tissue. In the case of legumes the complexity is greater where the symbiotic association of host plant and *rhizobia* are interdependent and the identification of RNE is difficult. This was shown clearly in the work of Jones (1974) in measuring the P response of the genus *Stylosanthes*.

The above physiological processes are themselves dependent upon complex biochemical reactions within the plant. All are interdependent, with a change in one setting off a chain reaction subsequently affecting other processes and morphological characteristics. For this reason it seems improbable that one single mechanism will be found to control nutrient efficiency. Nevertheless it is possible that one or several mechanisms may be identified which correlate well with nutrient efficiency, are measureable, and would act as a tool in the selection and breeding of more efficient species.and strains.

### (ii) Factors controlling nutrient uptake.

A study of nutrient efficiency requires a detailed knowledge of plant nutrition in order that processes can be identified that warrant further investigation in the search for mechanisms that correlate well with demonstrated nutrient efficiency. For this reason the following sections consider some of the more important processes that control plant nutrition, with special reference to the nutrition of N and P.

#### (a) Nutrient availability.

Aquatic plants are surrounded by their nutrient medium which is continuously replaced by diffusion. With higher plants a more elaborate process is involved before nutrients can be absorbed into the plant. Plants.in soil depend firstly upon the concentration of nutrients in the soil solution and the rate at which they can be replaced from the non-labile pool. As roots absorb nutrients in their immediate vicinity these nutrients need to be replaced. Barber (1962) put forward the theory, recently quantified by Wiersum (1973), that nutrient ions are replaced in the root vicinity by mass flow and diffusion. Which of these two has the greater effect is dependent upon (i) the concentration of nutrients in the soil solution which moves towards the root following the uptake of water by the plant (mass flow), and (ii) the rate of uptake - which creates a concentration gradient along which nutrients can diffuse towards the root. Factors governing the supply of nutrients from soil to plant root will not be examined further here. The important point to note is that plants can modify their nutrient environment in the immediate vicinity of a root surface. A plant with a greater ability to absorb nutrients may not be able to manifest this greater ability if the soil cannot replenish sufficient of the absorbed nutrient. Thus nutrient efficiency is likely to vary depending upon the chemical nature of the soil in which it grows, (Barrow 1975a).

#### (b) Root morphology.

Troughton (1963), in a review of the underground organs of herbage grasses, pointed to variation between species in the distribution of roots down the soil profile - in terms of root weight, number, length and vertical and horizontal distribution. Further he drew attention to inter-strain differences. Therefore plants with root distribution coinciding with the mineral distribution will have an advantage over those with other distributions (Troughton and Whittington, 1968). Nevertheless it has long been known that plants can modify their rooting habit. Wilkinson (1961) demonstrated the proliferation of roots of soybean in a zone of soil fertilised with N and P as did Drew and Ashley (1971) in a study of nitrate uptake by barley. In a study of the absorption of phosphate and calcium from different depths of soil by swards of perennial ryegrass Newbould, Taylor and Howse (1971) showed after the initial few months of a sward's life the proportion of both elements absorbed from different depths for a whole grazing season varied little with age of the sward up to 3½ years. They further demonstrated that changes in the relative contribution of different depths of soil to uptake occurred within a season and was considerably modified by soil-water content and the application of fertiliser.

The distribution of roots within the soil profile is not the sole determinant of uptake. The ability of different portions of the root itself to take up nutrients varies. Rovira and Bowen (1968) in a study of P uptake by seminal roots of wheat showed that uptake at root apices was high and declined markedly in the region immediately behind the apices. However, total uptake was dominated by the contribution from laterals. Work of Clarkson and Sanderson (1970) has provided evidence that active absorption of phosphate and potassium (but not calcium) can occur in older root tissue and is not confined exclusively to younger root tissue as formerly believed.

Root weight has been found to be a poor index of the functional size of roots (May, 1960; Alcock, 1964; May _et al_, 1965; Bouwer, 1966). Cowan (1965) showed that the uptake of water from soil correlated well with root length. According to Vose (1963), Troughton (1959) found in perennial ryegrass that the uptake of radioactive P by branched roots can be 30% greater than the uptake by unbranched roots. Later Clarkson and Sanderson (_loc.cit._) produced evidence to show that the relative contribution of root members (such as laterals and axes) to P uptake varies much more closely with volume than with surface area or length.

From the foregoing it is apparent that a number of root morphological factors affect nutrient uptake, and a clearer understanding of these factors is required before they can be related to nutrient efficiency. To some extent the problem has been overcome by measuring the efficiency of the root system as a whole. Such a method was originally used by Williams (1948) in which the unit absorption rate was calculated as the instantaneous rate of uptake I, of a mineral nutrient M, per unit weight of root. It took the following form:

$$I_M = \frac{1}{R} \cdot \frac{dM}{dt}$$

where R is the dry weight of root.

Using this method, but replacing dry weight with fresh weight of root, Longeragan and Asher (1967) studied the P absorption of eight annual pasture species grown in solution culture. By this method they were able to differentitate between species that exhibited greater nutrient efficiency in terms of growth response. They also found that their ability to absorb P at relatively high concentrations (> 1uM) was not closely related to their ability to absorb P at low concentration (0.04 - 1uM). This is probably a reflection of the operation of the dual mechanism of uptake, as will be discussed later. In a review of nutrient flow, Brewster and Tinker (1972) calculated unit absorption rate from a range of experimental data reported in the literature where a number of species were examined. They found close similarity, being about $10 \times 10^{-13}$, $1 \times 10^{-13}$ and $10 \times 10^{-13}$ g atoms $cm^{-1}$ $sec^{-1}$ for N, P and K respectively. From this it appears that variation in the absorptive capacity of roots is more a reflection of size of the system rather than the absorptive capacity per unit of root tissue.

(c) Nitrogen and phosphate ions absorbed by plant roots.

Nitrogen. Available nitrogen occurs in the soil as nitrous oxide ($N_2O$) nitric oxide (N O), nitrogen dioxide ($NO_2$), ammonia ($NH_3$), ammonium ($NH_4$), nitrate ($NO_3^-$) and nitrite ($NO_2^-$) (Black, 1968). The first three gaseous forms are present in barely detectable amounts, while the latter three forms are present in ionic form. Nitrite and nitrate ions occur as freely diffusable ions in the soil solution and can move through the soil to roots by mass flow (Milthorpe and Moorby, 1969) while ammonium ions are present in exchangeable and non-exchangeable forms. Both nitrate and ammonium ions are taken up by plants. Lychlama (1963) working with perennial ryegrass grown in solution culture, showed that nitrate absorption increased with increasing temperature (5° to 35°C) and to be optimum at pH 6.2. Ammonium absorption was greatest at 22°C and was not influenced greatly by pH levels in the 4.0-7.5 range. Lycklama found that the absorption of ammonium is little affected by the presence of nitrate whereas the uptake of nitrate is inhibited by the uptake of ammonium - a phenomenon he ascribed to the inhibiting effect of ammonium uptake on nitrate reduction.

While both nitrate and ammonium ions are taken up by plants the former is the main ion entering plants grown in soil (Epstein, 1972). This is due to the presence of large quantities of nitrate ions in the soil as Lycklama (1963) and Haunold et al (1968) have shown. When adequate ammonium and nitrate ions are present in solution culture the uptake of ammonium ions was two to three times greater than the uptake of nitrate ions.

On entry into the plant, nitrate is reduced to ammonium ions and incorporated into organic compounds, as reviewed by Beevers and Hageman (1969). In the plant, nitrogen is an essential constituent of protein, amino acids, nucleotids and co-enzymes. Stocking and Ongun (1962) have reported as much as 70% of the total leaf N to be located in the chloroplasts.

Phosphorus. Most of the phosphate absorbed by plants from the soil exists in the soil solution as inorganic orthophosphates (Black, loc.cit.). It can only move short distances (Milthorpe and Moorby, loc.cit.) so that sustained root growth into zones of higher concentration is necessary for its absorption as zones become depleted. The ionic form in which is is absorbed is as yet unresolved although the weight of research evidence favours the $H_2PO_4^-$ ion rather than both the $H_2PO_4^-$ and $HPO_4^-$ ions. This matter will be discussed further in the next section.

(d) Entry of nutrient ions into plant cells.

Nutrient ions cannot move freely by diffusion from soil solution into plant cells, otherwise the higher concentration in plant cells than in the soil solution could not be maintained. Entry is controlled by two impermeable membranes - the plasmalemma lying immediately inside the cell wall and containing the cytoplasm, and the tonoplast which forms an internal lining around the vacuole. The current theory explaining the movement of ions into plant cells depends firstly on the movement by diffusion of ions into the extracellular plant tissue and then into the cell wall fibrils. This was shown to be the case by Conway and Downey (1950) and was called the 'outer space' of the tissue. Epstein (1955) developed the general equation that -

$$\text{Outer space} = \frac{\text{diffusable ions}}{\text{external concentration}}$$

where diffusable ions are expressed as micromoles per g fresh weight of tissue and external concentration as micromoles per ml.

Cation exchange has been demonstrated by many workers, following the initial work of Epstein and Legget (1954), to be a property of the carboxyl groups on the outer wall of root cells. Cations in the soil solution exchange with these electronegative sites. The region in which this exchange occurs was described by Briggs, Hope and Palmer (1958) as the 'Donnan free space'. Epstein (1972) provides good reason to believe that this space is outside the plasmalemma.

The root tissue into which ions are free to diffuse are the extra-plasmalemma components of the epidermis, cortex and endodermis, with the Casparian band preventing movement into the stele (Van Fleet, 1961). Kramer (1969) has shown in perennial plants absorption of water in older, partially suberized roots: a finding in agreement with more recent work of Clarkson and Sanderson (<u>loc.cit</u>.).

In earlier years it was thought that cation exchange provided the selectivity of cations absorbed by plant roots. However Epstein (1972) argues convincingly that this is not so, excepting when the concentration of a cation in the soil solution is exceedingly low and contact exchange occurs between the electronegative sites of the carboxyl groups of the cell wall and the electronegative sites of clay and organic colloids in the soil (Jenny and Overstreet, 1939a and b). His argument is firstly that the ionic composition of plants is not compatable with the exchange properties of carboxyl groups. Secondly he argues that if all monovalent ions are to be exchanged equally then the cation exchange theory cannot allow for the selectivity that exists between ions such as potassium and sodium.

The foregoing serves to demonstrate that ions come into contact with the outer impermeable cell membrane - the plasmalemma, by both diffusion and cation exchange involving the cell wall.

Gauch (1972), Epstein (1972), Anderson (1972) and Higinbotham (1973) have each reviewed and provided their interpretation of the current state of knowledge of the absorption process whereby ions are transported across the impermeable membranes of the plasmalemma and tonoplast. Three possible hypotheses have been put forward (a) the cell membrane theory, (b) the carrier theory and (c) the binding theory. The reviewers mentioned above provide argument and counter-argument for the existence of each mechanism. There is as yet no unequivocal answer to the problem.

In simple terms these theories are (i) according to the membrane theory ion movement can be both active and passive. When passive their movement is governed by the laws of diffusion. When active (implying the expenditure of energy through respiration) movement is against an electrochemical gradient. Under the latter condition the transfer sites for ions are thought to be part of the membrane itself. Because these sites of ion exchange within the membrane are not carriers of the absorbed ion there need not necessarily be undirectional transport. Higinbotham (loc.cit.) gives reason to favour this theory. (ii) The carrier theory, first put forward by Van der Honert (1936), postulates that ions are transported by combining with organic substances at one surface of the membrane and being carried, by rotation of the ion complex or through spatial re-arrangement, across the membrane for release at the other side. These carriers have not yet been identified (Epstein, 1972). This theory, which currently appears to gain most support, is based on the theoretical kinetics of absorption applied to rate of absorption measurements. (iii) The binding theory explains the accumulation, retention and ion selectivity by electrostatic attraction (Ling and Hope, 1969., Hiatt, 1968).

Whatever the true mechanism or mechanisms of absorption it is already well founded (following the initial work of Epstein, Rains and Elzam (1963)with barley) that absorption is under the control of two mechanisms. The latter for example showed that the absorption of potassium at low concentrations in the solution (up to 0.20uM) follows the Michaelis-Menton kinetics in which

$$V = \frac{S_o V_{max}}{S_o + K_m}$$

V being the rate of ion uptake, $S_o$ the ionic concentration in the solution, $V_{max}$ the maximum uptake occurring and $K_m$ the Michaelis-Menton constant - a value found to be very similar for K uptake in plants so far studied (Epstein, 1972). Where $V = \frac{1}{2}V_{max}$ then $K_m = S_o$. Epstein found in his study of K uptake by barley roots that the $K_m = 0.021$mM and $V_{max} = 11.9$ micromoles per g fresh weight per hour. When the concentration of the solution was raised from 0.50 to 50mM potassium the observed rate of potassium absorption markedly exceeded the theoretical maximum. Epstein (1972) has documented evidence from a number of workers confirming this dual mechanism where absorption under low concentrations is described as Mechanism 1 and absorption under high concentrations as Mechanism 2. Pertinent to this thesis is the finding that phosphate (Legget, Galloway and Gauch, 1965; Andrews, 1966; and Edwards, 1968) and ammonia have been observed to follow this dual mechanism of uptake. It has yet to be demonstrated with $NO_3^-$.

There is currently conjecture as to the species of phosphate ion involved in absorption, and whether the dual mechamism arises through absorption of the $H_2PO_4^{-1}$ ion at different sites in the membrane or whether it involves $H_2PO_4^{-1}$ and $HPO_4^{-2}$ ions at the one site (Hagen and Hokins, 1955; Bieleski, 1973; Fageria, 1974).

From the work of Fageria (loc.cit.) with rice (Oryza sativa L.) it is apparent that the mechanism operating under the low concentration is the most important one in phosphate absorption by plants grown in soil. However, Fageria quotes the concentration of phosphate in the vicinity of a monocalcium phosphate pellet and concludes that roots within a distance of 3-4cm from the pellet will be absorbing phosphate via the high concentration and low affinity mechanism. Fageria, (loc.cit.) and Barber and Frankenburg (1971) and Barber (1974) provide another dimension to the problem of understanding the mode of ion absorption when they suggest, with sound argument, that the mechanism for absorption under low phosphate concentrations may be attributable to the involvement of micro-organisms on the root surfaces. The role of micro-organisms will be dealt with in Section 2(e).

Interspecific differences in $K_m$ values for P uptake by legumes has been demonstrated by Andrews (1966). At low substrate concentrations ($1 \times 10^{-6}$M $KH_2PO_4$) he found absorption rates in the order - barley (Hordeum vulgare L.) > Phaseolus lathyroides L. > Desmodium uncinatum (Jacq.) D.C. > lucerne (Medicago sativa L.). At high concentrations ($2 \times 10^{-4}$ M $KH_2PO_4$) they were equal. The P absorption rate of Stylosanthes humilis H.B.K. (a plant naturally invading low P soils of subtropical Australia) was greater than that of the other species at all concentrations. Andrews, in explaining the differences considered that they could be partially explained in terms of the kinetics of absorption, i.e. the rate of transfer of phosphate ions across the cell membrane, the carrier concentration and the apparent dissociation constant. However he does point out that other physiological factors such as root morphology could account for the differences - at least in part.

(e) Micro-organisms and ion absorption.

Mycorrhizal root fungi, both ectrophic and endotrophic, can exert a profound effect on plant growth and uptake of N and P. This was demonstrated firstly by Finn (1942) who showed a 47% increase in dry weight of coniferous tree seedlings and a 75% increase in their N content. There are several theories to account for the improved N supply to mychorrizal plants on low N soil (Black, 1968). One is that the surface area of roots plus fungus of mycorrhizal roots is much greater than the absorbing surface of non-mycorrhizal roots. Harley (1959) for example, showed that the total root volume of ectotophic mycorrhizal rootlets was increased by 30% because of the sheath of fungus surrounding the rootlet. A second theory is that mycorrhizal fungi can utilize soil N unavailable to higher plants. A third theory is that elemental N is fixed by the association of mycorrhizal fungi and the roots of higher plants.

Bieleski (loc.cit.) has reviewed literature on the effects and possible mechanisms of infection of roots by mycorrhiza on plant growth in relation to P nutrition. He quotes examples of ectotrophic mycorrhiza increasing growth two or three times, reducing P deficiency symptoms and increasing P uptake. He also quotes endotrophic mycorrhiza as increasing growth by three to six times, abolishing P deficiency symptoms and increasing the P content of the host plant. According to Bieleski mycorrhiza exert some influence on plant uptake of a number of nutrients but their effect is largely on P uptake. He explains the possible mechanism by which they have their effect as (a) having an accumulation mechanism with a higher affinity for P than in normal roots, (b) being able to obtain P from insoluble salts in the soil and (c) presenting a larger surface area for P absorption as described above for N.

In recent studies on the effects of mycorrhiza on the P requirement of a number of genera Baylis (1970a, 1970b, 1972) noted differences in the minimum level of P required for plant growth. The threshold of P availability required for growth was directly related to the extent of the root-soil interface - those with numerous root hairs, such as perennial ryegrass, did not require added P whereas those of woody species such as Coprosma robusta with no root hairs did. This concurs with the findings of Sanders and Tinker (1971) who provide evidence to show that increased P uptake by onion seedlings inoculated with endotrophic mycorrhiza was not through rendering available non-isotopically exchangeable P but through the ramifying hyphae absorbing P beyond the immediate zone of depletion in the soil and transporting it to the roots as root hairs do.

Mosse (1973a) reported findings similar to Baylis when she showed that mycorrhizal roots of onions take up more P than non-mycorrhizal roots of onions and may reach supra-optimal concentrations with smaller amounts of added P. In a later experiment with Melinus minutiflorum, Paspalum notatum and Centrosema pubescens Mosse (1973b) came to the conclusion that non-mycorrhizal roots of some species do not utilize P present at extremely low concentrations in the soil solution but that mycorrhizal roots with fungal hyphae do.

Asai (1944) showed that several legumes grew poorly and failed to nodulate in autoclaved soil unless they were mycorrhizal. Recently Crush (1974) has demonstrated in a P-deficient soil that the tropical legumes Centrosema pubescens and Stylosanthes guyanensis were more dependent for growth and nodulation on vesicular-arbuscular mycorrhiza than the temperate species Trifolium repens and Lotus pendunculatus. This he related to the less developed root hair systems of the tropical legumes and the resultant improvement in P supply and enhanced nodule formation of infected plants. Lotus, with a well developed root hair system was able to absorb sufficient P to nodulate and grow under P-deficient conditions in the absence of mycorrhiza. White clover was intermediate between the tropical legumes and Lotus in its response to mycorrhiza.

Other work of Crush (1973) with grasses with well developed root hair systems showed that they were less dependent upon mycorrhiza for P absorption. When ryegrass was grown with mycorrhizal white clover Crush (1974, loc. cit.) demonstrated that white clover could compete more successfully for P than could non-mycorrhizal white clover. These findings open up a number of promising avenues of research into the RNE of pasture species and strains in which apparent efficiency in P absorption and resultant growth needs to be distinguished between that inherent in the plant and that due to associated mycorrhiza.

Barber and Frankenburg (loc. cit.) also found that the effects of mycorrhiza were particularly pronounced at low concentrations in solution culture when they studied rubidium and P uptake by barley roots. This lead them to question the 'dual mechanism' of uptake - a proposal that was rejected by Epstein (1972) on the grounds that the population of mycorrhiza surrounding a root would be too small to make a significant contribution to absorption and thereby invalidate the 'dual mechanism'. Barber (1974) disproved this objection to his proposal when he showed micro-organisms to be present in sufficient numbers. As the situation currently stands it appears that there is good evidence from which to believe that Mechanism 1 in the 'dual mechanism' of uptake of ions under conditions of low external concentrations may be due in large part to the effects of mycorrhiza.

It is not the author's intention to deal with this subject in greater depth. It is considered sufficient to acknowledge that mycorrhiza can exert a marked effect on N and P uptake, particularly at low levels of nutrient availability in the soil, and in plants with few root hairs. Further it is to be recognised that while intergenetic and interspecific differences in N and P nutrition can be attributed to the presence of mycorrhiza, the effects have not yet been well evaluated with strains of grasses and legumes.

### (f) Effects of plant environment.

Species and strains of pasture plants are adapted to the environment in which they occur naturally or to which they have been bred. Examples are numerous and recently published results of comparisons of perennial ryegrass genotypes in New Zealand (Rumball and Armstrong, 1974) illustrate the point. They showed (under the New Zealand environment) that New Zealand bred strains invariably proved superior to overseas strains - which had been bred as superior to New Zealand strains in their own environment.

Of the factors making up a plant environment edaphic conditions have a considerable bearing on RNE. This has been discussed in Section 2 (ii) (a). The remaining factors are climatological - light, temperature, $CO_2$ and moisture. To review these factors in depth is a study in itself. However they all have a direct bearing on RNE as they individually and collectively determine the rate of photosynthesis, translocation and the storage of assimilates and their utilization for growth in the respiratory process. This provides the energy necessary for active absorption, translocation and metabolism of inorganic ions. They also bring about changes in morphological characteristics such as shoot: root ratio, rate of tiller initiation, flowering and so on. These changes in turn affect the competitive ability of individual species and strains and thereby their RNE.

The effects of light on plant growth have been reviewed by Black (1957), Cooper and Tainton (1968) and Went and Sheps (1969). A study demonstrating the varying demands for light can be found in reports of Blackman and Wilson (1951a and b) who, using the growth analysis technique, established that net assimilation (NAR) increased as the amount of light increased. Leaf area ratio (LAR) on the other hand increased as shading was increased - a response of the plant to increase its light interception when light absorption per unit area decreased. In a later experiment (Blackman and Black, 1959) it was shown that the theoretical concept - that relative growth rate is the product of NAR and LAR, held when applied to results of their experiment. They showed that NAR was related to the logarithm of light intensity for red clover (T. pratense L.) but for white clover, alsike clover (T. hybridum L.) and Italian ryegrass (L. multiflorum Lam.) (among others) the relationship, though curvilinear, was not logarithmic. They concluded that for all species examined assimilation was limited by light up to full day light (20 440 - 23 670 lux) in their experiment. Taking light intensities to greater levels Cooper and Tainton (loc.cit.) reported no further increase in NAR of L. multiflorum beyond 32 300 lux intensity, although the tropical species Paspalum dilatatum increased in NAR up to 64 000 lux without sign of light saturation. They suggested, from their work as well as that of others, that temperate species reach light saturation at around 27 - 30 000 lux - a level well below the maximum light intensity of 100 000 lux occurring under full sunlight.

More recent work with genotypes of perennial ryegrass (Wilson and Cooper, 1969a) has shown that genetic variation exists among them in efficiency of light utilization (apparent photosynthesis) under conditions of varying light and $CO_2$ concentrations. At a given $CO_2$ concentration apparent photosynthesis increased with increasing light intensity from 10 760 to 32 280 lux as the $CO_2$ concentration was increased from 'low' to 300 p.p.m. to 'high' concentrations. The authors explained the effects of light intensity (Wilson and Cooper, 1969b) on subsequent photosynthesis in terms of increased stomatal size and a negative association with size of mesophyll cells. In a subsequent experiment Wilson and Cooper (1969c) performed a half-diallel cross among perennial ryegrass genotypes. In this experiment they were able to clearly demonstrate additive genetic variation for light saturation and light-limited photosynthesis. The fastest rate of photosynthesis occured in thin, narrow leaves with small mesophyll cells and much chlorophyll per unit volume.

The effects of different constant temperatures on the growth of a number of pasture grasses and clovers under a 12 hour light intensity of 29 050 lux (Mitchell, 1956) showed optimum temperatures of 29.4°C for paspalum, 25°C for white clover and L. pendunculatus and 19-25°C for perennial ryegrass, L. perenne X L. multiflorum, browntop (Agrostis tenuis Sibth.), Yorkshire fog and subterranean clover (Trifolium subterraneum L.). The rate of growth of individual tillers was affected by lowering the day and night temperatures independently (Mitchell, 1960) with the former change bringing about a greater relative reduction.

In a study of the influence of temperature on five forage legumes Smith (1970) showed that the rate of growth of sweet clover (Melilotus alba L.) and lucerne was highest between 27/21 and 21/15°C day/night temperatures. For alsike (Trifolium hybridum L.) it was highest at 21/15°C and for red clover at 15/10°C. L. corniculatus was not markedly affected by temperature within this range, having a low rate of growth throughout except at 32/27°C where its rate of growth was slightly greater.

Temperature exerts an effect on leaf anatomy and morphology of grasses (Mitchell, 1953; Mitchell and Soper, 1958; Forde, 1966; Luxmore and Millington, 1970) and on mesophyll size (Wilson and Cooper, 1969d). Both Mitchell (1953) and Wilson and Cooper (1969d) working with Lolium spp. have shown the speed and pattern of morphological development and rate of light saturated photosynthesis adjusts rapidly to contemporary temperature and light conditions.

Not only have differences been demonstrated in interspecific requirements for temperature to enable optimum growth, but it has also been shown to exist between genotypes of perennial ryegrass (Wilson and Cooper, 1969d) and white clover (Hoglund and Brock, 1974).

The variation between species and strains of pasture plants in response to soil moisture regime is common knowledge and needs no review here. The classification of a large number of species according to their moisture requirements (Levy, 1925) is considered sufficient to illustrate the point.

Climate also affects the nodulation and N fixing activity of legumes. Temperature requirements for root hair infection and nodulation in subterranean clover differ between genotypes (Gibson, 1967) although in general terms it is improved with increasing temperatures up to 30°C but retarded above 33°C and below 7°C. Other legume species were shown to require temperatures greater than 18°C for satisfactory nodulation and they can tolerate temperatures up to 35-40°C.

Nitrogenase activity of well established legumes varies over a wide range of temperatures from 10-35°C (Dart and Day, 1971). Within the optimum range of 20-35°C established by these workers there were differences in optimum temperature requirements between the broad bean (*Vicia faba* L.), tares (*V. sativa* L.), red clover, subterranean clover, barrel medic (*Medicago trunculata* L.), soybean. Lucerne and the cow pea (*Vigna sinensis* L.) gave maximum nitrogenase activity at 35°C. Dart and Day (*loc.cit.*) also found that temperate species (*Vicia, Medicago* and *Trifolii*) reduced considerable amounts of acetylene (an index of nitrogenase activity) at 2-5°C.

Light exerts a variable effect on nodulation, as shown by Grobelaar, Clark and Hough (1971). They found that the exposure of roots to white light prior to inoculation had a stimulatory effect whereas exposure immediately after inoculation suppressed nodulation.

In the case of nodulated plants an increase in photo-period from 8 to 12 hours is claimed by Gibson (1973) to increase the rate of N fixation. Pate (1962) demonstrated increasing activity with increased light intensity and Gibson (1973) found in a transfer experiment that plants transferred from 8 600 to 32 300 lux at the commencement of the light period increased in nitrogenase activity by 50% within five hours. This he took to indicate that N fixing activity at low light intensities is limited by the supply of photosynthesates.

Soil moisture and N fixing activity of legumes are strongly correlated (Sprent, 1972), with activity greatest at field capacity. Engin and Sprent (1972) compared the N fixing activity of white clover and soybean under moisture stress and found the former was more tolerant of moisture stress than the latter. This they ascribed to the greater ability of white clover to resume N fixing activity on rewetting the soil.

This section dealing with the effects of plant environment on plant processes has pointed to inter and intraspecific differences. While the literature is singularly devoid of reports on the RNE of different species and strains as affected by environment there can be little doubt that is must be markedly affected. This is likely to arise largely through the effect environment exerts on the supply of photosynthesates necessary to provide the energy for absorption, translocation and metabolism of inorganic ions. A study of RNE with the objective of identifying efficiency and of understanding its underlying cause must take into account the possibility of inter and intraspecific variation in response to the environment in which the plants are grown.

### (iii) Translocation of metabolites and the source-sink concept of distribution.

It would be inappropriate for the author to attempt to review this very wide topic extensively. Extremely comprehensive reviews of the literature have been provided by Mason and Phillis (1937), Williams (1955), Esau (1969), Wardlaw (1968), Biddulph (1969), Zimmerman (1969), Beevers (1969), Craft and Crisp (1971), Epstein (1972), and Bieleski (1973). Therefore this present review only gives a concise account of phloem transport and the operation of the source-sink mechanism as a background for discussion of the hypothesis put forward later and tested in this experimental programme - that the ability of plants to retranslocate nutrients has a large bearing on their relative efficiency of utilization of nutrients.

The work of Mason and Maskell (1931) indicated that N and P and other minerals ascend in the xylem and any excess not currently used in the leaves is re-exported down the plant in the phloem. The ratios of N, P, and carbohydrates moving down in the phloem were in excess of that required in the roots and it was suggested that the excess was liberated into the xylem sap to re-ascend the stem. Later Biddulph and Markel (1944) were to show this to be so when they demonstrated phloem to xylem migration of P. P and N are both extremely mobile elements in the phloem, as shown by Biddulph (1941) for P and by Gregory (1953) for P and N.

There are several theories still incompletely resolved to explain the mechanism of transport in the phloem viz. mass flow, protoplasmic streaming, metabolic movement, activated diffusion, and surface migration. Craft and Crisp (1971) discuss the evidence that can be assembled to support each of these theories and in their view the mass flow mechanism originally proposed by Munch (1930) is the most rational explanation. They contend that it can now, with recent advances in electron microscopy, be reconciled with phloem anatomy, with various aspects of phloem exudates and with the variety of distribution patterns observed with labeled tracers.

Movement of assimilates within phloem bundles can be bi-directional, as demonstrated by Biddulph and Cory (1960) with the Red Kidney bean (Phaseolus vulgaris L.). Later they gave evidence (Biddulph and Cory, 1965) to suggest that it occurred in separate phloem bundles although the work of Trip and Gorman (1968) with the squash plant (Curcubita melopepo Bailey) provides observations to suggest that bi-directional flow occurs within the same seive tube of the phloem. Craft and Crisp (1971) are doubtful that this is so and attribute the results of Trip and Gorman to the extended time over which they measured movement and the possibility of a complete change in direction of flow within the petiole during the three hour measurement period rather than simultaneous bi-directional flow. Craft and Crisp claim that bi-directional flow within the same sieve tube does not allow for the velocity of flow observed in many experiments or allow for the mass flow mechanism of solute transport.

The source-sink concept was originally proposed by Mason and Maskell (1928) in relation to the movement of carbohydrates. A very clear diagrammatic representation of movement of $^{14}C$ in 15 day old squash plants has been prepared by Webb and Gorham (1964). In this concept a source is considered to be an exporting organ and a sink a receiving organ. They concluded that 'the translocation of assimilated carbon in the squash plant is a distinctly channeled multidirectional movement associated with the phloem. This movement appears to be determined by the demands of growth and is readily switched for leaf to leaf as each matures and ceases to require carbon assimilates synthesised elsewhere'.

A lucid account of the movement of $^{14}C$ labelled assimilated in soybean was presented by Thrower (1962). She identified the expanding leaf as the major apical sink and showed that it imports it from the expanded leaves below. The importation fell to almost zero when the leaf was half expanded and then it commenced to export assimilates to younger leaves. Some of the export went down the stem to the root until the leaf was fully expanded when some slight import may occur. She also found that export of assimilates from an expanded leaf to the apex and root was proportional to its distance from these sinks. The concentration of assimilates in the stem derived from the source leaf decreased with distance from the source leaf, and the maximum concentration occured at the node. Defoliation between source leaf and the root caused more assimilates to move to the root and less to the apex.

While the pathway of entry of P and N compounds into the phloem from the roots is via the xylem, and is from chloroplasts via mesophyll cells to the phloem for carbohydrates, once inside the phloem they follow a similar pattern of movement (Hopkinson, 1964). Williams and his colleagues (Williams, 1948) provided one of the first attempts at partitioning P among plant organs. Biddulph et al (1958) studied this in the Red Kidney bean and their results will be detailed as an example of the distribution from the source to sink. They placed the bean seedlings in a nutrient solution containing $^{32}P$ for a period of one hour and traced the movement of the radioactive tracer by radioautography for the ensuing 96 hours. They found that a large amount of the labelled P which entered the plant remained sufficiently mobile to supply continuously growing young leaves and stem apexes (where the highest concentration accumulates) for the 96 hours over which measurements were made. The mature primary leaves showed their peak concentration after six hours although this P was subsequently withdrawn, i.e. they changed from being a sink to a source of P as it moved into the stem apices to meet their growth requirements. All plants showed a marked retention of $^{32}P$ within the root system. Koontz and Biddulph (1957) have demonstrated that there is also movement into the roots and Biddulph and Cory (1957) have shown that much of this is liable to re-ascension in the xylem.

In an experiment with Ladino clover (Trifolium repens L.) Wilkinson and Gross (1965a) grew seedlings in solution culture with varying amounts of P and withdrew them at 14 day intervals up to 42 days. They found that plants grown with adequate P accumulated sufficient to maintain rapid growth for several weeks. As the accumulated P was depleted shoot growth was first to suffer and good root growth continued for some time. This serves to illustrate the operation of the source-sink mechanism, with re-distribution providing initial shoot growth but with the greatest demand for limited P being exerted by the roots. A similar finding was also demonstrated with graminaceous plants by Williams (1948). In the same experiment referred to earlier, Wilkinson and Gross (1965b) measured the concentration of a number of mineral elements in different structures of Ladino clover. They found that increasing the P concentration of the nutrient solution increased the P content of leaflets at all levels of maturity but that the highest concentration of P occurred in immature leaflets. They quoted an example where 30% of the total leaflet uptake of P was contained in 19% of the total leaf weight at the highest level of P. Mature leaflets on the other hand made up 34% of leaflet weight but contained only 24% of the leaflet uptake. The actual content of P according to stage of maturity were:- immature = 49%, recent = 32%, older = 26%. Withdrawal of P did not alter the relative distribution of P among leaflets of varying maturity, although there was a marked decline in P concentration in all leaflet maturity classes. This gives further evidence that P is readily translocated from older mature tissue to immature tissue. Yet further evidence of this pattern of movement was found in cucumber by Hopkinson (1964) and Sansing (1963).

The distribution of dry matter and P among different plant organs was also reported by Wilkinson and Gross (1965b). The following data are extracted from their published work and the percent P distribution calculated from the data.

| Plant part | P (percent) | Dry matter (percent distribution) | P distribution (percent calculated) |
|---|---|---|---|
| Leaflets | .34 | 30.4 | 38.7 |
| Petioles | .16 | 25.9 | 15.5 |
| Stolons | .22 | 20.8 | 17.2 |
| Roots | .33 | 18.5 | 22.9 |
| Flowers | .62 | 0.8 | 1.9 |
| Peduncle | .34 | 1.0 | 1.3 |
| Crowns | .25 | 2.7 | 2.5 |

The above data serve to illustrate the extremely high concentration of P in the flower and the relatively low concentration in structural organs, where less meristematic tissue is present. Calculation of the percentage P distribution in the plant as a whole shows that the flower represents a small demand in absolute amounts of plant P with leaves retaining the bulk of it.

In a paper on the effects of P supply on the rate of intake of P and N in oats (Avena sativa L.) and Phalaris tuberosa L. Williams (1948) discussed the results of several previously reported experiments in terms of P metabolism. He concluded that the major determinants of the rate of P intake by plants are (a) the demand set up by growth and normal functioning of various plant parts and (b) the concentration of the nutrient in the medium. He considered (a) above to be the more important of the two. The core of his argument was the fact that P deficient plants derive 30% of their inflorescence P from other plant parts whereas those plants with an excessive supply derived no less than 93% of the inflorescence P from these sources. This suggests that P supplied by redistribution from other plant parts (from leaves, roots and finally stems) is more readily available, or possibly involves the expenditure of less energy in its acquisition than does its provision by root uptake.

The movement of N follows a similar pattern to that of P already described. Petrie (1940) measured the movement of N and P in the leaves of wheat (Triticum aestivan L.) and Sudan grass (Sorghum sudanese Piper Stapf). He showed that while the amounts exported from leaves differed between species (which they ascribed to the difference to demand of the inflorescence), net export of N and P was similar. Watson and Petrie (1940), working with tobacco plants removed the inflorescence in order to examine the re-distribution of N. They suggested that the rise of competing sinks represented by organs higher in the acropetal series was an important factor leading to net export of N by leaf groups. The final stage in the re-distribution occurred when there was a net export to the stems and roots, i.e. they were the ultimate sinks if nitrogen was not taken up by the inflorescence or leaves.

In a comparison of results from two separate experiments conducted by Tivers, 1942 and Tivers and Williams, 1948 (one with Liral Crown flax (Linum species) and one with linseed (Linus usitatissimum L. variety Punjab), the latter authors came to the conclusion that 'the distribution within the plant of carbohydrates and other metabolites is largely determined by the changing demand set up by competitive meristematic tissue. The basic pattern of this dynamic balance is an inherent characteristic of the species or variety under consideration, but the balance can be modified by many factors of the environment'.

The source-sink mechanism has been shown to operate in symbiotic N fixation. Lawn and Brun (1974) were able to show a decline in N fixation of, and between, two varieties of soybean during podfilling. This they showed (by manipulating the source-sink demands through shading and defoliation) to be due to an inadequate supply of assimilates to the nodule. In another experiment in which reciprocal grafts were made of eight soybean genotypes Lawn, Fisher and Brun (1971) showed shoot genotype differences in total nodule activity which related to the genotype's ability to fix $CO_2$.

From the foregoing two points are clear. Firstly, plant organs can be placed in a 'pecking order' in which their requirements for N, P and carbohydrates are met. Secondly, the distribution and re-distribution of these metabolites are dependent upon the demands of the sinks and the ability of different sources to meet these demands. The questions then arise - what creates a sink? What is the mechanism that switches a sink to a source? Obvious reasons are: (a) changes in plant structures as occurs when a plant is defoliated, (b) changes in environmental conditions such as temperature or shading, (c) nutrient supply, (d) ontogeny, and so on. Examples of these abound in the reviews referred to at the outset of this section. It could be argued that the shift is under hormonal control in certain tissue and the metabolic reactions they initiate are themselves brought about by changes in source-sink activity. What is cause and what is effect? These questions seem to have no clear answer at present. One can only come to the rather inconsequential conclusion that plant nutrition is complex - a complexity brought about by the interdependence of all metabolic processes.

Attempts have been made to quantify the source-sink concept (de Wit et al, 1970; Watson, 1971; Wilson, 1972). The latter proposed that it be defined in terms of losses and gains of a substance in a particular plant part. It has already been pointed out that a plant part can act at the same time both as a source and a sink. Wilson allowed for this by proposing that a source be defined as positive and a sink as negative.

Wilson firstly defined the change in weight M for a particular plant part as:

$$M = E_g - E_1 + T_g - T_1 + T_1 + C_g - C_1$$

where $E_g$ and $E_1$ are the gains and losses by influx and efflux to the environment, $T_g$ and $T_1$ are the import and export by translocation and $C_g$ and $C_1$ the synthesis and utilisation by chemical conversion. Next he divided the source-sink status between transport of the substances and its involvement in metabolism. Thus the following equations were developed:

Transport. Source-sink status = $(E_1 - E_g) + (T_1 - T_g)$

Metabolism. Source-sink status = $(C_g - C_1)$

The change in weight of the substance is given by the net loss or gain by metabolism less the net loss or gain by transport into or out of the plant:

$$(C_g - C_1) - [(E_1 - E_g) + (T_1 - T_g)]$$

By the above definition if a plant part is synthesizing, (metabolically +ve) and the amount removed by transport (-ve) are equal, then the plant is in a state of equilibrium and its source-sink status is zero.

The foregoing indicates whether a plant part is a source or a sink and the value obtained would indicate the degree to which it is one or the other. Wilson (1967) also examined ways of quantifying the 'strength' of sources and sinks. He proposed the following:

Source strength = source size x source activity

and Sink strength = sink size x sink activity.

In this definition 'strength' is the absolute rate of change of weight in a plant part, 'size' is the weight of the substance, and 'activity' is the rate of change in weight per unit of weight (size). He gave examples of this using net assimilation rate as the 'strength', leaf area as the 'size' and rate of assimilation per unit leaf area as the 'activity'.

There has been insufficient time since the postulation of these concepts to test their validity. Present day interest in systems stimulation make this type of approach a likely candidate for intensive research. It perhaps offers an approach which will provide a better understanding of plant metabolism and the factors governing it. This may serve to orientate the direction of physiological and biochemical research more accurately towards the mechanisms controlling nutrient efficiency.

(iv) Conclusions.

This review has traced the main factors involved in plant nutrition from ion uptake by the roots to their movement to sites of metabolism in growing tissue. At all points along this chain of events one could argue that each factor taken separately is the one controlling nutrient uptake and as a consequence the efficiency of uptake and utilization. A treatise on biochemical mechanisms controlling plants metabolism could do likewise. The problem, as already stated, arises through the interdependence of physiological and biochemical events. One arrives at a circular argument in which the apparent initial cause of nutrient efficiency is itself modified by the effects it sets in train. Currently there does not appear to be any way out of this dilemma. From the practical viewpoint, which is the justification for seeking a basic understanding of plant physiological processes, attempts can be made to identify mechanisms which are indicative of RNE.

# SECTION 3

# EXPERIMENTAL MATERIALS AND METHODS

## (i) Introduction

Many experimental details were similar in all trials and for this reason a full discussion of materials and methods will be given in this section. An abbreviated section dealing with experimental methods is given in the reports of individual experiments.

## (ii) Growth medium and potting procedure

In all experiments plants were grown in a 2:1 soil-sand mix. There was the alternative choice of growing plants in solution culture, but this was rejected on the grounds that (a) there would be no test of the dependence of plants on their root morphological characteristics for ion uptake when bathed in nutrient solution. Asher and Loneragan (1967) have demonstrated differences in the type of root development of plants grown in nutrient solution compared to soil, (b) moisture stress (one of the treatments examined in Experiment 7) could not be imposed.

The soil selected was a Tokomaru silt loam, a transitional yellow-grey yellow-brown earth (Anon, 1954). It was removed from a site which had been sown to pasture circa 1930 and has not, according to available information, been topdressed. The top 2.5cm of soil and turf was removed and the experimental soil was taken from the 2.5 to 10cm depth of the $A_{11}$ horizon. The soil for Experiments 5 and 6 unavoidably contained the top 2.5cm of soil and turf, although the majority of the turf was removed by sieving. For Experiment 7, access was obtained to an undisturbed site.

Mechanical analysis of the soil (Pollock, pers.comm.) gave the following structural composition - coarse sand 1%, fine sand, 55%, silt 23% and clay 21%.

Chemical analysis (for methods see Section 3(vii) (c)) results were as follows:-

| | | Percent | | | ppm extractable |
|---|---|---|---|---|---|
| | pH | C | N | C/N | P |
| All Exps excluding 5 and 6 | 5.6 | 3.6 | 0.25 | 14.4 | 11.4 |
| Experiments 5 and 6 | 5.3 | 4.0 | 0.30 | 13.3 | 22.5 |

For all experiments the soil was air-dried and passed through a 1.3cm seive. To 1 600g of soil 800g of plasterer's sand was added to provide improved aeration. The soil was placed in 1.9L square plastic pots of 182cm$^2$ surface area. The soil was consolidated during potting by dropping a 4.5 kg weight onto a plate covering the surface. It was dropped once from a height of 30 cm when the pot was half full of soil and again when full.

### (iii) Basal nutrients

These were applied to the soil (contained in plastic bags) in solution and then mixed in a small mixer similar in design to a concrete mixer. During the course of several experiments potassium sulphate was added and this is detailed in relevant sections.

The basal nutrients added were:-

$K_2SO_4$ at 0.531 g per pot; equivalent to 190 kg K/ha and 39 kg S/ha.

$Na_2MoO_4.2H_2O$ at 391 mg per pot; equivalent to 175 g/ha of sodium molybdate.

### (iv) Seed germination and inoculation

To enhance even and rapid germination seeds were imbibed in water and stored in a refrigerator at 8°C for five days before germinating on filter paper in petri dishes at 22°C.

In the case of legumes, inoculations were made prior to imbibition This was done by scraping the rhizobial culture from the surface of the agar slopes, adding distilled water and then pouring onto the seed in petri dishes.

Inoculum was supplied by Dr R.M.Greenwood[1]. For white clover Rhizobium trifolii Hasting's[2] strain No. 2153 was used, and for L. pendunculatus Greenwood's strain No. CC8154 was used. In Experiment 4 a L. pendunculatus x L. corniculatus hybrid was included. The inoculum used for this hybrid was a mixture of strain No. CC8154 and N.Z. P2238. The selection of strains of Rhizobia was made on the advice of Dr Greenwood.

Over a two day period prior to planting, the soil in the pots was wetted to about field capacity (f.c.). Germinated seedlings, selected for uniformity of size, were pricked out. Nine seedlings were planted in each pot and any considered to be unlikely to survive were replaced within the first three days.

### (v) Moisture regime

Field capacity determinations were made on six pots prepared by the standard procedure described in section (ii). These pots were placed in a tray of water for 24 h during which time they were twice daily

1. Grasslands Division, D.S.I.R., Palmerston North.
2. Plant Diseases Division, D.S.I.R., Auckland.

watered from the top. They were removed and allowed to drain for 48h whilst covered on top with a film of plastic to prevent surface evaporation. The moist soil was weighed then air dried at 105°C then re-weighed, and the field capacity (f.c.) calculated. The f.c. varied little between experimental batches of soil-sand, and was in the range of 30 to 32%.

In all experiments (excluding Experiment 7) pots were watered to 80% of their f.c. This level was considered to provide optimum conditions for plant growth yet avoid leaching losses. Pots were watered every two days initially and when (by daily check weighing) they were found to fall below 60% f.c. daily watering was adopted. At a later stage twice daily watering was necessary. In Experiment 7 one treatment was watered to 80% f.c. and the above procedure was followed. Another treatment was designed to grow the plants under a moisture stress. A level of 30% f.c. was chosen as the upper limit of moisture availability. Pots were watered to this level and permitted to cycle down to 20% of their f.c. before re-watering to 30% f.c. This cycle took two to three days initially, then one day until finally twice daily watering became necessary - depending upon plant production.

### (vi) Environments

### (a) Glasshouse conditions

Experiments 1 to 4 were grown in an automated glasshouse in which there was a degree of control over temperatures. Heating maintained temperatures above 15°C and cooling, which followed a stepwise procedure of opened louvres, fan extraction of air then evaporative cooling, held temperatures below 25°C. During the conduct of Experiments 3 and 4 ambient temperatures were in the vicinity of 35°C on four days and glasshouse temperatures could not be held below about 30°C.

Conditions used in Experiments 5, 6 and 7 will be detailed in the sections of the text describing each of these experiments.

### (b) Controlled environmental conditions

The climate rooms of Plant Physiology Division, DSIR, Palmerston North were used for Experiments 5, 6 and 7. In these rooms the lighting system consisted of 4 x 1 000 watt Sylvania metal arc high pressure discharge lamps, together with 4 x 1 000 watt Philips tungsten iodide incandescent lamps. Where necessary wire or nylon mesh screens were used to adjust light intensities to the required values. Light eveness of both lamp types were adjusted to within $\pm 12 Wm^2$.

The photoperiods were changed with abrupt light/dark or dark/light changeovers.

Temperatures were controlled within ±0.3°C, relative humidity within ±1% and $CO_2$ concentrations were maintained at ambient levels of 320-340ppm. Airflow down through the plants was 0.3-0.5m/sec$^{-1}$ as measured at the top of the canopy.

### (vii) Analytical

### (a) Nitrogen fixing activity

Measurement technique. Acetylene reduction assay was adopted as a well established and reliable method of estimating nitrogenase activity. The technique has been comprehensively reviewed by Hardy et al (1973). Techniques commonly employed range from measurement of the activity of excised nodules, the activity of root systems, the activity of entire plants removed from their growth medium and finally the activity of plants-in-soil. The plant-in-soil technique reported by Sinclair (1973) was adopted for this study, and the same type of incubation vessel was used. The method was shown by Sinclair, and indeed by other recent reports (Dart and Day, 1970) to provide a high correlation of ethylene production with N fixed when assessed by chemical analysis for N present in plant tissue and soil.

In Sinclair's study (loc.cit.) there was variation in the incubation time found necessary. It was dependent upon the clay content (or compaction) of the soil and upon the moisture regime. At saturation levels of moisture acetylene reduction did not proceed. This was probably due to reduced or zero movement of acetylene into, and ethylene out of the soil matrix. As the soils in the experiments reported here were well compacted and as watering was to 80% of the f.c. it was decided to incubate the plants-in-soil for 24 hours. Prior to the commencement of Experiment 7 two studies were made of ethylene production over a period of 24 hours. Briefly, the studies involved the sampling of gases in the pots at two hourly intervals over the 24 h period. One study was conducted in dark and the other with 12h light and 12h dark. Results of both studies were similar. They showed a near linear production of ethylene over the entire period. From these studies it was concluded that the 24h period of incubation adopted in Experiments 3, 4 and 6 would have given a reliable estimate of N fixing activity, but that a shorter period could have been adopted. In Experiment 7 a 4h incubation was employed.

The incubation procedure was to place the soil containing the plant into a 5l opaque plastic vessel and covering with a snap-on lid. A silcone rubber bung was fitted in each pot for injection and removal of gases. 250ml of air was removed and replaced with 250ml of acetylene. At the completion of the incubation period about .8ml of the gases was removed for assay. The temperatures and barometric pressures at the time of injection were recorded.

Gas liquid chromatography. Measurement of ethylene production were made with a Shimadzu Model GC-4BD chromatograph fitted with a Model FID4 flame ionization detector and a 30cm x 3mm diameter stainless steel column packed with 80-100 mesh Poropak T. The temperature of the injection port was 110°C, column 90°C and detector 105°C. N was used as the carrier gas at a flow rate of 30ml/minute. The gas sample size injected was 0.5ml. The same volume of acetylene was added to each incubation vessel, and was used as an internal standard in the analysis. The calculation of moles of ethylene produced was by the following formula:-

$$\text{moles } C_2H_4 = \frac{X \frac{a}{b} KF}{1 + \frac{a}{b} K}$$

where $X$ = volume of $C_2H_4$ used in the incubation, $a$ = $C_2H_4$ peak height, $b$ = $C_2H_2$ peak height in sample, and

$$K = \frac{\text{ml } C_2H_4 \text{ in standard}}{\text{Peak height } \frac{(C_2H_4)}{(C_2H_2)} \times C_2H_2 \text{in standard}}$$

and $$F = \frac{P \text{ atmos.} \times 273 \times 1\,000}{22.4 \times (273 + T^{\circ}C)}$$

(b) Harvesting.

Plants were harvested by removal of plant tops to soil level and then oven drying at 80°C in a forced-air drying oven in the case of Experiments 1 to 4, and at 40°C under 3mm Hg vacuum for Experiments 5, 6 and 7.

Pots containing soil and roots were stored in a cool room at 5°C for periods of about three weeks. Roots were removed by washing with a high pressure solid-cone water spray after over-night soaking. During washing the material was held on a 5mm mesh sieve. Remaining debris was removed by hand washing. Extremely clean root samples were obtained by this procedure - a factor considered more important than the minor loss of root material observed.

(c) Carbon, Nitrogen, Phosphorus and sugar analyses.

Analyses of soil organic carbon content was by the colorimetric of Blackmore et al (1972). Total N in both soil and plant material was determined by the Kjedahl method in which digestion was with $H_2SO_4$, $K_2SO_4$ and $CuSO_4$ with selenium as catalyst, followed by an automated distillation procedure for $NH_4$ determination based on the method of Keay and Manage (1969).

Available soil P was determined by the Truog method in which soil P was extracted with .002N $H_2SO_4$ containing 3g $(NH_4)_2SO_4$ per l. P in the filtrate was determined by the automated method of Murphy and Riley (1962), using antimony to catalyse the formation of molybdenum blue colour with ascorbic acid reducing agent.

Total P in plant tissue was determined on the same digest used for N determinations, using an automated molybdo-vanadate method developed by Grigg (pers.comm.) and using the reagents of Barton (1948).

Sugar analyses was based on the extraction of sugars from dried plant material with 62.6% methanol, with soluble sugars being determined on aliquots of the extract by the phenol-sulphuric acid method of Dubois et al (1956).

(viii) Statistical

(a) It is difficult to make valid comparisons of the yield of plants according to level of applied nutrient when they differ markedly in yield. To facilitate meaningful comparisons the scale differences in some experiments were reduced by making a logarithmic ($l_n$) transformation of yield, nutrient absorption and ethylene production. This treatment also has the advantage of stabalising the variance. The square root of the independent variable (level of nutrient applied) was taken for all variables in order to provide response curves approximating linearity. For other variables such as P and N concentrations, which did not differ markedly between strains, no such transformations were made. Which of these methods of treating the data was used before the analysis of variance will be indicated in the relevant section of the text.

# SECTION 4

# SCREENING OF GRASSES AND LEGUMES FOR RNE

# GENERAL INTRODUCTION

Reasons for the selection of Yorkshire fog, perennial ryegrass, white clover and Lotus for study have been briefly touched on and will be elaborated further in the introduction to each of the four experiments. So too was the choice of strains where information was available. Where no information was available the selections were made on the basis of soil fertility and climate of the region of origin with the aim of including a range of material of diverse origin.

At the commencement of this study few strains within the above species had been demonstrated to exhibit differing nutrient efficiency. For this reason the experiments reported in this section were designed as <u>screening</u> experiments from which to select material for further study in more precise experiments. Details of subsequent investigations by other workers will be given later.

The number of strains included in each experiment represent a compromise between examining a wide and diverse range of material on the one hand and containing the number to a level that would ensure reasonable precision on the other. The latter consideration was considered to be important as the differences between strains were likely to be small.

In experiments examining grass species, both N and P were applied as the independent variables. This restricted the number of levels of each nutrient which could be applied to three - if the size of the experiments were to be held within manageable bounds. Results will show that no outstanding differences in RNE were apparent and in retrospect it is now clear that many more levels of nutrients needed to be included to enable clear differentiation of interstrain differences in RNE. Attempts were made to fit quadratic and inverse polynomial (Nelder, 1966) curves to the data but in each case strains were present which gave spurious results. Some for example gave zero yields at minus 500 kg/ha N and obviously indicated that the model was inadequate. For this reason curve fitting was abandoned and strains were finally compared by analysis of variance and on the basis of relative strain performance.

With the legume experiments it was possible to include five levels of applied P and curve fitting was possible as an aid to the identification of RNE and its Type. This was performed by use of 'experience curve' regression (Jowett, <u>pers.comm.</u>) in which the 'experience curve' was the mean strain yields to which individual strain responses were fitted by the least square method. While no claim is made that the results present a perfect representation of strain responses, it appeared to be the most appropriate method of handling the data.

# EXPERIMENT 1

## RELATIVE NUTRIENT EFFICIENCY OF STRAINS OF YORKSHIRE FOG IN RESPONSE TO APPLIED NITROGEN AND PHOSPHORUS

# INTRODUCTION

Yorkshire fog has been described by Saxby (1956) as 'one of the most maligned of New Zealand's common grasses in spite of its being regarded as undesirable, a very large amount of New Zealand's butterfat producing pastures contain a considerable amount of it'. There is documented evidence to support this observation in the work of McMeekan and Walshe (1963) in the Waikato, where three dairy cows/ha were grazed on a sward in which Yorkshire fog was a major constituent. They observed that Yorkshire fog was the main contributor to yield in autumn and winter.

Because of its presence in untopdressed pasture (Saxby, loc.cit.; Harlan, 1965; O'Connor, 1961; During et al, 1962; Jacques, 1962; Whittet, 1964; Smith, 1972; White et al, 1972 and Vartha, 1973) Yorkshire fog is generally regarded as a low fertility species. An attempt was made by Miles and Miles (1970) to utilise its tolerance of low fertility. They found it promising when sown into heather (Callunetum) sites in N.E. Scotland. In experiments into the reseeding of natural pasture Yorkshire fog has proved to be one of the most successful introductions in Wales and Scotland. (Davies, 1940; Hughes and Nicholson, 1961a, 1961b; Hunt, 1964), and in Australia (Anon, 1971). In New Zealand it was included in early oversowing on natural pasture (Burnett, 1927) and the presence of Yorkshire fog today is a reflection of its ability to survive and produce under adverse climatic and edaphic conditions.

Recent experimentation in New Zealand provides conflicting evidence on the relative merits of oversowing Yorkshire fog on low fertility hill country. Dunbar (1971) found on exposed high altitude sub-soils that topdressing was necessary for the survival of 10 herbaceous species including a bred cultivar (Massey Basyn) of Yorkshire fog. In the presence of fertiliser he found Yorkshire fog superior to all other species (including 'low' and 'high' fertility grasses) in their ability to make rapid growth. In another study (Dunbar, 1970) Yorkshire fog showed less vigour than cocksfoot (Dactylis glomerata) in the first season but more ground cover in the third season.

On moderately topdressed mid-altitude sites in Canterbury Vartha (1973) recorded comparable establishment of Massey Basyn Yorkshire fog and Ruanui ryegrass. White et al (1972) on the other hand reported poor establishment of the former compared to other 'high fertility' grasses sown on low fertility hill country.

From the foregoing review it is apparent that Yorkshire fog has an ability to survive competitively with indigenous and introduced grass species under both low and high fertility conditions. Other factors apart, this provides evidence of a considerable degree of efficiency in the uptake and utilisation of nutrients which could bear further investigation.

Reference has been made to levels of fertility but not to the relative requirements for specific nutrients. Little research has been reported on this aspect. Vartha (1960) showed a lower need for S by browntop than by Yorkshire fog, while O'Connor (1961) found Yorkshire fog to be more responsive to applied N than sweet vernal (Anthoxanthum odoratum L.) and browntop. In a glass-house study of Massey Basyn Yorkshire fog grown in sub-soil taken from an exposed mountain site, Dunbar (1972) recorded poor growth in the absence of applied N and P but a very large N X P interaction.

Because of the paucity of information on the relative nutrient requirements of Yorkshire fog and in view of the promise of high yields and improved palatability following selection (Jacques, loc.cit.), a study was made of its requirements for N and P when grown as a monoculture.

## EXPERIMENTAL

The following treatments were examined in a two replicate 8 x 3 x 3 factorial design, giving a total of 144 experimental pots;-

S1 Massey Basyn (N.Z. Bred)
S2 Dipton (N.Z. natural ecotype)
S3 Abbotsford (N.Z. natural ecotype)
S4 Rotorua (N.Z. natural ecotype)
S5 Glen Innes (N.S.W. Tablelands natural ecotype)
S6 Almeida de Sayago (Spanish natural ecotype)
S7 Stratford (N.Z. natural ecotype)
S8 Tara Hills (N.Z. natural ecotype)

Massey Basyn was the only known bred strain of Yorkshire fog.

P was applied in solution as sodium dihydrogen phosphate at 10, 60 and 180kg per ha calculated on a superficial basis. N was applied as ammonium nitrate in solution at 0, 50 and 100kg per pa (calculated on a superficial basis) and re-applied at half the initial rates on days 22, 36, 47 and 59. The experiment was sown on 26 September, 1972.

Details of soil used, basal nutrients applied and cultural condition have been given in Section 3.

## RESULTS AND DISCUSSION

Total shoot yields are shown in Appendix I, shoot absorption of N and P in Appendices II and III and shoot utilization of N and P in Appendices IV and V. To aid interpretation of data, results are presented on a relative basis in Table I. They are based on mean values as no interactions were recorded between strains and levels of N or P. An exception was N absorption according to level of applied N where, because of a significant positive interaction, relative results are presented in the presence of high levels of P.

Results failed to show any outstanding differences between strains and because of this it was decided to restrict the selection of strains for further examination to two; one exhibiting high RNE and another exhibiting low RNE. Further, as no large differences were apparent in RNE according to P applied it was considered desirable, in view of the importance of N for grass production, to restrict the subsequent study to an examination of the RNE of strains in response to applied N.

In view of the above decisions Massey Basyn was selected for further study on the grounds that it gave high shoot yields and was among the highest yielding strains on a relative basis in the absence of applied N. Accepting the limitations of the data in providing only three points on the response surface, there was reason to believe that Massey Basyn possessed the properties of RNE Type 1 in that its yields at 50kg/ha N relative to 100kg/ha N were low compared to other strains; it had not approached its yield asymptote as closely as other strains.

Tara Hills was selected as a strain for further study because of its low relative yields in the absence of applied N; it was a strain differing most markedly from Massey Basyn. In addition, the Tara Hills strain gave high yields at 50kg/ha N relative to 100kg/ha N - indicating it was more closely approaching its yield asymptote than was Massey Basyn. This suggests it possessed the properties of a Type 2 RNE curve but, because of its lower absolute yields compared to Massey Basyn, would be classed as Type 3 RNE. As no strains exhibited Type 2 RNE (none were clearly superior to Massey Basyn at any point in the response range), Tara Hills appeared to be the most appropriate strain to compare with Massey Basyn.

Table 1. Relative yields, absorption and utilization of N and P by Yorkshire fog in response to applied N and P.
(100 kg N/ha = 100, 180 kg P/ha = 100)

| | Applied N (kg/ha) | | Applied P (kg/ha) | |
|---|---|---|---|---|
| | 0 | 50 | 10 | 60 |
| Relative yields ...... (D.M./pot) | of N | | of P | |
| Massey Basyn | 47 | 75 | 70 | 87 |
| Dipton | 41 | 79 | 74 | 94 |
| Abbotsford | 46 | 88 | 67 | 98 |
| Rotorua | 45 | 91 | 63 | 88 |
| Glen Innes | 42 | 85 | 61 | 89 |
| Almeida de Sayago | 48 | 96 | 59 | 99 |
| Stratford | 46 | 92 | 62 | 90 |
| Tara Hills | 40 | 88 | 76 | 83 |
| Relative absorption ..... (N or P/pot) | of N | | of P | |
| Massey Basyn | 25 | 59 | 33 | 58 |
| Dipton | 22 | 62 | 31 | 60 |
| Abbotsford | 25 | 65 | 27 | 63 |
| Rotorua | 24 | 72 | 24 | 52 |
| Glen Innes | 25 | 74 | 31 | 66 |
| Almeida de Sayago | 31 | 75 | 30 | 63 |
| Stratford | 25 | 72 | 30 | 56 |
| Tara Hills | 23 | 72 | 34 | 53 |
| Relative utilization ...... (D.M./unit N or P absorbed) | of N | | of P | |
| Massey Basyn | 181 | 127 | 209 | 142 |
| Dipton | 182 | 127 | 248 | 159 |
| Abbotsford | 187 | 140 | 247 | 159 |
| Rotorua | 186 | 128 | 251 | 166 |
| Glen Innes | 168 | 116 | 202 | 135 |
| Almeida de Sayago | 162 | 129 | 207 | 159 |
| Stratford | 188 | 130 | 217 | 170 |
| Tara Hills | 176 | 122 | 220 | 162 |

# EXPERIMENT 2

## RELATIVE NUTRIENT EFFICIENCY OF STRAINS OF PERENNIAL RYEGRASS IN RESPONSE TO APPLIED NITROGEN AND PHOSPHORUS

# INTRODUCTION

Perennial ryegrass is a species indigenous to the humid-temperate regions of north-western Europe and is one of the most widely sown grasses in temperate regions of the world. It is a species that quickly adapts to its geographic or ecological environment as shown in the early years of plant breeding (Jenkin , 1930; Levy, 1933; Beard and Hallowell, 1952).

In view of the importance of perennial ryegrass to world agriculture it is surprising that little has been done to identify and select strains efficient in their nutrient requirements. A programme of research into the genetic control of N and P uptake of populations and strains of perennial ryegrass has been undertaken at the Welsh Plant Breeding Station, Aberystwyth. There the initial work of Holmes (1967), who grew S.24 and Melle perennial ryegrasses under two levels of N, showed (by parent-progeny regression) that the heritability was greater than 0.3 in dry weight of shoot and of root, percentage N in the shoot and total N yield in shoot and in the whole plant. He also found lower N concentrations in S.23 and S.101 than in New Zealand (Ruanui) and Irish perennial ryegrasses.

Goodman and Shurety (1971) reported a response to selection within S.23 perennial ryegrass for dry matter production at limiting P levels (12 ppm). The heritability was 0.68. At high levels of P (186 ppm) these differences were not present. They found that the population showing efficiency at low levels of P also contained low concentrations of P in the herbage. Further, the effect of selection was passed on from the parent to seedling. Later Goodman and Hughes (1972) reported a study of parents and $F_1$ progeny of a diallel cross between contrasting perennial ryegrass cultivars. They recorded a high heritability of 0.63 for N and 0.56 for P for yield at low and optimal levels of these nutrients. They did not find tolerance to high levels of N or P to be heritable. Differences were recorded in the response to high levels of N and P between Lior, S.22 and Aberystwyth and strain Bb 1277 perennial ryegrasses. The same authors also found heritabilities of 0.79 and 0.36 for both N and P concentrations in the herbage. These glasshouse results were subsequently confirmed (Goodman and Hughes, 1973) in the field.

A study of differences in response to P of populations of perennial ryegrass by Crossley and Bradshaw (1968) showed considerable differences in response of populations from contrasting soil types. This they considered, suggests specific physiological adaptation to P level. Cultivated varieties did not perform as well as specifically adapted populations under extreme nutrient conditions. They also showed a change in nutrient response within populations in the course of seed multiplication.

The foregoing illustrates the existence of varying nutritional efficiency in perennial ryegrass and a relatively high heritability but with physiological adaptation occurring over a short period of time. Up to the present time no cultivars have been bred specifically for efficiency in response to mineral nutrients. The prospects of breeding for nutrient efficiency appear promising in view of the foregoing research. Further, the practice of plant breeders in confining their selection work and national testing to high fertility soils or to ones on which adequate basal dressings of deficient nutrients are applied will have meant the selection of cultivars that need not necessarily be efficient at sub-optimal levels of nutrients.

The selection of strains in the experiment to be reported here was based firstly on the availability of seed and secondly on the desire to include strains from diverse geographic and environmental situations. Seed from two strains (Rosnovsky and Norlea) reported by Goodman, P.J.(pers. comm.) to possess a degree of efficiency in response to N, P and K was not available.

## EXPERIMENTAL

The following strains were examined in a two replicate 7 X 3 X 3 factorial experiment:-

| | | |
|---|---|---|
| S1 | R.v.P. Hay pasture: | Rijksstation voor planten veredeling, Merelbeke (Lemberge), Belgium. |
| S2 | W.W. Virus: | Plant Breeding Institute, Landskrona, Sweden. |
| S3 | Manhattan: | Carey Strcme, California. |
| S4 | Medea: | Growers seed, Adelaide, Australia. |
| S5 | Aberystwyth S.23: | National Seed Development Organization, Cambridge, U.K. |

S6 Grasslands Ruanui: Breeders stock, Grasslands Division, Palmerston North, N.Z.
S7 Mangere[1]: Grasslands Division G4711, Palmerston North, N.Z.

Seed was supplied by Grasslands Division, D.S.I.R. Palmerston North.

The experiment was sown on 26-5-72. Phosphorus was applied as sodium dihydrogen phosphate at 0, 60 and 180kg/ha calculated on a superficial basis. On 21-6-72 the 'No P' treatments were exhibiting P deficiency symptoms and plant mortality appeared imminent. To these pots was added 10kg/ha P to alleviate the problem.

Nitrogen was applied as ammonium nitrate at 50 and 100kg/ha at sowing and re-applied at half the initial rate on days 26, 35, 41 and 48.

Harvesting took place on days 46 and 60.

Details of soil used, basal nutrients and cultural conditions have been given in Section 3.

## RESULTS AND DISCUSSION

Total shoot yields are shown in Appendix VI, absorption of N and P in Appendices VII and VIII and utilization of N and P in Appendices IX and X. Relative strain performance in these paramaters are shown in Table II where data was derived from mean values unless significant positive interactions occurred. In this event the effect of N was taken from values in the presence of the highest level of P.

Results show high yields from S.23, Ruanui and Mangere. The latter two strains gave low relative yields in the absence of N and did not reach their yield asymptotes at 50kg/ha N as did other strains (excluding S.23). Thus these two strains appeared to possess Type 1 RNE in that they continued to respond to high levels of applied N. Absorption of N by these strains at the lower levels of applied N did not differ from the absorption by the others and does not account for their low RNE. Utilization gave variable results for these two strains, with the value for Ruanui being difficult to explain in view of the low absorption. However values for relative utilization taken from the mean of all levels of P show Ruanui providing similar high utilization to Mangere at low levels of applied N. It was because

---

1. Subsequently released as 'Grasslands Nui' perennial ryegrass (Boyd, 1973; Walton, 1973).

**Table II:** Relative yields, absorption and utilization of N and P by perennial ryegrass in response to applied N and P (100kg/ha N = 100, 180kg/ha P = 100).

| | Applied N (kg/ha) | | Applied P (kg/ha) | |
|---|---|---|---|---|
| Relative yields | 0 | 50 | 0 | 60 |
| (D.M./pot) | of N | | of P | |
| R.v.P. | 40 | 103 | 29 | 76 |
| W.W.Virus | 46 | 100 | 34 | 79 |
| Manhattan | 38 | 102 | 29 | 73 |
| Medea | 46 | 106 | 27 | 86 |
| S.23 | 42 | 72 | 26 | 85 |
| Ruanui | 34 | 83 | 29 | 76 |
| Mangere | 37 | 86 | 26 | 67 |
| Relative absorption | of N | | of P | |
| (N or P/pot) | | | | |
| R.v.P. | 26 | 95 | 20 | 64 |
| W.W.Virus | 25 | 94 | 22 | 58 |
| Manhattan | 23 | 90 | 20 | 61 |
| Medea | 27 | 97 | 19 | 65 |
| S.23 | 22 | 67 | 19 | 68 |
| Ruanui | 19 | 69 | 21 | 61 |
| Mangere | 19 | 77 | 19 | 64 |
| Relative utilization | of N | | of P | |
| (DM/unit N or P absorbed) | | | | |
| R.v.P. | 154 | 107 | 150 | 120 |
| W.W.Virus | 184 | 108 | 151 | 137 |
| Manhattan | 167 | 113 | 149 | 125 |
| Medea | 167 | 109 | 149 | 133 |
| S.23 | 194 | 109 | 140 | 123 |
| Ruanui | 177 | 120 | 157 | 133 |
| Mangere | 190 | 111 | 142 | 107 |

of these attributes described above together with the fact that Ruanui and Mangere (recently released as Nui) are of considerable significance to New Zealand agriculture, that these strains were selected for further study.

R.v.P. was a strain showing relatively high yields at low levels of applied N. It reached its yield asymptote at 50kg/ha - at which level it also gave yields comparable to Ruanui and Mangere. Thus R.v.P. appeared to be a strain possessing the properties of Type 2 RNE, accountable by relatively high absorption in spite of relatively low utilization of N. It was therefore selected for further study.

The inclusion of an additional strain showing high RNE at low levels of applied N in the subsequent experiment was also considered desirable. The choice was between W.W.Virus and Medea. The former was selected because of its high relative utilization of N - which further investigation could well show was a particular attribute of W.W.Virus which may account for its high RNE.

Basically the relative yield response of W.W.Virus was of Type 2, but because it was not clearly superior to Ruanui or Mangere (both Type 1 RNE) at any point over the range of N application rates it is classified as possessing Type 3 RNE.

Relative strain responses in yield, P absorption and utilization in response to applied P showed little variation. For this reason, and for the reason of the greater importance of N nutrition for grass production, it was decided to confine the subsequent experiment to a study of the N nutrition of strains of perennial ryegrass.

# EXPERIMENT 3

## THE RELATIVE EFFICIENCY OF STRAINS OF LOTUS IN RESPONSE TO APPLIED P

## INTRODUCTION

There are few comparisons reported in the literature between strain of Lotus pendunculatus ('Lotus'). In New Zealand a breeding programme of Barclay (1957) and Barclay and Lambert (1966) resulted in the production of an induced tetraploid, Grasslands 4702. It was bred from Grasslands 4701, a diploid selection from a wide range of New Zealand material, for improved winter growth. Subsequently an autotetraploid, Grasslands 4705 was released to farmers under the name Grasslands Maku, (Boyd, 1973). This new strain is reported (Barclay and Lambert, loc.cit.) to possess the attributes of improved seedling vigour and higher yields except under hard grazing.

Overseas reports on the relative performance of strains of Lotus are few. In Welsh hill country Davies (1969) conducted an experiment comparing a number of strains, including one from New Zealand, but failed to obtain useful results owing to poor seedling establishment and survival. Other reports in the literature provide claims as to the superiority of one strain over another, but these reports do not provide data to substantiate claims made.

There have not been any experiments reported examining the RNE of Lotus strains. Accordingly the experiment reported here had the aim of providing information to enable selection of material for further study. The number of strains available for comparison within New Zealand collections were few. For this reason one hybrid (L.pendunculatus X L. corniculatus L.) was included, and will be referred to as L.hybrid.

## EXPERIMENTAL

The experiment was conducted in a temperature controlled glasshouse, and compared eight strains of Lotus at five levels of P application in a four replicate randomized block layout. Strains compared were as follows:

1. Lotus hybrid ex Grasslands Division.
2. Marshfield ex Soil Conservation Service, Corvallis.
3. Lotus tetraploid ex Grasslands Division (Grasslands 4702).
4. Dunedin natural ecotype.
5. Los Lagos ex southern Chile.
6. Chilean strain of unknown origin (referred to as 'Chile').
7. Wairoa natural ecotype.
8. Timaru natural ecotype.

Phosphorus was applied as sodium dihydrogen phosphate at 15, 30, 60, 100, and 200kg/ha P (calculated on a superficial basis) and mixed with the soil. Nine seeds were sown per pot on 16-10-72 following inoculation. Two harvests were made - the first on 11-12-72 (day 56) and the second on 5-1-73 (day 82). Further experimental details have been given in Section 3.

Nitrogen fixing activity was measured on days 55 and 81 and root weights were measured on day 82. As they do not add any information to alter selections based on data reported, these results will not be reported.

## RESULTS AND DISCUSSION

Shoot yield, absorption and utilization of P results are presented in Appendix XI. Fitted curves for yields are shown in Fig. 3 where, to aid examination, results are shown in two graphs with the curve for one (L.hybrid) being shown on both to facilitate comparison. Relative yields of strains, comparing fitted yields at 60 and 100kg/ha P against yields at 200kg/ha P are shown in Table III. Fitted curves for absorption and utilization of P are presented in Fig. 4.

Yield results (Table III, Fig. 3) do not show any strain reaching its yield asymptote at 200kg/ha P and this is to some extent a reflection of the effects of fitting curves to the strain mean yields. However in spite of this limitation it is apparent that two strains - L.hybrid and Timaru, were not approaching their yield asymptote as closely as the remainder. This indicates that they possessed the properties of a Type 1 curve more than the other strains.

The strains other than L.hybrid and Timaru had the properties of a Type 2 curve in that at low levels of applied P they were superior to these two. Of this Type, L.tetraploid gave the highest yields at all levels of P and is classified as a Type 2 RNE whereas Marshfield, Los Lagos, Wairoa and Dunedin strains differed little in their response surface, were at no point superior to L. tetraploid, and are accordingly classified as Type 3.

Examination of absorption and utilization results shows that L.hybrid and Timaru both gave low relative yields at low levels of P due to poor utilization of absorbed P. The superior yields of L. tetraploid were a function of its ability to utilize absorbed P at a high level, particularly at the low level of P input. Remaining strains gave similar patterns of absorption and utilization of P which is in conformity with

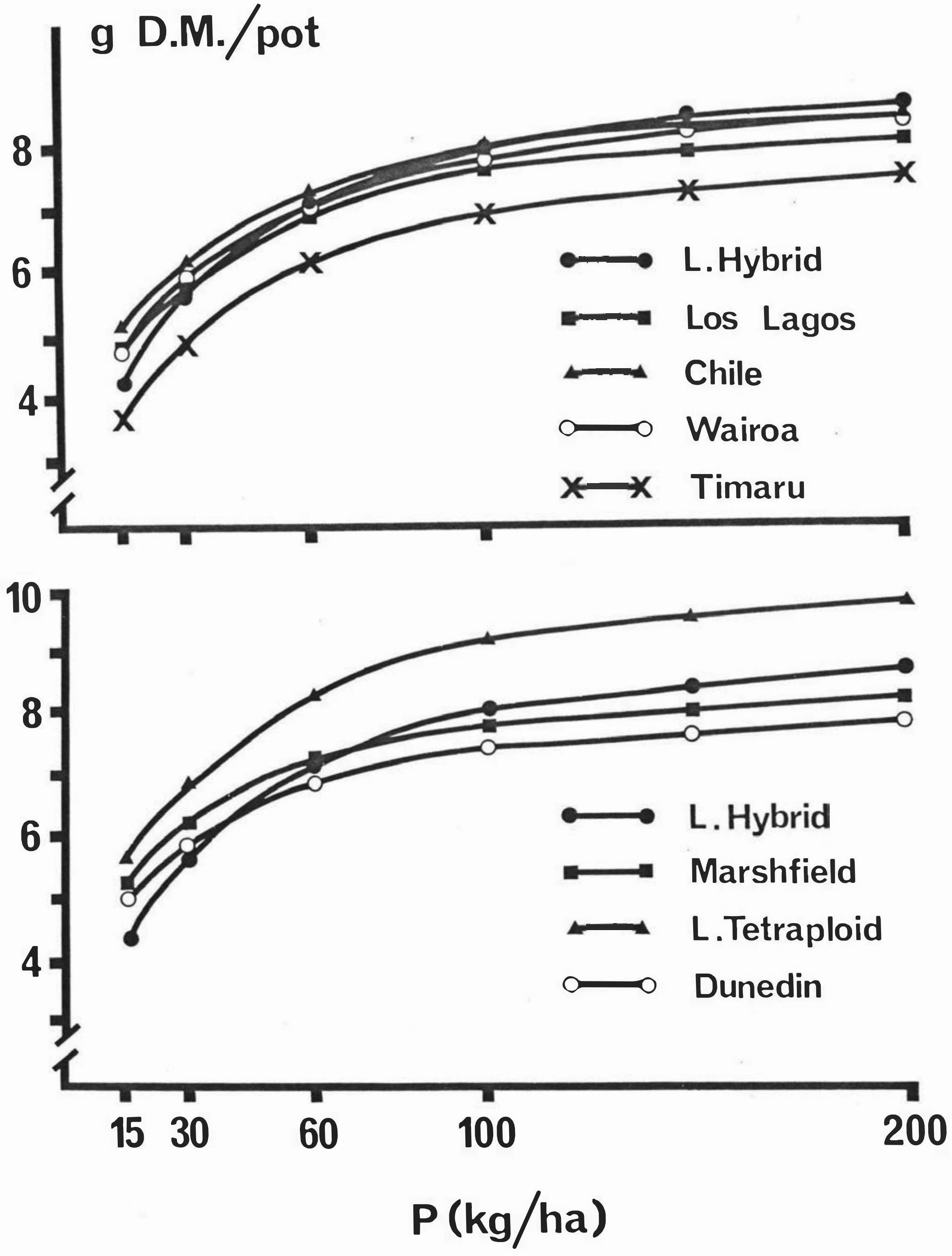

Fig. 3. Dry matter yields of strains of Lotus according to level of P using fitted values.

Table III. Relative yields of strains of Lotus
(200kg/ha P = 100)

| Strain | Level of P (kg/ha) | |
|---|---|---|
| | 60 | 100 |
| L.hybrid | 81.4 | 92.2 |
| Marshfield | 83.5 | 94.3 |
| L. tetraploid | 83.6 | 93.1 |
| Dunedin | 86.5 | 94.3 |
| Los Lagos | 85.0 | 93.7 |
| Chile | 85.3 | 93.8 |
| Wairoa | 83.8 | 93.2 |
| Timaru | 81.4 | 92.2 |

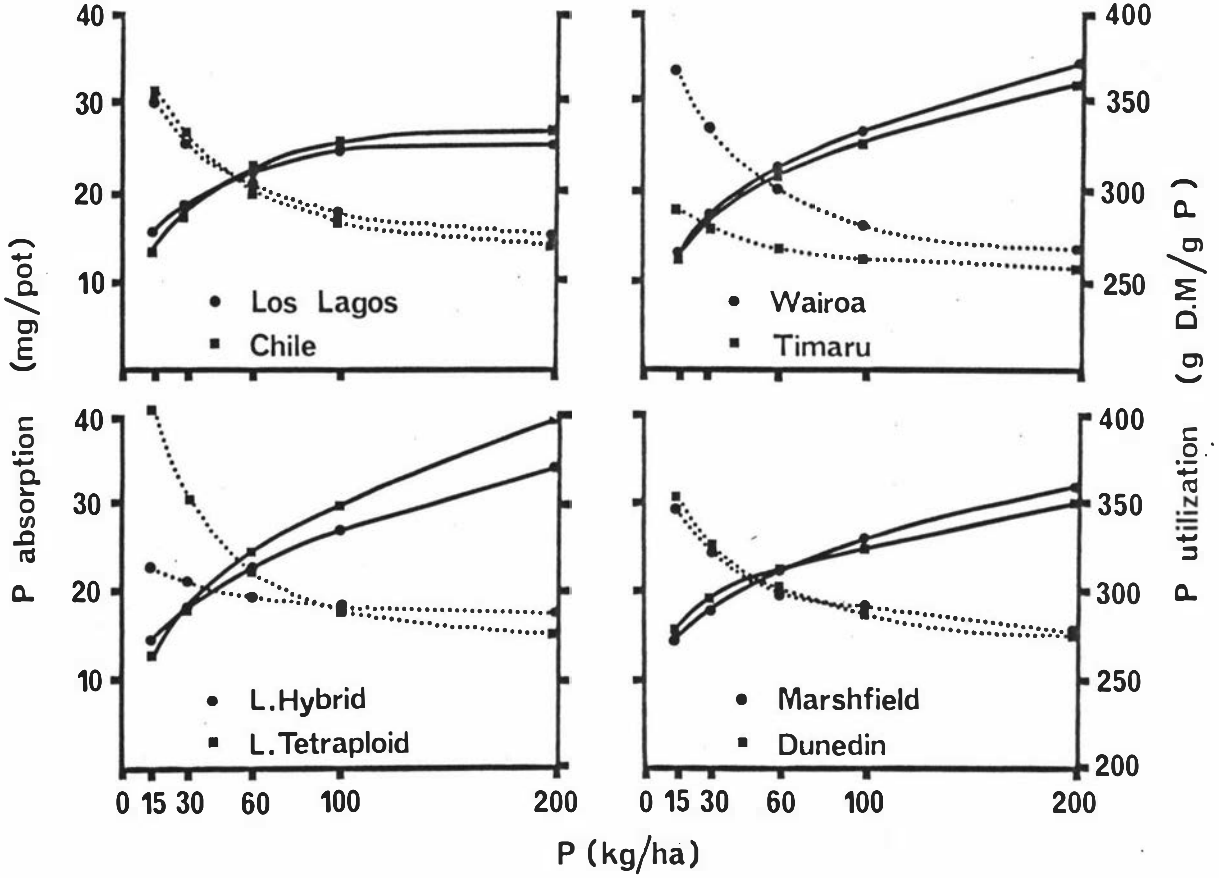

Fig. 4 Fitted curves for P absorption (———) and utilization (·····) by strains of Lotus according to level of P.

their relative yield results.

As only two strains differed markedly in their relative yields and in utilization of P it was decided to include only these in the subsequent experiment. They were the Type 1 strain L.hybrid and the Type 2 strain L. tetraploid.

# EXPERIMENT 4

# THE RELATIVE EFFICIENCY OF STRAINS OF WHITE CLOVER IN RESPONSE TO APPLIED P

## INTRODUCTION

There is little doubt from the work of Sears and Evans (1953) that breeding white clover for improved growth results in increased nitrogen fixation. To further increase the supply of nitrogen to associated grasses, the aims in white clover breeding have been mainly to increase cool season activity (Barclay, 1969). Little attention has been given to selecting strains for high RNE to improve their ability to compete for nutrients in the grass/clover sward. Selection and breeding has probably militated against this in that plant breeding and regional testing experiments have been confined to high fertility soils or to low fertility soils to which adequate basal nutrients have been applied (Barclay, 1969; Lambert et al, 1969; Weeda et al, 1969; Cullen, N.A., pers.comm.re basal treatments).

The experiment reported here was designed to identify strains of white clover exhibiting varying degrees of nutrient efficiency. This step was made necessary by the absence of published data identifying efficient strains, apart from the recent work of Brock and Hoglund (1974) which was published after this experiment was completed. They found that Pitau white clover made relatively more growth than Huia under low levels of P. Phosphorus was selected as the nutrient for which efficiency was sought because superphosphate is the fertiliser applied in largest quantities to New Zealand soils.

The selection of strains of white clover to be examined was based upon the need to include material of diverse geographic origin and upon the need to include strains (such as the Tekapo strain) which are known to survive under low fertility conditions together with strains used in high producing pastures.

## EXPERIMENTAL

The following strains of white clover (supplied by Grasslands Division, Palmerston North) were compared in a 10 x 5 factorial arranged in three randomized blocks:

1. N.Z. Certified Grasslands Huia.
2. " " " Pitau
3. Ecotype from Lake Tekapo from a site not previously topdressed, and referred to hereafter as 'Tekapo'.
4. Bituanai white clover ex Lithuanian Institute of Agriculture, U.S.S.R.

5. S.100 white clover ex National Seed Development Organization Ltd, Cambridge.
6. S.184 white clover ex National Seed Development Organization Ltd, Cambridge.
7. Tamar (culture type) white clover ex Zwaan and De Wiljas, Scheemda, Holland.
8. Spanish white clover - a Spain X Spain cross made by Grasslands Division.
9. Kentish wild white clover ex John Harvey Ltd, Dover, referred to hereafter as K.W.W.
10. Louisiana S-1 white clover ex U.S.D.A. Plant Introduction Section, Beltsville.

P was applied as sodium dihydrogen phosphate at 15, 30, 60, 100 and 200kg/ha P (calculated on a superficial basis) and mixed with the soil. Nine seeds were sown per pot following inoculation on 16-10-72 and were grown in a temperature controlled glasshouse and plants were harvested on days 59 and 87. Further experimental details have been given in Section 3.

Nitrogen fixing activity was measured on days 48 and 84 and root dry matter yields were measured on day 87. As these results add little further information as a basis for selection of strains they will not be presented.

## RESULTS AND DISCUSSION

Shoot yields, absorption and utilization of P are shown in Appendices XII, XIII, and XIV respectively. Fitted curves for yields are shown in three line drawings in Fig. 5 where, to aid comparisons, Huia is represented in each. Relative yields, in which yields at 60 and 100kg/ha P are compared relative to yields at 200kg/ha P using fitted values, are presented in Table IV.

Fitted yields (Fig. 5) failed to show any marked differences in shape of the response surfaces of strains. However there were some differences in degree of curvature. Most noticeable in this respect was the lack of response to increasing levels of P by Tekapo. This finding is in agreement with those of Snaydon and Bradshaw (1962), who found that populations from soils low in P did not respond to P as much as populations coming from soils high in P.

Table IV. Relative yields of strains of white clover (200kg/ha P = 100)

| Strain | Level of P (kg/ha) | |
|---|---|---|
| | 60 | 100 |
| Huia | 74.0 | 86.5 |
| Pitau | 76.8 | 87.8 |
| Tekapo | 76.6 | 87.8 |
| Bituanai | 72.9 | 86.5 |
| S.100 | 75.5 | 88.0 |
| S.184 | 68.6 | 83.6 |
| Tamar | 70.4 | 84.5 |
| Spanish | 70.9 | 84.8 |
| K.W.W. | 69.3 | 84.0 |
| Louisiana | 74.3 | 86.5 |

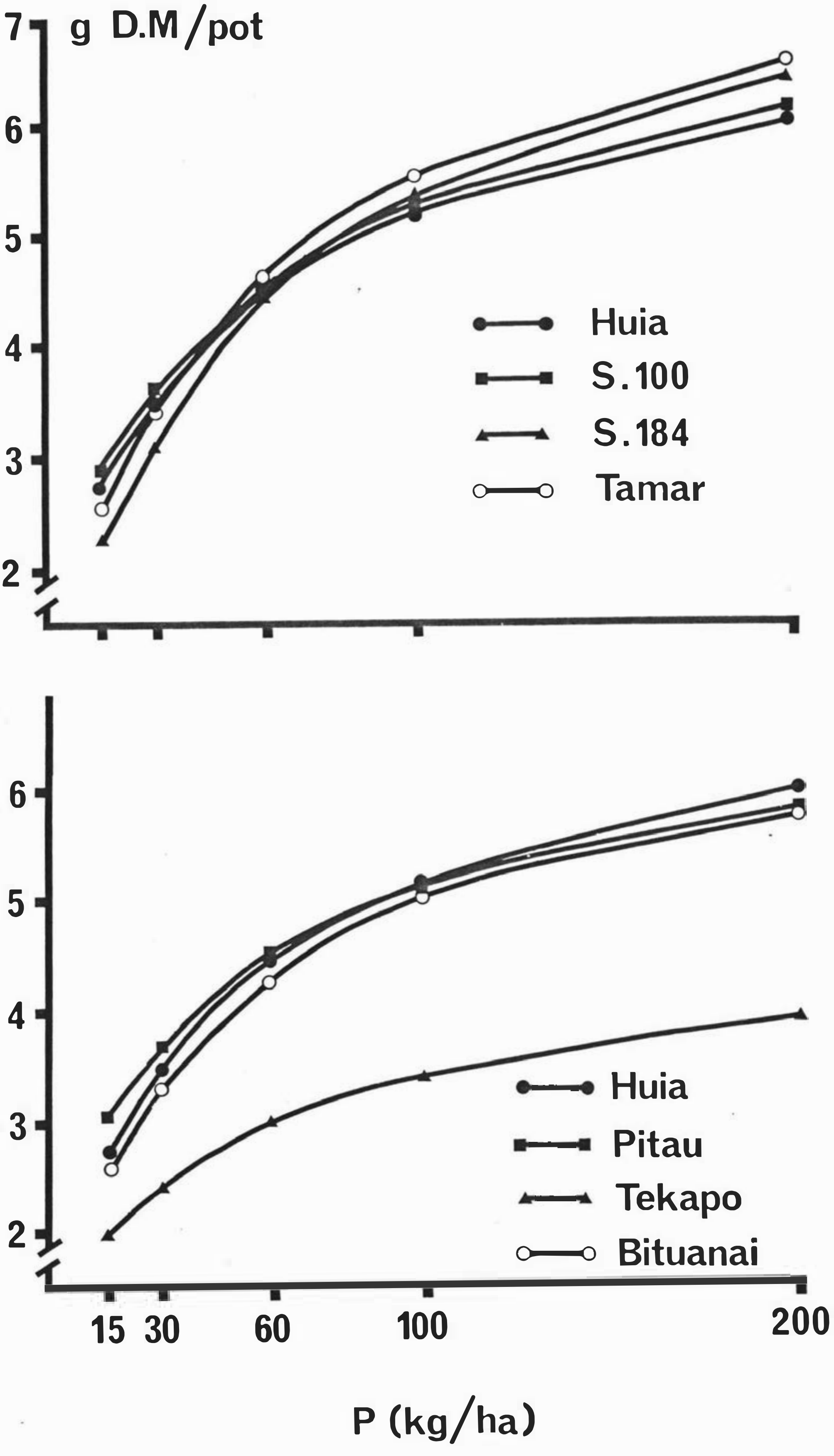

Fig. 5. Dry matter yields of strains of white clover according to level of P using fitted values.

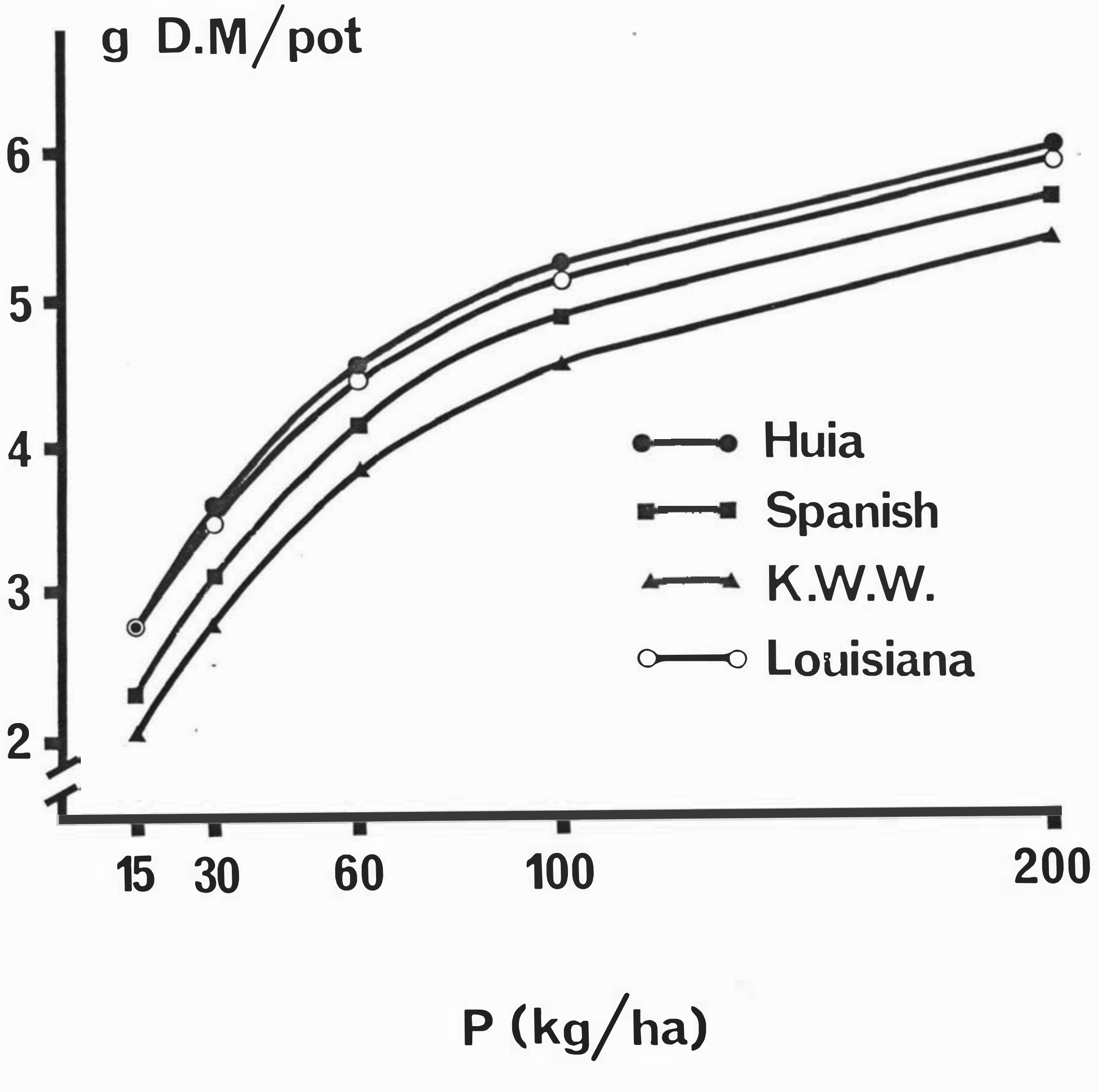

Fig. 5. Dry matter yields of strains of white clover according to level of P using fitted values.

Available information on strains used in this experiment indicates that the Tekapo strain was from a soil low in available P and one which had not been topdressed. The reason for its low overall yields lay in its low level of P absorption while its poor relative yield performance at low levels of applied P was due to its relative inability to utilize absorbed P (Fig. 6 and Appendix XIV). The remaining strains, including K.W.W. (see Snaydon and Bradshaw's classification), had been bred and cultivated under high P conditions.

Results did not provide statistically sound evidence to confirm the superiority of Pitau compared to Huia at low levels of P as found by Brock and Hoglund, loc.cit. although the actual yields and fitted curves suggests their finding may have been reproduced. The data provides evidence that the superiority of Pitau was due to its greater ability to utilize absorbed P (Fig. 6 and Appendix XIV).

Some distinction needed to be made between strains if promising material was to be selected for further study. Accordingly it was decided to classify strains into Type 1 RNE if their yields at 100kg/ha P were less than 85 percent of their yields at 200kg/ha P. On this basis (Table IV) Tamar, Spanish S.184 and K.W.W. would be classified as possessing Type 1 RNE. From among these Tamar was selected for further study as it gave highest yields at 200kg/ha P and among the lowest yields at 15kg/ha P (Fig. 5). Tamar also differed from other strains (with the exception of Louisiana) in that it gave high utilization as the level of P was increased. Its absorption of P differed little from that of other strains.

From among the remaining strains Huia was selected for further study. It was typical of strains with a tendency towards Type 2 RNE, (Table IV). Fitted curves (Fig. 5) showed Huia gave high yields relative to Tamar at 15kg/ha P and lower yields at 200kg/ha P although actual yields (Appendix VII) indicated that the differences may have resided more at the higher levels of P. Absorption and utilization of P by Huia (Fig. 6 and Appendices XIII and XIV) was generally similar to that of other strains. One further reason for the selection of Huia was its importance to New Zealand agriculture.

Louisiana was selected as a third strain for further study. It gave yields similar to Huia but differed substantially in its utilization of absorbed P (Fig. 6 and Appendix XIV). Thus it held promise of possessing genetic variability in P utilization which could bear further examination.

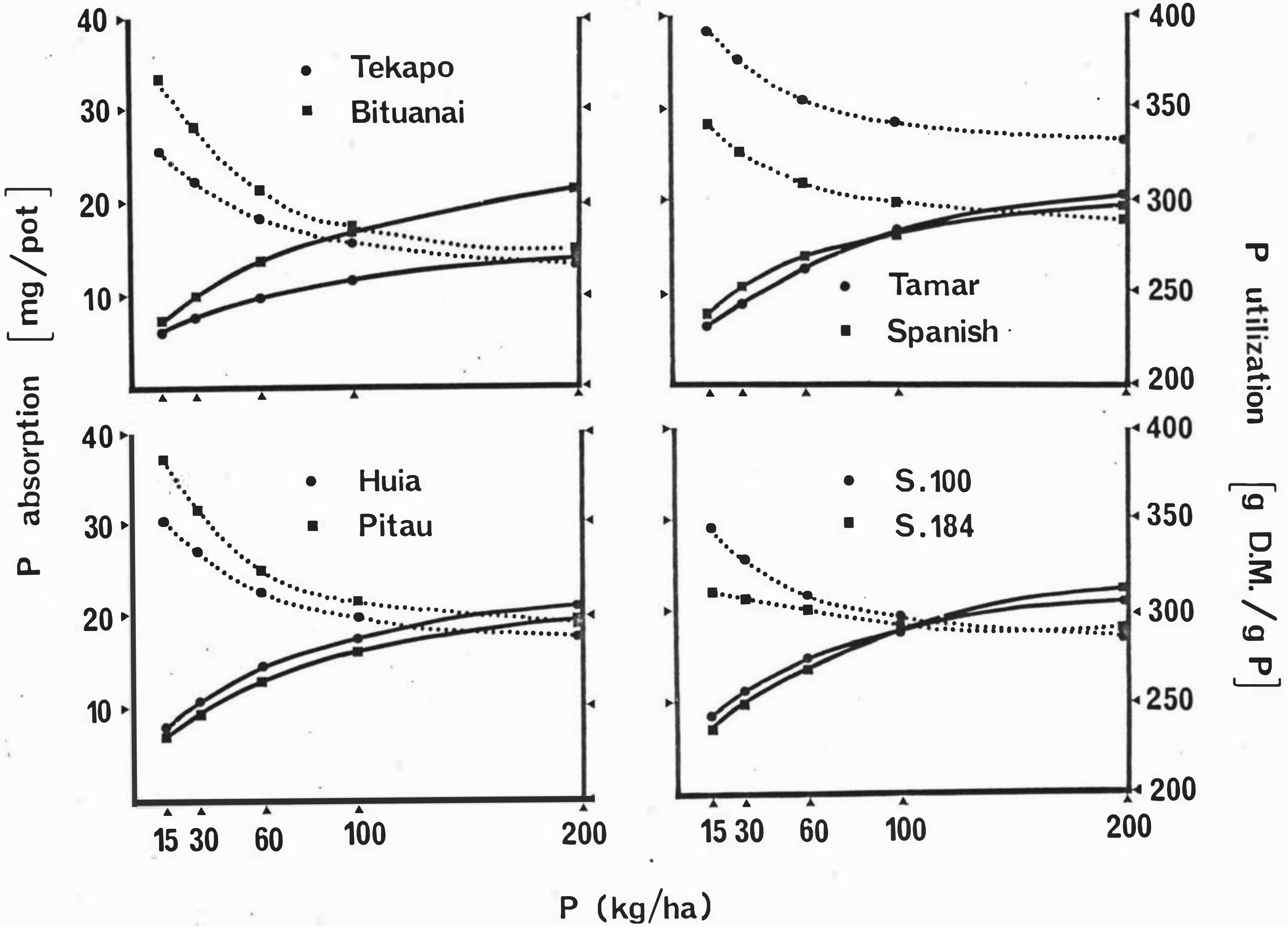

Fig. 6 Fitted curves for P absorption ( ____ ) and utilization ( .....) by strains of white clover according to level of P (cont. over).

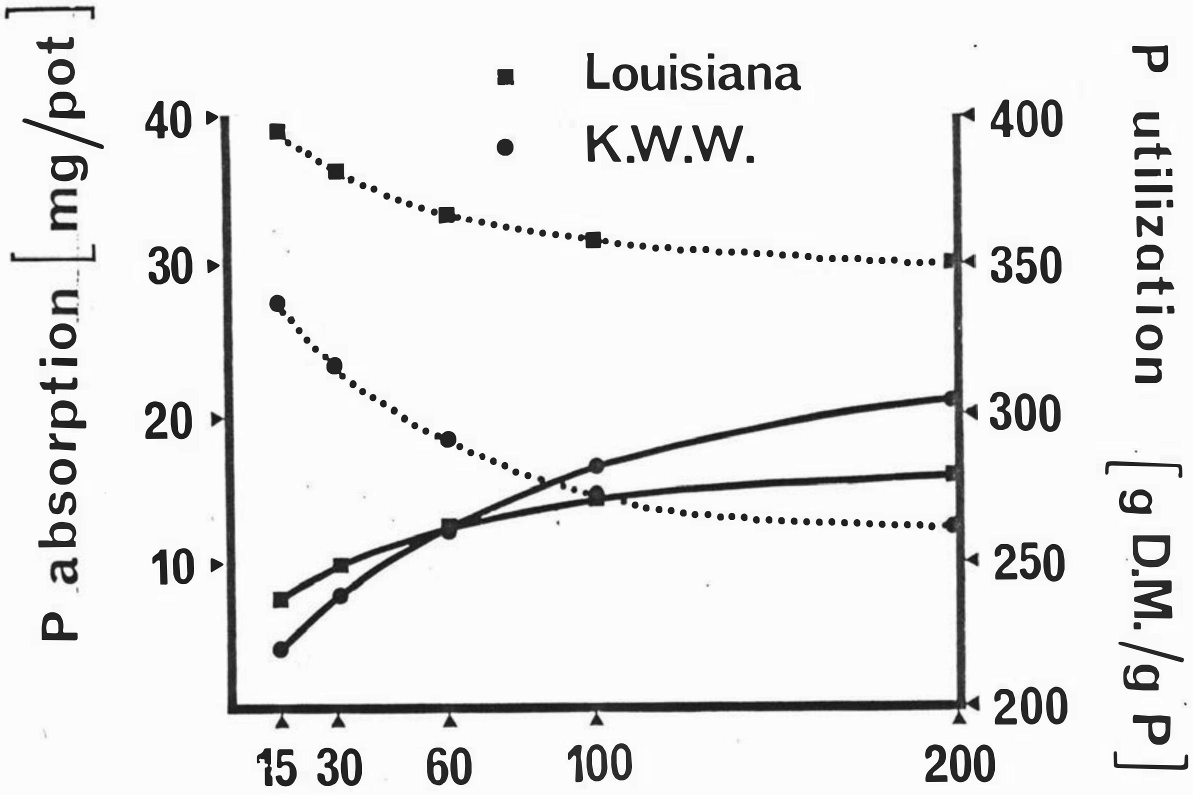

Fig. 6 (cont.) Fitted curves for P absorption ( ——— ) and utilization (·····) by strains of white clover according to level of P.

# SECTION 5

## INTER AND INTRASPECIFIC COMPARISONS OF EFFICIENT AND INEFFICIENT GRASSES AND LEGUMES UNDER THREE CLIMATIC REGIMES.

## General Introduction

It has already been pointed out that a study of RNE must take into account environmental factors because of the marked influence they exert on the production and utilisation of assimilates and the consequential effects on the uptake of inorganic ions.

It was the objective of experiments described in this section to study RNE of strains of the two grasses (Yorkshire fog and perennial ryegrass) in one experiment and the two legume species (white clover and Lotus) in another. The experiments were designed to provide a more precise measurement of interstrain and interspecific differences in RNE. This latter comparison was desirable to distinguish between the contributions of RNE and environment to the relative superiority between the two grasses and between the two legumes. This question of relative superiority has been discussed in the introductory section.

The climatic paramaters mainly affecting plant growth are temperature, light and moisture. To examine these satisfactorally would necessitate the use of four controlled environment rooms; two light comparisons x two temperature comparisons and within-environment comparisons of moisture. As only three controlled environment rooms were available it was decided to simulate three distinct climatic environments. This approach had the disadvantage of not enabling the identification of the effects of different environmental parameters on RNE. It did have some practical advantage in enabling the simulation of climatic conditions in regions of New Zealand. Should this approach lead to the detection of inter or intraspecific differences then the logical next step would be to examine the separate effects of light, temperature and moisture in subsequent experiments.

To simulate a climate in totality is not possible owing to the large variations that occur in daily, seasonal and annual conditions. The best approximation that can be made is to simulate mean seasonal conditions. This was the approach taken in the experiments reported in this section. Data on mean monthly temperatures was extracted for the period 1960/70 from New Zealand meteorological records. In the absence of sufficient data on light intensities and durations the selection of light intensities had to be based on records of the number of hours of sunshine and the practical application of this into the broad categories of 'low' and 'high' light intensities.

# EXPERIMENT 5

## RELATIVE NUTRIENT EFFICIENCY OF YORKSHIRE FOG AND PERENNIAL RYEGRASS IN RESPONSE TO APPLIED NITROGEN

## INTRODUCTION

Justification for the selection of strains of Yorkshire fog for study in this experiment were given in the discussion of Experiment 1 and for perennial ryegrass in the discussion of Experiment 2. Briefly, Massey Basyn Yorkshire fog was selected because its yield response to N demonstrated Type 1 RNE and Tara Hills was selected because it exhibited Type 3 RNE - there being no strain identified as Type 2. Of the perennial ryegrasses Ruanui and Mangere were selected because they showed characteristics of Type 1 RNE and R.v.P. and W.W.Virus because they were of Type 2 RNE.

The first objective of this experiment was to provide confirmatory evidence of results obtained in the previous two experiments through restricting the nutrients studied to nitrogen and through increased replication. The second and main objective was to measure the effects of climate on RNE of the above species/strains. There have been no reports of studies into this subject. As has been discussed in the review of literature, climate exerts a large effect on photosynthesis and the resultant energy status of plants, and species and strains vary in their response to climate. It therefore seemed reasonable to expect climate to affect the RNE of species/strains as their energy supplies for the absorption and utilization of nutrients varied. This experiment was designed to test this hypothesis.

## EXPERIMENTAL

The six species/strains named above and detailed earlier were compared in the presence of five levels of applied N in four randomized blocks, each within three distinct climatic environments.

Nitrogen was applied as ammonium nitrate at the equivalent of 0, 5, 10, 30 and 60kg/ha N (calculated on a superficial basis) and mixed with the soil. Re-application of these rates were made on days 21, 31 and 36.

Reasons for the simulation of distinctly different climates were given in the general introduction. These climates were as follows:-

| Climate | | Tara Hills<br>(summer) | Palmerston North<br>(spring) | Taieri<br>(winter) |
|---|---|---|---|---|
| Temperature ($^{\circ}$C) | Day | 22.5 | 15 | 12 |
| | Night | 7.5 | 10 | 3 |
| Light (Wm$^2$) | | 150 | 180 | 90 |
| Day length (hours) | | 14 | 12 | 7 |
| Day/night changeover (hours) | | 1.5 | 1 | 2 |
| Vapour pressure deficit (mb) | | 10 | 5 | 2 |
| Soil moisture (% field capacity) | | 50 cycling to 30 | 80 | 80 |

$CO_2$ ambient

Further details of experimental proceedures have been given in Section 3. In addition a basal dressing of 1.647 g/pot sodium dihydrogen phosphate was applied and mixed with the soil. Germinated seedlings were sown on 3-4-72 and grown in a temperature controlled glasshouse (15-25$^{\circ}$C) before transferring to the climate rooms on day 21. Plants were harvested on day 52. Roots were removed by washing and their dry weight determined.

Statistical analyses were performed on transformed data to improve the homogeneity of the variance. A natural log ($l_n$) transformation was made on shoot yields and P absorption data, but no transformation was considered necessary on N utilization data. All data was analysed as orthogonal polynomials in which the square root of the level of N application was used. In the analyses, variance was apportioned among treatments and their linear, quadratic and residual components, as shown in Appendix XV.

To aid interpretation where significant strain X linear or quadratic effects were recorded, results will be presented as line drawings of the re-transformed data. This explains the apparent irregularity of some of the response surfaces shown.

## RESULTS AND DISCUSSION

Results of shoot yields, shoot:root ratios and N absorption and utilization according to levels of N are presented in Tables V, VI, VII and VIII respectively.

Table V. Effect of N on shoot dry matter yields of perennial ryegrass and Yorkshire fog. ($1_n$ g/pot)

| | Level of N (kg/ha) | | | | |
|---|---|---|---|---|---|
| **Tara Hills** | 0 | 5 | 10 | 30 | 60 |
| Ruanui p.r. | 1.545 | 1.674 | 1.821 | 1.932 | 2.161 |
| Mangere p.r. | 1.554 | 1.771 | 1.740 | 1.938 | 2.069 |
| R.v.P. p.r. | 1.548 | 1.705 | 1.849 | 2.026 | 2.004 |
| W.W. Virus p.r. | 1.495 | 1.604 | 1.662 | 1.878 | 1.979 |
| Massey Basyn Y.f. | 1.873 | 1.904 | 2.034 | 2.199 | 2.339 |
| Tara Hills Y.f. | 1.685 | 1.841 | 1.960 | 2.074 | 2.132 |

L.S.D. 5% 0.064, 1% 0.086, C.V.% 5.52

Sig. int: Strain X N n.s., Strain X lin. n.s., Strain X quad. n.s.

| **Palmerston North** | | | | | |
|---|---|---|---|---|---|
| Ruanui p.r. | 1.184 | 1.378 | 1.419 | 1.696 | 1.733 |
| Mangere p.r. | -1.105 | -0.461 | -0.942 | 0.389 | 1.859 |
| R.v.P. p.r. | 1.291 | 1.345 | 1.671 | 1.751 | 1.824 |
| W.W. Virus p.r. | 1.207 | 1.326 | 1.395 | 1.637 | 1.720 |
| Massey Basyn Y.f. | 1.473 | 1.605 | 1.700 | 1.811 | 2.003 |
| Tara Hills Y.f. | -1.108 | -1.674 | -2.286 | 0.906 | 1.438 |

L.S.D. 5% 0.43, 1% 0.57, C.V.% 72.94

Sig. int: Strain X N $p<0.001$, Strain X lin. $p<0.001$, Strain X quad.$p<0.05$.

| **Taieri** | | | | | |
|---|---|---|---|---|---|
| Ruanui p.r. | -0.077 | -1.241 | -0.865 | -0.942 | -1.930 |
| Mangere p.r. | -1.859 | -1.686 | -1.385 | -0.916 | -1.039 |
| R.v.P. p.r. | -1.054 | -1.520 | -0.719 | -0.887 | -0.742 |
| W.W. Virus p.r. | -1.295 | -0.921 | -0.789 | -0.847 | -0.913 |
| Massey Basyn Y.f. | -1.258 | -1.331 | -1.143 | -1.358 | -1.315 |
| Tara Hills Y.f. | -1.393 | -1.548 | -1.262 | -0.883 | -2.272 |

L.S.D. 5% 0.45, 1% 0.60, C.V.% 61.12

Sig. int: Strain X N n.s., Strain X lin. $p<0.05$, Strain X quad. n.s.

Table VI. The effect of N on the shoot:root ratio of strains of Yorkshire fog and perennial ryegrass.

| | Level of N (kg/ha) | | | | |
|---|---|---|---|---|---|
| Tara Hills | 0 | 5 | 10 | 30 | 60 |
| Ruanui | .783 | .869 | .812 | 1.114 | 1.215 |
| Mangere | .651 | .967 | .810 | .939 | 1.201 |
| R.v.P. | .648 | .808 | .955 | 1.056 | 1.350 |
| W.W. Virus | .619 | .500 | .837 | .982 | 1.023 |
| Massey Basyn | .857 | .814 | 1.001 | 1.155 | 1.459 |
| Tara Hills | .846 | .869 | .936 | 1.225 | 1.105 |

Strain X N interaction n.s. L.S.D. 5% .264, 1% .351

C.V.% 19.37

| Palmerston North | | | | | |
|---|---|---|---|---|---|
| Ruanui | .707 | .765 | .834 | 1.097 | 1.153 |
| Mangere | 1.454 | .951 | 3.051 | 2.720 | .935 |
| R.v.P. | .701 | .694 | .937 | 1.102 | 1.158 |
| W.W. Virus | .628 | .677 | .761 | 1.135 | 1.274 |
| Massey Basyn | .991 | .765 | 1.053 | 1.112 | 1.310 |
| Tara Hills | 3.241 | 2.887 | .673 | 2.284 | 1.264 |

Strain X N interaction $p<0.001$ L.S.D. 5% 1.094, 1% 1.455

C.V.% 61.02

| Taieri | | | | | |
|---|---|---|---|---|---|
| Ruanui | 1.229 | 1.931 | 2.739 | 2.440 | 2.790 |
| Mangere | 2.667 | 1.391 | 1.434 | 2.189 | 1.470 |
| R.v.P. | 1.515 | 1.647 | 1.236 | 1.360 | 1.531 |
| W.W. Virus | 2.114 | 1.184 | 1.069 | 1.197 | 1.075 |
| Massey Basyn | 1.552 | 1.956 | 1.631 | 1.240 | 1.930 |
| Tara Hills | .792 | 1.617 | 1.259 | 1.103 | 1.781 |

Strain X N interaction n.s. L.S.D. 5% .471, 1% .632

C.V.% 56.36

Table VII. Effect of N on N absorption in shoots of perennial ryegrass and Yorkshire fog.
($l_n$ g N/pot)

| | Level of N (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 0 | 5 | 10 | 30 | 60 |
| **Tara Hills** | | | | | |
| Ruanui p.r. | -2.503 | -2.325 | -2.403 | -1.594 | -1.337 |
| Mangere p.r. | -2.622 | -2.333 | -2.373 | -1.848 | -1.450 |
| R.v.P. p.r. | -2.651 | -2.447 | -2.239 | -1.737 | -1.590 |
| W.W. Virus p.r. | -2.703 | -2.518 | -2.271 | -1.849 | -1.464 |
| Massey Basyn Y.f. | -2.511 | -2.376 | -2.229 | -1.789 | -1.373 |
| Tara Hills Y.f. | -2.523 | -2.376 | -2.092 | -1.605 | -1.517 |

L.S.D. 5% .089, 1% .119, C.V.% 66.16

Sig.int.; Strain X N n.s., Strain X lin. n.s., Strain X quad. n.s.

| | | | | | |
|---|---|---|---|---|---|
| **Palmerston North** | | | | | |
| Ruanui p.r. | -2.487 | -2.369 | -2.135 | -1.791 | -1.318 |
| Mangere p.r. | -4.119 | -3.387 | -3.838 | -2.827 | -1.382 |
| R.v.P. p.r. | -2.536 | -2.318 | -1.914 | -1.699 | -1.436 |
| W.W. Virus p.r. | -2.522 | -2.307 | -2.137 | -1.645 | -1.416 |
| Massey Basyn Y.f. | -2.360 | -2.257 | -2.034 | -1.805 | -1.578 |
| Tara Hills Y.f. | -4.043 | -4.584 | -5.205 | -2.662 | -1.578 |

L.S.D. 5% .42, 1% .56, C.V.% 62.10

Sig.int.; Strain X N $p<0.01$, Strain X lin. $p<0.001$, Strain X quad. $p<0.05$

| | | | | | |
|---|---|---|---|---|---|
| **Taieri** | | | | | |
| Ruanui p.r. | -3.055 | -4.226 | -3.828 | -2.864 | -5.104 |
| Mangere p.r. | -4.965 | -4.719 | -4.371 | -3.895 | -3.980 |
| R.v.P. p.r. | -4.199 | -4.614 | -3.903 | -3.895 | -3.980 |
| W.W. Virus p.r. | -4.401 | -4.021 | -3.975 | -3.890 | -3.932 |
| Massey Basyn Y.f. | -4.202 | -4.501 | -4.074 | -4.318 | -4.184 |
| Tara Hills Y.f. | -4.330 | -4.441 | -4.217 | -3.778 | -4.394 |

L.S.D. 5% .45 1% .60, C.V.% 38.00

Sig. int.; Strain X N n.s., Strain X lin. $p<0.001$, Strain X quad. n.s.

Table VIII. Effect of N on N utilization in shoots of perennial ryegrass and Yorkshire fog.
(g D.M./g N absorbed)

| | Level of N (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 0 | 5 | 10 | 30 | 60 |
| **Tara Hills** | | | | | |
| Ruanui p.r. | 57.28 | 54.54 | 68.30 | 33.98 | 33.04 |
| Mangere p.r. | 65.10 | 60.58 | 61.12 | 44.07 | 33.75 |
| R.v.P. p.r. | 66.61 | 63.56 | 59.62 | 43.07 | 36.37 |
| W.W. Virus p.r. | 66.55 | 61.68 | 51.05 | 41.55 | 44.83 |
| Massey Basyn Y.f. | 80.15 | 72.24 | 71.02 | 53.94 | 40.93 |
| Tara Hills Y.f. | 67.22 | 67.82 | 57.51 | 39.60 | 38.43 |
| L.S.D. 5% 8.14, 1% 9.71, C.V.% 3.63 | | | | | |
| Sig.int; Strain X N n.s., Strain X lin. n.s., Strain X quad. n.s. | | | | | |
| **Palmerston North** | | | | | |
| Ruanui p.r. | 39.29 | 42.39 | 34.70 | 32.68 | 21.13 |
| Mangere p.r. | 20.36 | 18.65 | 18.10 | 24.92 | 25.55 |
| R.v.P. p.r. | 45.92 | 38.97 | 36.05 | 31.50 | 26.04 |
| W.W. Virus p.r. | 41.63 | 37.82 | 34.29 | 26.62 | 23.01 |
| Massey Basyn Y.f. | 42.20 | 47.56 | 41.84 | 37.18 | 35.90 |
| Tara Hills Y.f. | 18.82 | 18.85 | 18.50 | 35.44 | 20.40 |
| L.S.D. 5% 3.99, 1% 4.75, C.V.% 9.17 | | | | | |
| Sig.int; Strain X N $p<0.01$, Strain X lin. $p<0.001$, Strain X quad. $p<0.05$ | | | | | |
| **Taieri** | | | | | |
| Ruanui p.r. | 19.64 | 19.78 | 19.35 | 18.57 | 23.90 |
| Mangere p.r. | 22.33 | 20.75 | 19.80 | 19.66 | 18.93 |
| R.v.P. p.r. | 23.21 | 22.06 | 24.14 | 21.75 | 21.26 |
| W.W. Virus p.r. | 22.33 | 22.19 | 24.19 | 20.96 | 20.47 |
| Massey Basyn Y.f. | 18.99 | 23.80 | 18.74 | 26.04 | 17.61 |
| Tara Hills Y.f. | 18.85 | 18.04 | 19.20 | 18.08 | 8.34 |
| L.S.D. 5% 3.03, 1% 3.62, C.V.% 9.64 | | | | | |
| Sig.int.; Strain X N n.s., Strain X lin. n.s., Strain X quad. n.s. | | | | | |

Growth of species/strains were in the order: Tara Hills>Palmerston North> Taieri. Under the Taieri climate very little growth was present and plant growth within pots was observed to be extremely variable, with some plants barely surviving.

(i) Tara Hills climate. Results failed to show any species/strain X N interaction on shoot yields or shoot:root ratio. In view of the high level of experimental precision in the measurement of shoot yields, as indicated by the low coefficient of variation, it is concluded that under high temperatures and high light intensities, where photosynthetic activity and growth was greatest (irrespective of the high moisture stress), the RNE of the species/strains did not differ.

Results of N absorption measurements, although extremely variable, lend support to yield results in that no interaction between species/strains were recorded. Utilization of N provided significant species/strain X N interaction due largely to the high utilization of N by Massey Basyn. As there were no differences between species/strain in the shape of the utilization response surfaces (no linear or quadratic effects) this indicates that Massey Basyn behaved in the same manner as others over the entire range of N application. It is worth noting that Massey Basyn provided high yields due to a high level of N utilization rather than to differences in N absorption.

(ii) Palmerston North climate. There was a highly significant species/strain X N interaction on shoot yields, and the presence of linear and quadratic interactions indicates that they differed consistently in their response (RNE) to increasing levels of N. Fitted curves (see experimental) are shown in Fig. 7 and relative yields at 30kg/ha N (taking 60kg/ha N as $X_{max}$) are presented in Table IX. Results show Mangere and Tara Hills to be Type 1 RNE and the remainder Type 2. They are in line with glasshouse results where Mangere was found to be Type 1 and Tara Hills of Type 1 curve but classified as Type 3 because of its low overall yields. The discrepancy in comparative species/strain performance in this climate compared to the Tara Hills climate (where no differences were found) are explicable only in terms of differences in climate. Temperatures in the Palmerston North climate were below optimum for Yorkshire fog and perennial ryegrass (Mitchell, 1956) and light intensities were high. The rapid increase in yields as the level of N was increased shows that Mangere and Tara Hills strains were restricted in growth at low levels of N by their inability to absorb N (Table VII and Fig. 8) due to poor root development (Table VI) at low levels of N and to poor utilization of absorbed N (Table VIII and Fig. 8). Which factors among these were controlling factors

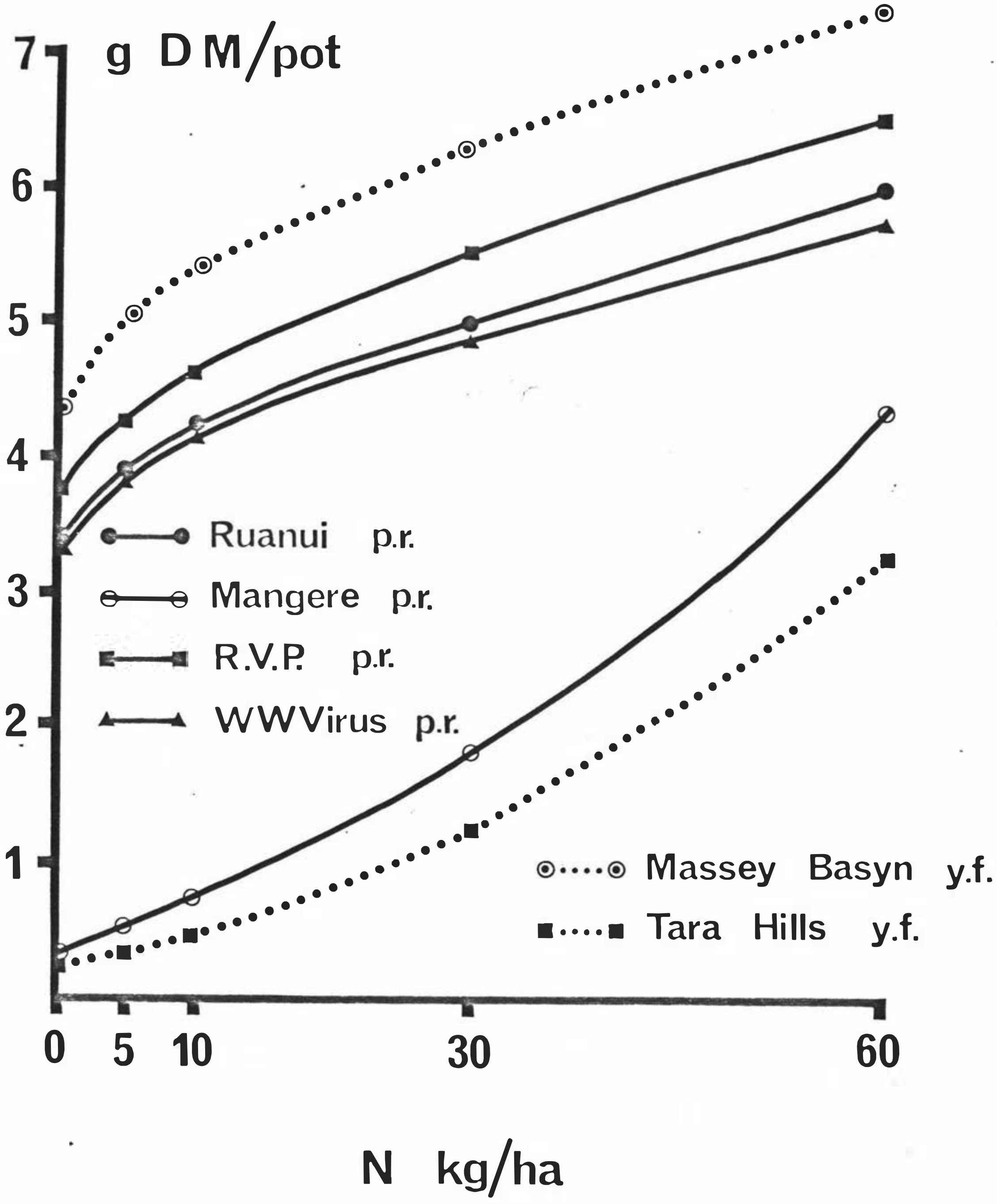

Fig. 7. Fitted curves for shoot dry matter yields of perennial ryegrass and Yorkshire fog under the Palmerston North climate, retransformed from $l_n$ yields and $\sqrt{N}$ applied.

Relative yields of perennial ryegrass and Yorkshire fog.

$(\frac{30}{60}$ kg/haN X 100)

| Climate: | Palmerston North | Taieri |
|---|---|---|
| Ruanui p.r. | 85 | 154 |
| Mangere p.r. | 42 | 74 |
| R.v.P. p.r. | 84 | 88 |
| W.W. Virus p.r. | 85 | 85 |
| Massey Basyn Y.f. | 86 | 102 |
| Tara Hills Y.f. | 38 | 117 |

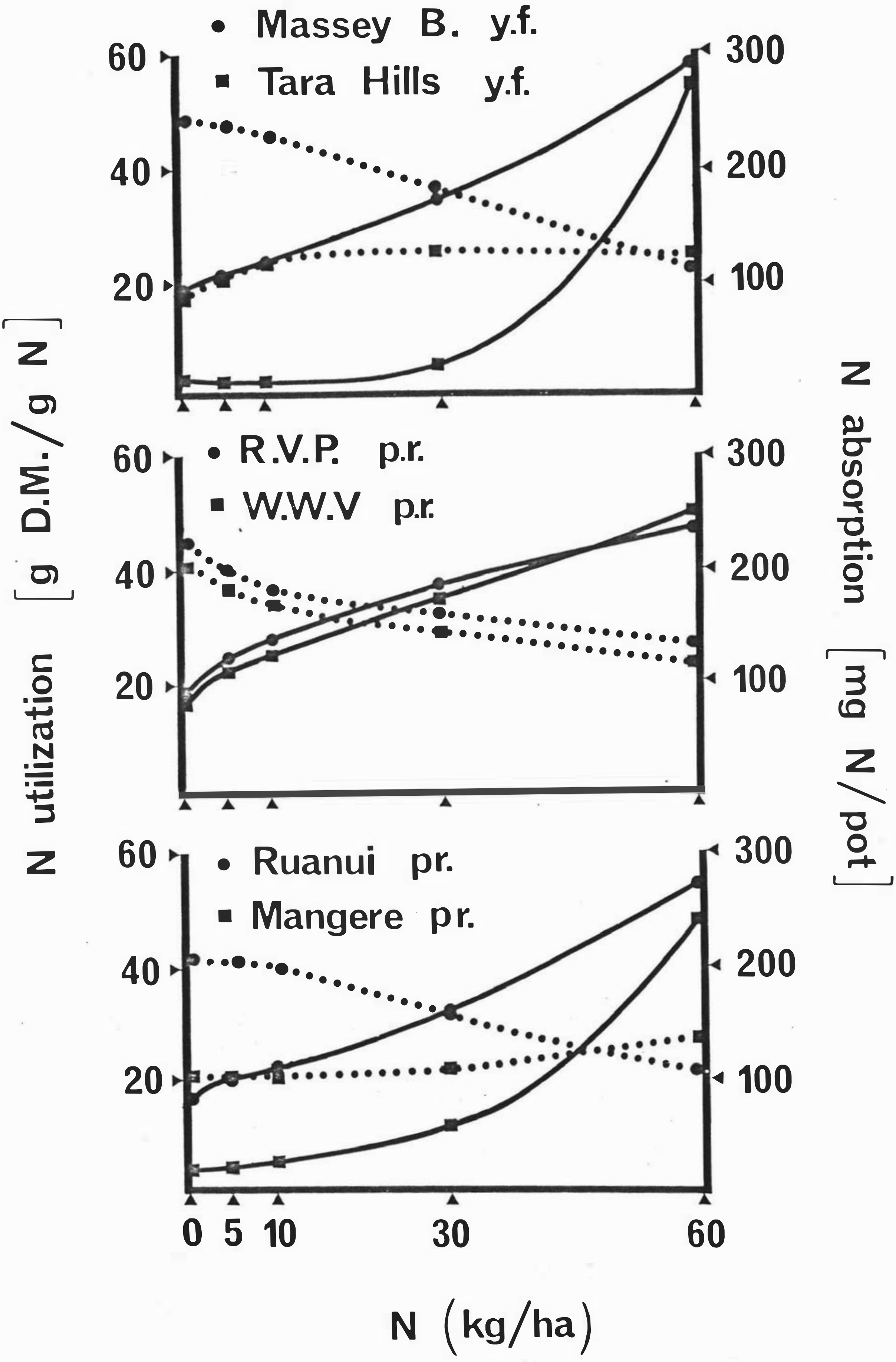

Fig. 8 Absorption ( ——— ) and utilization ( ····· ) of N by strains of perennial ryegrass and Yorkshire fog in response to N under the Palmerston North climate.

and which were dependent factors cannot be distinguished from results of this experiment.

The shape of the response surfaces of the remaining species/strains did not differ materially, i.e. they all exhibited Type 2 RNE. They differed in actual yields, with Massey Basyn providing highest yields - as was also the case under the Tara Hills climate, and again attributable to high utilization of N by Massey Basyn.

(iii) Taieri climate. Shoot yields showed a significant species/strain effect (Table V and Fig. 9). Results show Ruanui and Tara Hills giving unusual yield results in that they declined as the level of N was increased. Such an effect cannot be classified according to Types of RNE defined in the introductory section of this thesis. Their behaviour can be explained in terms of diminishing N absorption (Fig. 10) which closely followed the pattern of the yield curves. Utilization of N by these strains (particularly Ruanui) actually increased at high levels of N (Fig. 10) - a finding in contrast to the general downward trend noted for other species/strains. This effect can only be ascribed to climate where, in view of the findings of Blackman and Templeman (1938) with Festuca rubra and Agrostis tenuis and Murata (1961) with rice, conditions for growth were probably limited more by light than by temperature. Murata (1969) postulated that a high supply of N results in protein synthesis which is carried out at the expense of carbohydrate reserves and can result in actual decreases in dry matter production as the N supply is increased. He further stated that such plants would face the danger of ammonium toxicity as they would have insufficient carbohydrate to promote the conversion of amides to amino acids. If the suggestions of Murata are true, then the low N absorption of Ruanui and Tara Hills was probably due to low energy status of the plants under high levels of applied N - it having been pointed out previously that absorption is an energy demanding process. This is supported by the increasing shoot:root ratio which increased as the level of N was increased.

Massey Basyn yields did not alter as the level of N was increased and probably approached the limits of its carbohydrate reserves, as postulated in the cases of Ruanui and Tara Hills. Again this is supported by the shoot:root ratio increase which approached significance at the 5% level.

The remaining species/strains (Mangere, R.v.P. and W.W. Virus) did not differ in RNE but did nevertheless respond to increasing N. Thus they were better adapted to low light intensities and because of this were able to continue to absorb N (Fig. 10) and to make active growth. All three were of

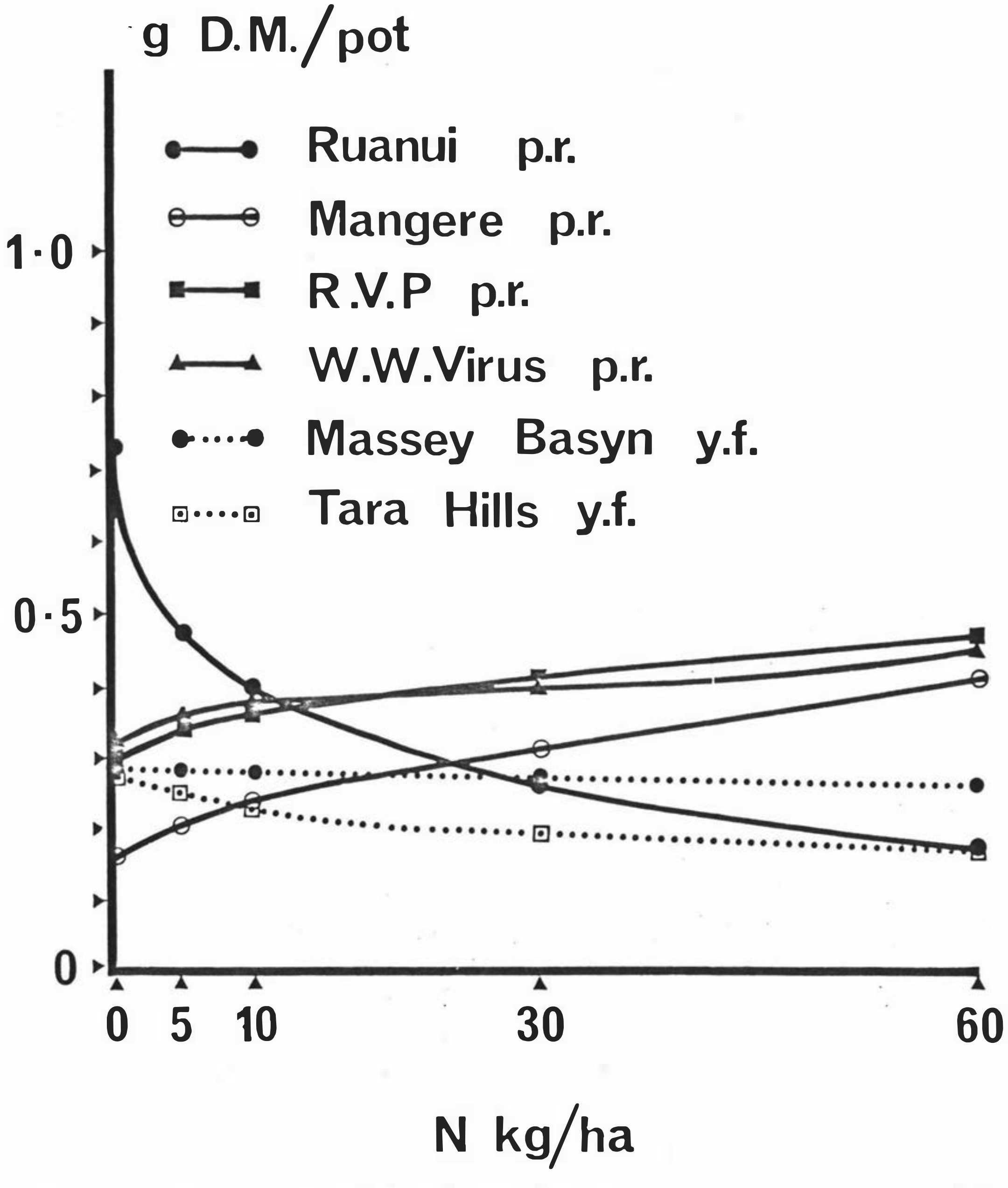

Fig. 9 Fitted curves for shoot dry matter yields of perennial ryegrass and Yorkshire fog under the Taieri climate, transformed from $l_n$ yields and $\sqrt{N}$ applied.

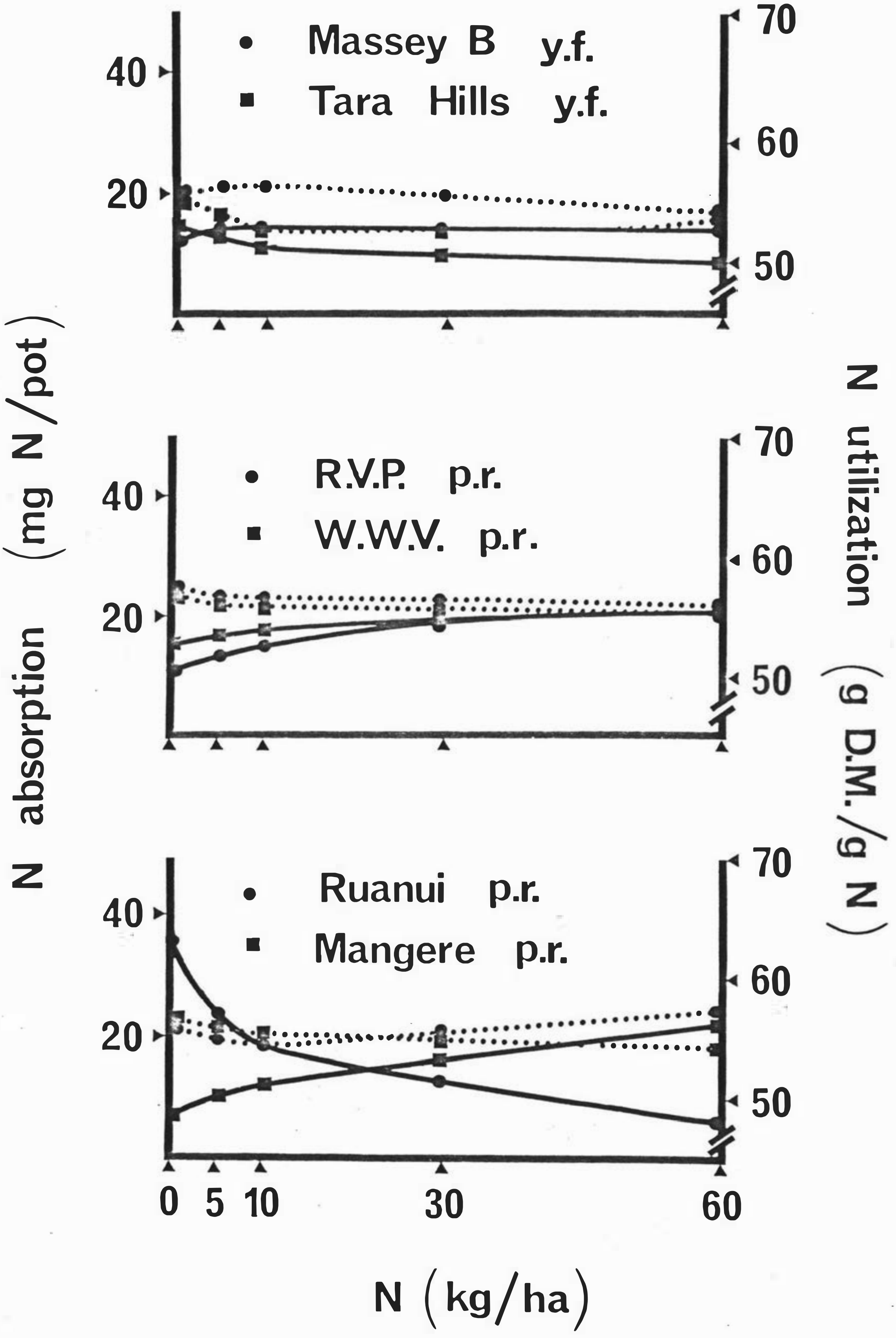

Fig. 10 Absorption ( —— ) and utilization ( ···· ) of N by strains of perennial ryegrass and Yorkshire fog in response to N under the Taieri climate using fitted values transformed from $l_n$ values.

Type 1 RNE (Table IX) and were capable of withstanding environmental stress and in that sense exhibited greater RNE than Ruanui, Tara Hills and Massey Basyn.

Apart from the identification of strains of Yorkshire fog and perennial ryegrass which differ in RNE, one important finding has arisen - that the RNE of species/strains in their response to N differ according to climate. Under conditions where adequate light and temperature prevailed their RNE did not differ. Where limitations were placed on light and/or temperature differences were apparent. These differences were associated with differences in shoot: root ratio and this in turn brought about differences in N absorption. Utilization of N also differed.

In a study of N responses by strains of rice, Osada and Murata (1962, 1965) and Osada (1964, 1966) showed that high N-response strains showed greater photosynthetic activity and lower respiratory activity than low N-response strains. Takahashi, Iwata and Baba (1959) showed that high N response strains accumulated more carbohydrate than low N-response strains. These findings applied to the present experiment explain results under the Palmerston North climate where the low N-response strains, Mangere and Tara Hills, had a greater shoot:root ratio than the remainding strains at lower levels of applied N, indicating that the products of photosynthesis were insufficient to meet the needs of the roots. This resulted in less root development and a smaller absorbing system. Hence absorption of N and yields suffered. The remaining strains on the other hand had a greater ability to photosynthesise under these conditions - resulting in a low shoot:root ratio. In other words their supply of C assimilates in the roots was greater and the energy available for N absorption and the development of a larger absorbing system was greater, resulting in higher yield increases.

Under the Taieri climate it is considered that much the same situation prevailed as under the Palmerston North climate except that an extra dimension to the situation may have been added in the cases of Ruanui and Tara Hills where it is suggested that protein synthesis was carried out at the expense of carbohydrate reserves and may have resulted in ammonium toxicity.

# CONCLUSIONS

This experiment has shown that species/strains of Yorkshire fog and perennial ryegrass differ in their RNE according to climate. Where conditions were favourable for most active growth differences in RNE were not recorded. Where conditions were sub-optimal, particularly temperature, one strain of Yorkshire fog (Tara Hills) and one strain of perennial ryegrass (Mangere) exhibited Type 1 RNE whereas the remainder exhibited Type 2 RNE. However those of Type 1 RNE were at no point better than those of Type 2 RNE and for this reason would have little to offer in the breeding of more efficient species/strains.

Under conditions of low temperature and light intensities Tara Hills Yorkshire fog and Ruanui perennial ryegrass provided declining yields and hence negative RNE. This effect is attributed to a low level of carbohydrate reserves resulting from low light intensities. Massey Basyn failed to respond to N under these conditions. Mangere, R.v.P. and W.W. Virus perennial ryegrass exhibited greater RNE than the two strains of Yorkshire fog included in this experiment.

# EXPERIMENT 6

## RELATIVE NUTRIENT EFFICIENCY OF WHITE CLOVER AND LOTUS IN RESPONSE TO APPLIED PHOSPHORUS

## INTRODUCTION

Experiments 3 and 4 examined the RNE of strains of Lotus and white clover in a heated glasshouse in which environmental conditions could not be clearly defined. Results of Experiment 5 showed that differences exist in RNE between grass species and strains according to climate. For this reason the main objective of this experiment was to study the effects of climate on RNE of selected strains of white clover and Lotus.

Since completion of this research programme Brock (1973) has published results of an experiment comparing strains of white clover and Lotus. He obtained a species X level of P X year interaction which he considered to be due to variations in environmental conditions - probably soil moisture. The strains Brock compared were not the same as those selected for study in this experiment. However they show, as was the case in the grass experiment, that climate exerts an effect on RNE.

Reasons for the selection of strains were discussed in reports of Experiments 3 and 4. Briefly, Huia white clover was selected because it gave Type 2 RNE response curve and because it was considered to be a useful strains to include because of its importance to New Zealand agriculture. Tamar white clover was included because it exhibited Type 1 RNE and because of the strong possibility that this may have been due to its greater ability to utilize absorbed P more efficiently - a finding worthy of further study. Louisiana white clover was included as another strain of Type 2 RNE and as one which gave exceptionally high utilization of absorbed P - a characteristic which could be of value in plant breeding.

Of the Lotus strains L. tetraploid was examined as it possessed the properties of Type 2 RNE and was higher yielding than other strains of its Type and had a greater ability to utilize absorbed P at low levels of applied P. L. hybrid was selected because it tended more towards Type 1 RNE and at low levels of applied P was less efficient in utilizing absorbed P.

## EXPERIMENTAL

The above named strains were compared under five levels of applied P in a four replicate randomized block layout under three distinct climates.

P was applied as sodium dihydrogen phosphate at 15, 30, 60, 100 and 200 kg/ha P (calculated on a superficial basis) and mixed with the soil. Details of basal dressings have been given in Section 3 and in addition a re-application of potassium sulphate was made on day 38.

Nine germinated seedlings were planted per pot on 11-6-73 and grown in a heated glasshouse (15-25°C) for 38 days before being transferred to climate rooms. Acetylene reduction assays were made on day 71, and harvesting took place on day 74. Roots were removed by washing and their dry weights determined. Herbage samples were analysed for N and P contents.

The three climates simulated were as follows:-

| Climate: | | Tara Hills<br>(summer) | Palmerston North<br>(spring) | Taieri<br>(winter) |
|---|---|---|---|---|
| Temperature (°C) | Day | 22.5 | 15 | 12 |
| | Night | 7.5 | 10 | 3 |
| Light ($Wm^{2}$) | | 200 | 200 | 100 |
| Day length (hours) | | 14 | 12 | 7 |
| Day/night changeover (hr) | | 1.5 | 1 | 2 |
| Vapour pressure deficit (mb) | | | | |
| | Day | 10 | 5 | 2 |
| | Night | 3 | 20 | 1 |
| $CO_2$ ambient | | | | |
| Soil moisture<br>(% field capacity) | | 50 cycling to 30 | 80 | 80 |

In the analysis of variance, degrees of freedom were apportioned among treatments and their linear, quadratic and residual components as shown in Appendix XVI. Other details of the statistical treatment of results were the same as those described for Experiment 5.

## RESULTS

### (i) Dry matter yields.

Results of shoot dry matter yields are presented in Table X . They show yields for all species/strains in the order Tara Hills>Palmerston North>Taieri.

Under the Tara Hills climate there were significant strain X linear and

Table X. Effect of P on shoot yields of dry matter of white clover and Lotus
($l_n$ g/pot)

| | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 100 | 200 |
| **Tara Hills** | | | | | |
| Huia | 1.556 | 1.561 | 1.677 | 1.583 | 1.983 |
| Tamar | 1.444 | 1.686 | 1.709 | 1.950 | 1.978 |
| Louisiana | 1.413 | 1.457 | 1.721 | 1.783 | 2.062 |
| L. tetraploid | 1.519 | 1.556 | 1.801 | 1.882 | 2.085 |
| L. hybrid | 1.588 | 1.598 | 1.763 | 1.864 | 2.005 |

L.S.D. 5% 0.152, 1% 0.201, C.V.% 6.23

Sig.int. Strain X P $p<0.05$, Strain X lin. $p<0.05$, Strain X quad. $p<0.05$

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| **Palmerston North** | | | | | |
| Huia | 1.105 | 1.279 | 1,504 | 1.604 | 1.803 |
| Tamar | 1.226 | 1.400 | 1.546 | 1.722 | 1.874 |
| Louisiana | 1.084 | 1.167 | 1.485 | 1.728 | 1.883 |
| L. tetraploid | 0.823 | 1.109 | 1.273 | 1.325 | 1.727 |
| L. hybrid | 0.864 | 1.289 | 1.266 | 1.419 | 1.664 |

L.S.D. 5% 0.191, 1% 0.254, C.V.% 9.67

Sig.int. Strain X P n.s., Strain X lin. n.s. Strain X quad. n.s.

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| **Taieri** | | | | | |
| Huia | 0.194 | 0.255 | 0.603 | 0.591 | 1.164 |
| Tamar | 0.422 | 0.528 | 0.746 | 0.957 | 1.302 |
| Louisiana | -0.001 | 0.126 | 0.395 | 0.825 | 1.189 |
| L. tetraploid | 0.063 | 0.207 | 0.361 | 0.612 | 0.936 |
| L. hybrid | -0.156 | -0.101 | 0.395 | 0.479 | 0.856 |

L.S.D. 5% 0.225, 1% 0.299, C.V.% 30.85

Sig.int. Strain X P n.s., Strain X lin. n.s., Strain X quad. n.s.

quadratic interactions on shoot yields, as shown for fitted values in Fig. 11. Relative yields at 100kg/ha P, taking 200kg/ha P as $X_{max}$, are given in Table XI. On the basis of these results Tamar is classified as Type 2 RNE as it reached 95% of its yield asymptote at 100kg/ha P and was superior to all others at some point in its response surface. In other words, at all but the lowest and highest levels of applied P it was the most efficient species/strain. The remaining species/strains did not approach their yield asymptotes at 100kg/ha P and are therefore classified as possessing Type 1 RNE. Of particular interest is the negative curve of Huia white clover. It was this result which was largely responsible for the significant interaction. Of all species/strains examined it was the furthest removed from its yield asymptote at 100kg/ha P. Further justification that the differences in yield were largely between Huia and Tamar is given by shoot and root yields; fitted values for which are shown in Fig. 12.

Under both the Palmerston North and Taieri climates no significant interactions were recorded in yields between species/strains, although by L.S.D. criteria the lower yields of Lotus strains at the 100 and 200kg/ha level of P were at times significant under the Palmerston North and Taieri climates.

(ii) Shoot:root ratio

Shoot:root ratios are shown in Table XII. Results show no interaction under the Tara Hills and Palmerston North climates so that it can be concluded that strains behaved in a similar manner. Under the Taieri climate there was a significant interaction which arose due to an increase in shoot:root ratio of Louisiana as the level of P was increased. The other strains did not alter in their shoot:root ratio as P was increased.

(iii) P absorption and utilization

P absorption data is presented in Table XIII and utilization data in Table XIV. Results show that there were significant species/strain X linear and quadratic interactions on both absorption and utilization under all climates. Fitted values for these are shown in Figs. 13-15.

Under the Tara Hills climate the interaction arose largely because of the negative P absorption curve for Huia compared to the positive curve for the remaining strains. Absorption of P by Huia did not increase until the 60kg/ha level of P whereas other species/strains responded at lower levels of P. At 100kg/ha P Huia had a lower absorption of P than other strains whereas at 200kg/ha P it had a greater absorption.

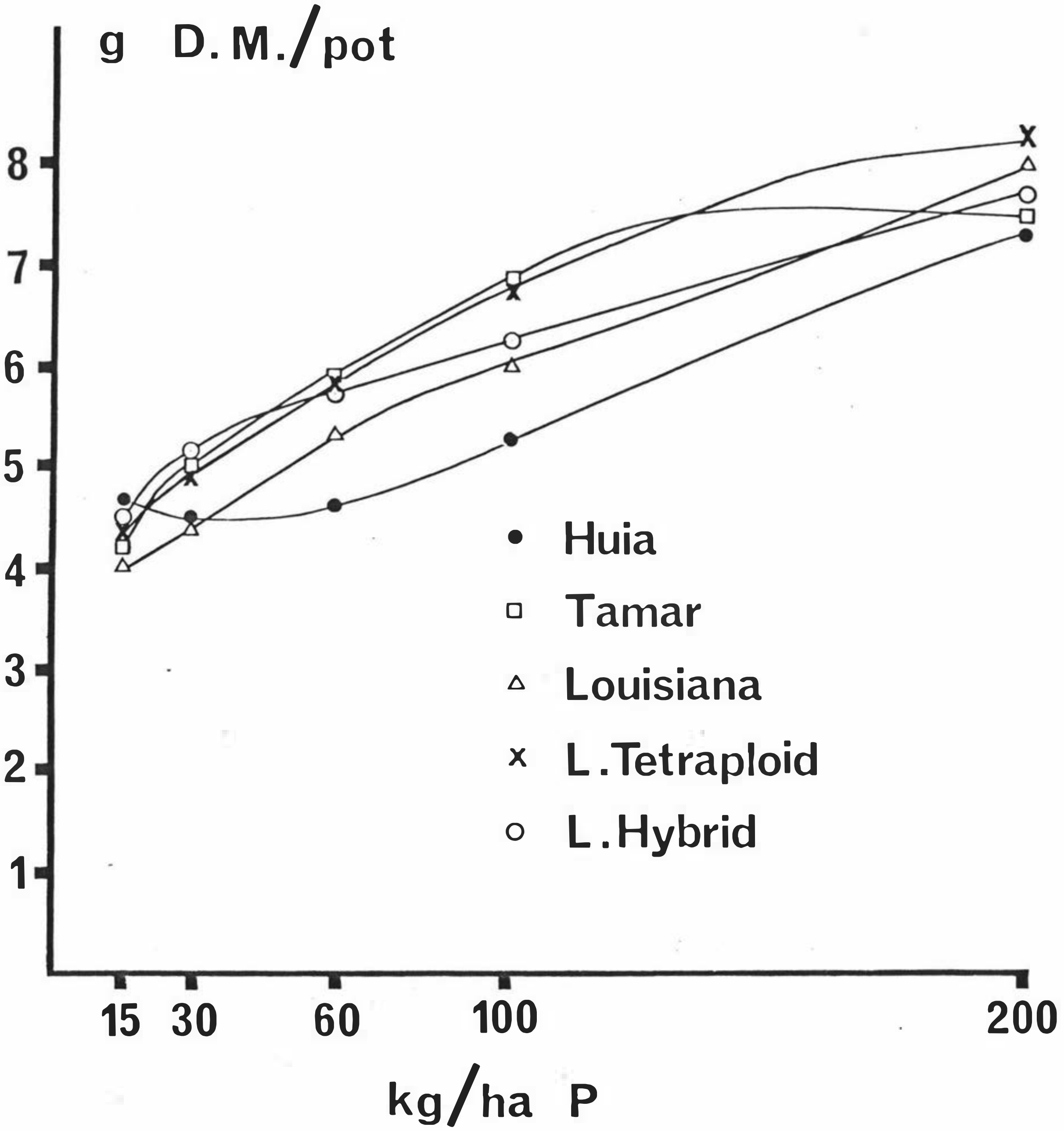

Fig. 11 Dry matter yields of shoots of white clover and Lotus strains in response to applied P under the Tara Hills climate.

Table XI. Relative yields of strains of white clover and Lotus. (100kg/ha P compared to 200kg/ha P = 100)

| | |
|---|---|
| Huia | 80.3 |
| Tamar | 98.0 |
| Louisiana | 82.8 |
| L. tetraploid | 83.3 |
| L. hybrid | 89.6 |

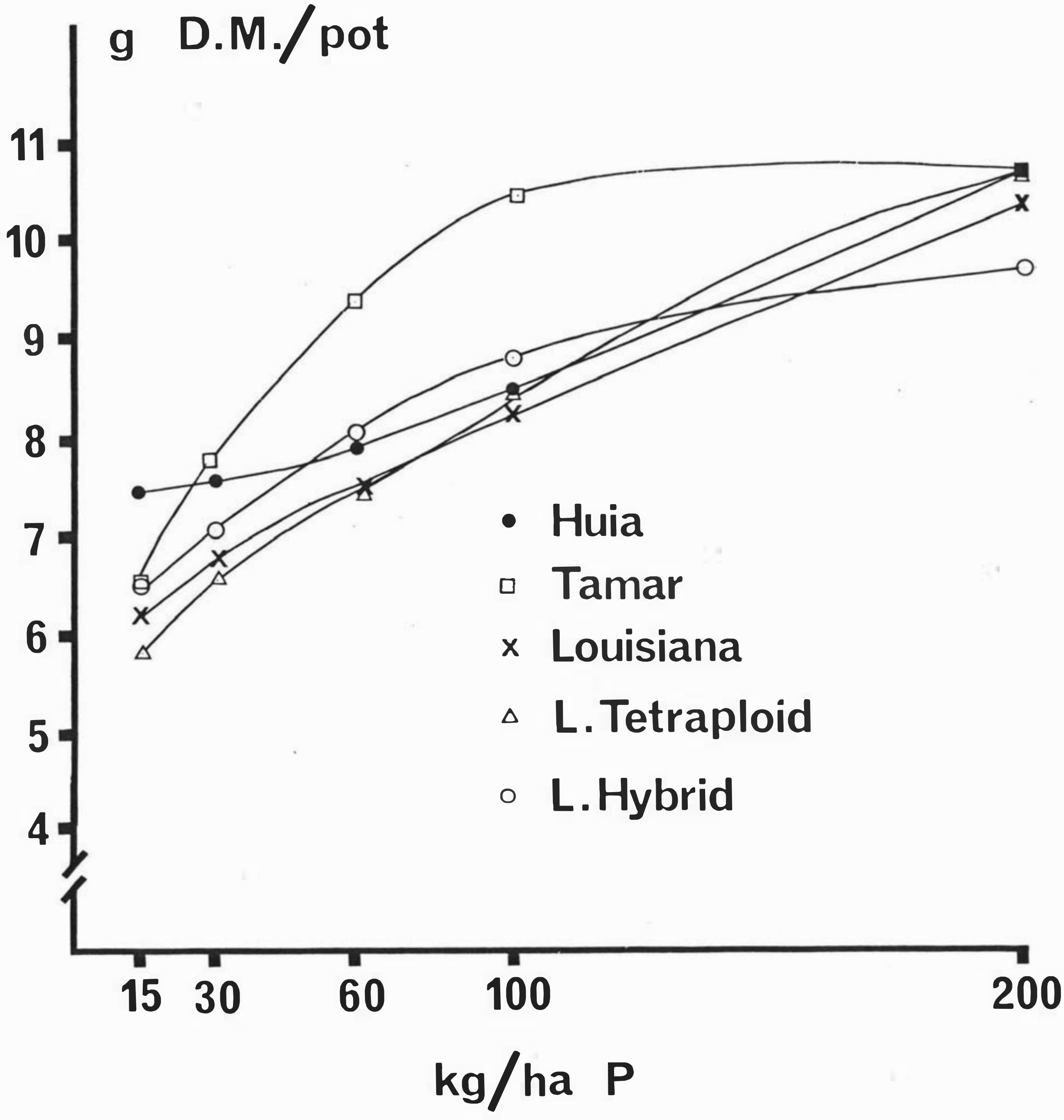

Fig. 12. Dry matter yields of shoots + roots of white clover and Lotus in response to applied P under the Tara Hills climate.

Table XII. Shoot:root ratios of strains of white clover and Lotus.

| | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 100 | 200 |
| **Tara Hills** | | | | | |
| Huia | 1.93 | 1.47 | 2.70 | 1.59 | 1.96 |
| Tamar | 1.82 | 1.97 | 1.84 | 1.99 | 1.99 |
| Louisiana | 2.06 | 2.38 | 2.35 | 2.37 | 3.00 |
| L. tetraploid | 2.65 | 2.56 | 3.06 | 3.32 | 3.98 |
| L. hybrid | 2.95 | 2.48 | 2.54 | 2.71 | 3.17 |
| **Palmerston North** | | | | | |
| Huia | 1.24 | 1.17 | 1.36 | 1.25 | 1.59 |
| Tamar | 1.45 | 1.47 | 1.61 | 1.70 | 1.75 |
| Louisiana | 1.51 | 1.54 | 1.83 | 2.10 | 2.26 |
| L. tetraploid | 1.95 | 1.83 | 2.14 | 1.67 | 2.21 |
| L. hybrid | 1.37 | 1.38 | 1.54 | 1.34 | 1.48 |
| **Taieri** | | | | | |
| Huia | 1.43 | 1.24 | 1.50 | 1.53 | 1.41 |
| Tamar | 1.72 | 1.42 | 1.46 | 1.49 | 1.69 |
| Louisiana | 1.29 | 1.52 | 1.88 | 1.70 | 1.92 |
| L. tetraploid | 1.79 | 2.84 | 2.03 | 1.98 | 2.03 |
| L. hybrid | 1.49 | 1.14 | 2.03 | 1.89 | 1.79 |

Tara Hills:
Strain X P interaction : n.s.
L.S.D. 5% 0.49, 1% 0.65, C.V.% 20.43

Palmerston North:
Strain X P interaction : n.s.
L.S.D. 5% 0.40, 1% 0.53, C.V.% 17.42

Taieri:
Strain X P interaction : $p<0.05$
L.S.D. 5% 0.39, 1% 0.52, C.V.% 23.35

Table XIII. Effect of P on P absorption in shoots of white clover and Lotus. (mg P/pot)

| | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 100 | 200 |
| <u>Tara Hills</u> | | | | | |
| Huia | 8.0 | 8.9 | 10.1 | 9.9 | 17.5 |
| Tamar | 6.4 | 8.9 | 9.6 | 13.0 | 14.3 |
| Louisiana | 6.1 | 6.7 | 9.4 | 10.6 | 15.7 |
| L. tetraploid | 7.0 | 7.8 | 9.8 | 16.7 | 15.1 |
| L. hybrid | 7.2 | 8.0 | 10.2 | 11.4 | 15.3 |

L.S.D. 5% 1.6, 1% 2.0, C.V.% 11.11

Sig.int. Strain X P $p<0.01$, Strain X lin. n.s., Strain X quad. $p<0.001$

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| <u>Palmerston North</u> | | | | | |
| Huia | 7.5 | 8.7 | 12.9 | 15.3 | 24.3 |
| Tamar | 7.4 | 8.6 | 10.7 | 16.4 | 26.7 |
| Louisiana | 7.9 | 8.3 | 11.5 | 16.6 | 25.3 |
| L. tetraploid | 6.1 | 8.2 | 10.4 | 11.3 | 19.5 |
| L. hybrid | 6.7 | 9.3 | 10.7 | 12.3 | 17.3 |

L.S.D. 5% 2.3, 1% 2.9, C.V.% 12.89

Sig.int. Strain P $p<0.001$, Strain X lin. $p<0.001$, Strain X quad. n.s.

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| <u>Taieri</u> | | | | | |
| Huia | 4.6 | 5.4 | 7.8 | 8.4 | 11.1 |
| Tamar | 5.3 | 6.1 | 7.7 | 9.5 | 14.6 |
| Louisiana | 3.5 | 4.4 | 5.9 | 8.4 | 12.6 |
| L. tetraploid | 3.6 | 4.3 | 5.1 | 6.3 | 9.9 |
| L. hybrid | 3.3 | 3.7 | 6.4 | 7.0 | 10.0 |

L.S.D. 5% 1.7, 1% 2.2, C.V.% 18.11

Sig.int. Strain X P n.s., Strain X lin. $p<0.05$, Strain X quad. n.s.

Table XIV. Effect of P on utilization of P by strains of white clover and Lotus.
(g D.M./g P absorbed)

| | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 100 | 200 |
| **Tara Hills** | | | | | |
| Huia | 598 | 534 | 526 | 507 | 413 |
| Tamar | 657 | 606 | 571 | 540 | 507 |
| Louisiana | 666 | 636 | 598 | 564 | 500 |
| L. tetraploid | 657 | 606 | 613 | 564 | 534 |
| L. hybrid | 680 | 617 | 571 | 564 | 483 |

L.S.D. 5% 33, 1% 44, C.V.% 5.89

Sig. int. Strain X P n.s., Strain X lin $p<0.01$, Strain X quad. n.s.

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| **Palmerston North** | | | | | |
| Huia | 400 | 413 | 348 | 325 | 250 |
| Tamar | 460 | 465 | 434 | 344 | 245 |
| Louisiana | 374 | 381 | 381 | 338 | 259 |
| L. tetraploid | 369 | 369 | 342 | 333 | 289 |
| L. hybrid | 350 | 389 | 331 | 336 | 305 |

L.S.D. 5% 38, 1% 50, C.V.% 7.35

Sig. int. Strain X P $p<0.001$, Strain X lin. $p<0.001$, Strain X quad $p<0.05$.

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| **Taieri** | | | | | |
| Huia | 259 | 239 | 232 | 212 | 240 |
| Tamar | 288 | 277 | 273 | 273 | 251 |
| Louisiana | 280 | 258 | 248 | 168 | 259 |
| L. tetraploid | 298 | 289 | 218 | 289 | 258 |
| L. hybrid | 258 | 256 | 234 | 234 | 235 |

L.S.D. 5% 24, 1% 32, C.V.% 6.81

Sig. int. Strain X P n.s., Strain X lin n.s., Strain X quad $p<0.05$.

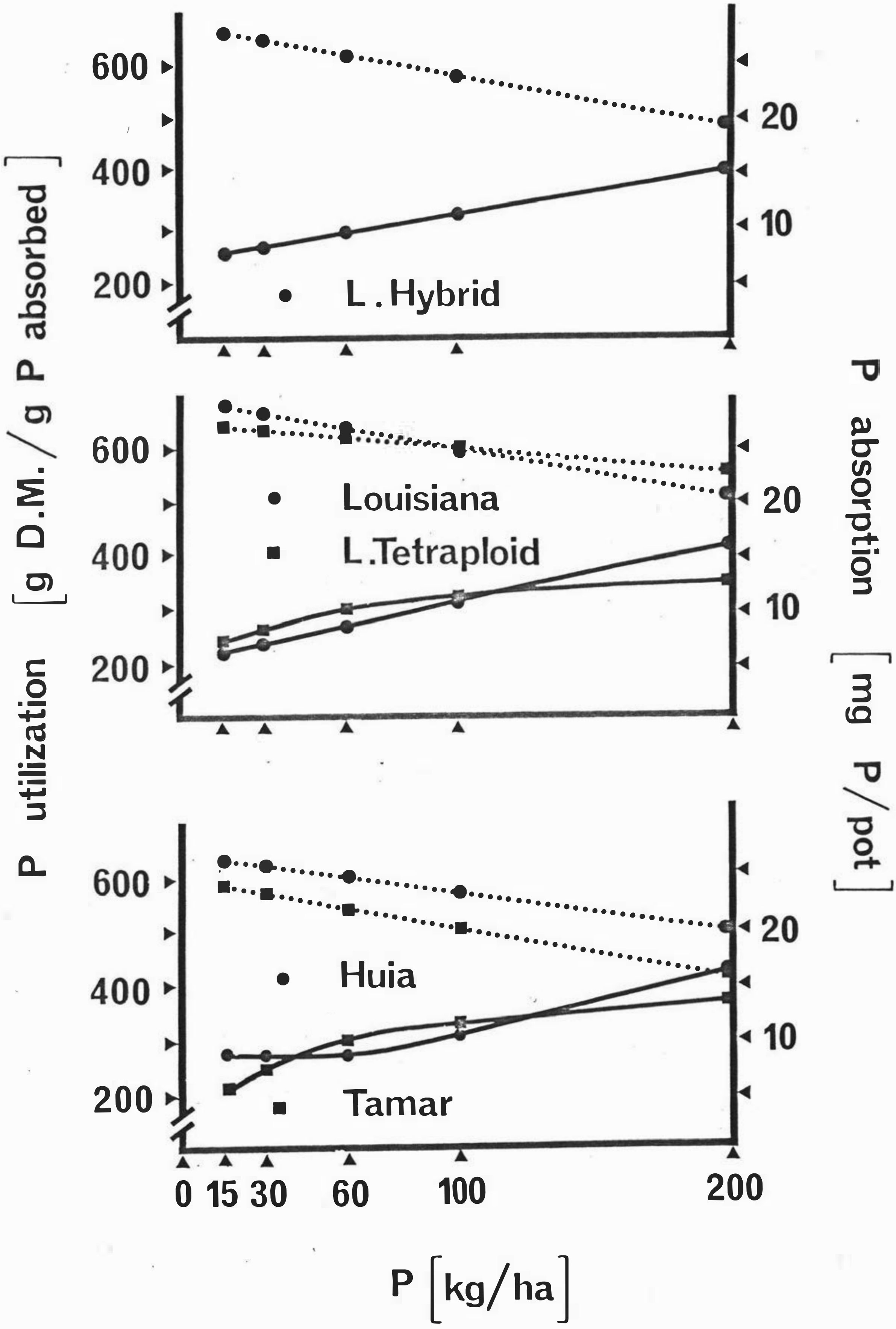

Fig. 13 P absorption ( —— ) and utilization (·····) by white clover and Lotus under the Tara Hills climate using fitted values.

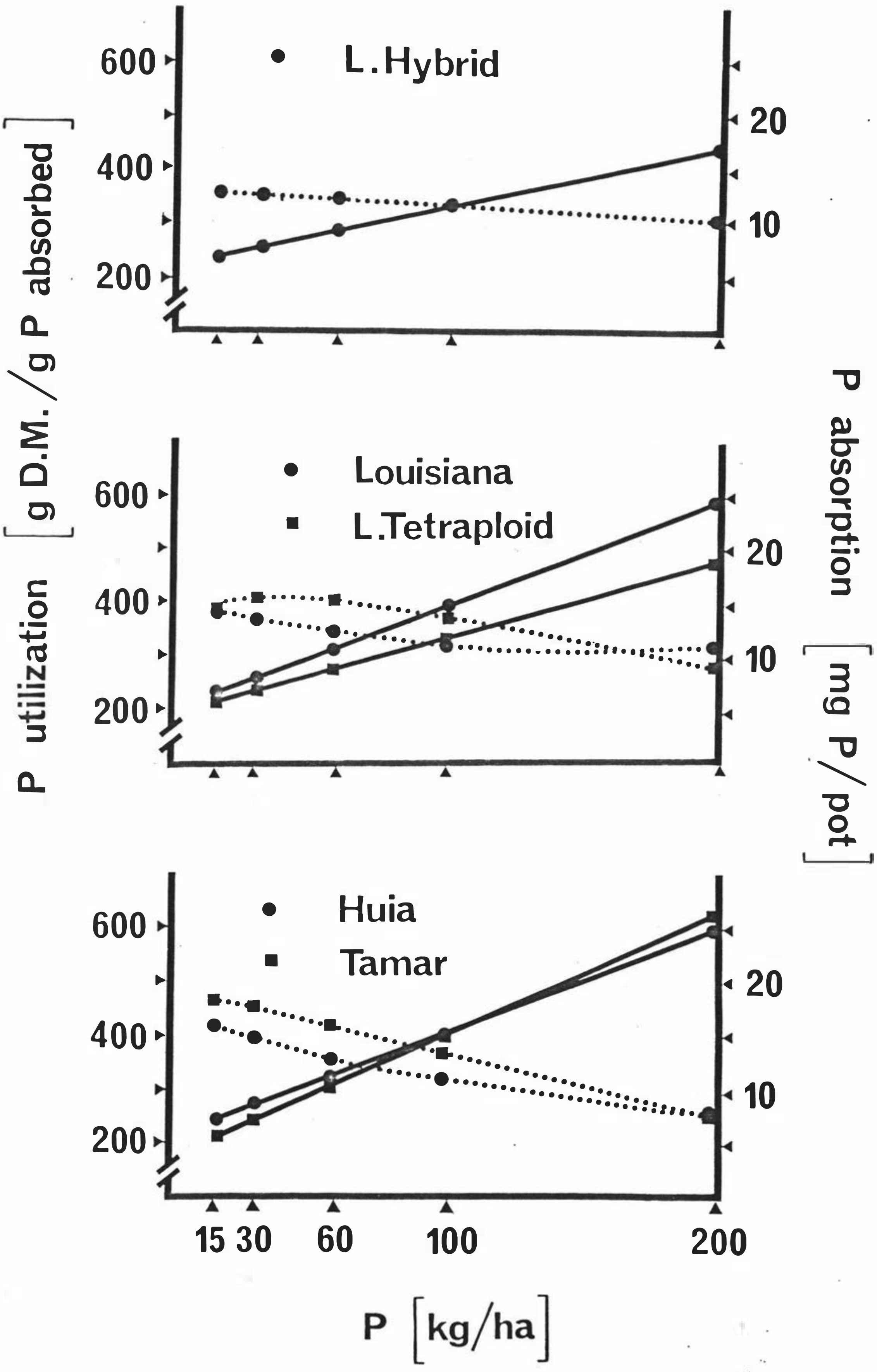

Fig. 14 P absorption ( —— ) and utilization (·····) by white clover and Lotus under the Palmerston North climate using fitted values.

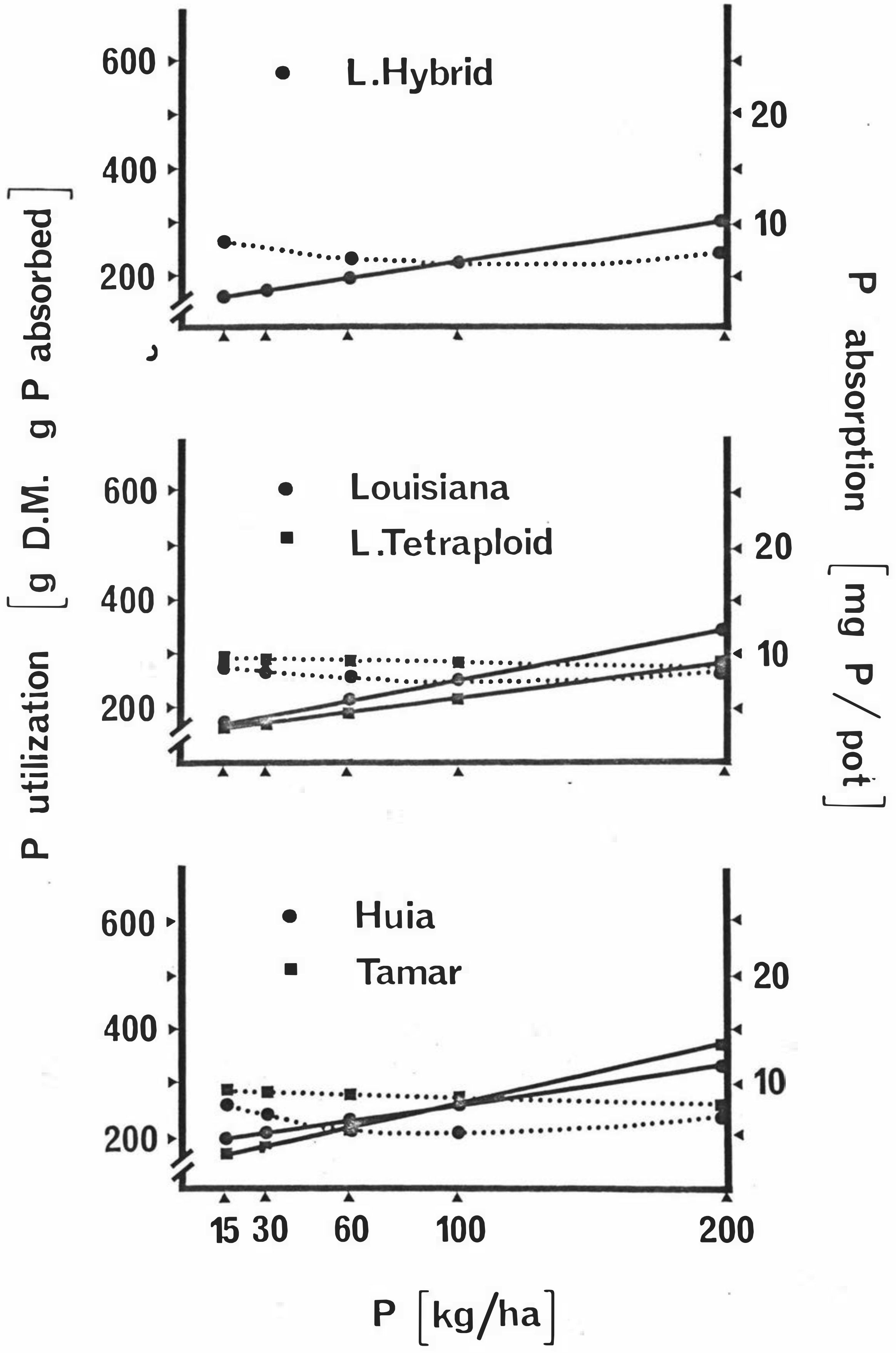

Fig. 15 P absorption ( ——— ) and utilization ( ···· ) by white clover and Lotus under the Taieri climate using fitted values.

Differences in P utilization were largely between Huia and the remaining species/strains, with Huia showing lower overall utilization and a greater decline in utilization as the level of P was increased. There was a trend towards a lesser utilization of P by Lotus than by white clover as the level of P was increased.

Under the Palmerston North climate the interaction showed low absorption of P by Lotus strains which increased little compared to white clover strains as the level of P was increased. Utilization of P by Lotus strains was less than that of white clover (with the exception of Louisiana) at low levels of applied P and decreased as the level of P was increased.

Between the two Lotus strains, L. tetraploid gave greater absorption than L. hybrid at 200 kg/ha P, but their utilization of P did not differ. Within strains of white clover no differences in P absorption were recorded. Utilization differed at the two lowest levels of P where Louisiana showed a lesser ability to absorb P than did Huia or Tamar.

Under the Taieri climate Lotus strains gave lower absorption of P than did white clover strains. The main cause of the interaction was the high absorption of P by Tamar white clover compared to other species/strains at high levels of applied P. Utilization of P gave a significant interaction due to low utilization by Huia at intermediate levels of P. There were no differences in utilization between species.

(iv) Nitrogen fixing activity.

Results of acetylene reduction assay as a measure of nitrogen fixing activity are presented in Table XV. They show a highly significant species/strain x linear and quadratic interaction under the Tara Hills climate. A line drawing of fitted values is presented in Fig. 16.

Differences in ethylene production were recorded bewteen species, where mean production was greater for white clover than for Lotus. The levels of ethylene production also increased at a greater rate for white clover as the level of P was increased.

Within white clover species ethylene production differed little between Huia and Tamar. Louisiana gave lower production than these two at low levels of P, but similar production at high levels of P.

Table XV. Effect of P on ethylene production of white clover and Lotus. ($l_n$ micromoles $C_2H_4$/pot)

| | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 100 | 200 |
| **Tara Hills** | | | | | |
| Huia | 5.410 | 5.622 | 5.869 | 5.712 | 6.564 |
| Tamar | 5.175 | 5.678 | 5.950 | 6.152 | 6.314 |
| Louisiana | 4.584 | 4.484 | 5.263 | 5.573 | 6.350 |
| L. tetraploid | 4.637 | 5.180 | 5.027 | 5.164 | 5.402 |
| L. hybrid | 3.683 | 3.882 | 4.413 | 4.580 | 5.366 |

L.S.D. 5% 0.574, 1% 0.763, C.V.% 7.71

Sig. int. Strain X P n.s., Strain X linear $p<0.001$, Strain X quad. $p<0.001$.

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| **Palmerston North** | | | | | |
| Huia | 5.205 | 5.636 | 6.283 | 6.439 | 6.740 |
| Tamar | 5.185 | 5.882 | 6.262 | 6.868 | 6.180 |
| Louisiana | 4.403 | 5.083 | 5.846 | 6.623 | 6.763 |
| L. tetraploid | 4.155 | 4.521 | 5.095 | 5.355 | 6.279 |
| L. hybrid | 3.665 | 4.846 | 5.162 | 5.191 | 5.841 |

L.S.D. 5% 0.539, 1% 0.716, C.V.% 6.83

Sig. int. Strain X P n.s., Strain X lin. n.s., Strain X quad. n.s.

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| **Taieri** | | | | | |
| Huia | 2.882 | 3.458 | 3.831 | 4.103 | 5.104 |
| Tamar | 3.511 | 3.962 | 4.354 | 4.656 | 5.286 |
| Louisiana | 3.115 | 3.027 | 3.343 | 4.438 | 5.134 |
| L. tetraploid | 2.948 | 3.076 | 4.006 | 3.836 | 4.974 |
| L. hybrid | 2.613 | 2.746 | 2.711 | 3.018 | 4.061 |

L.S.D. 5% 0.529, 1% 0.703, C.V.% 9.96

Sig. int. Strain X P n.s., Strain X lin. n.s., Strain X quad. n.s.

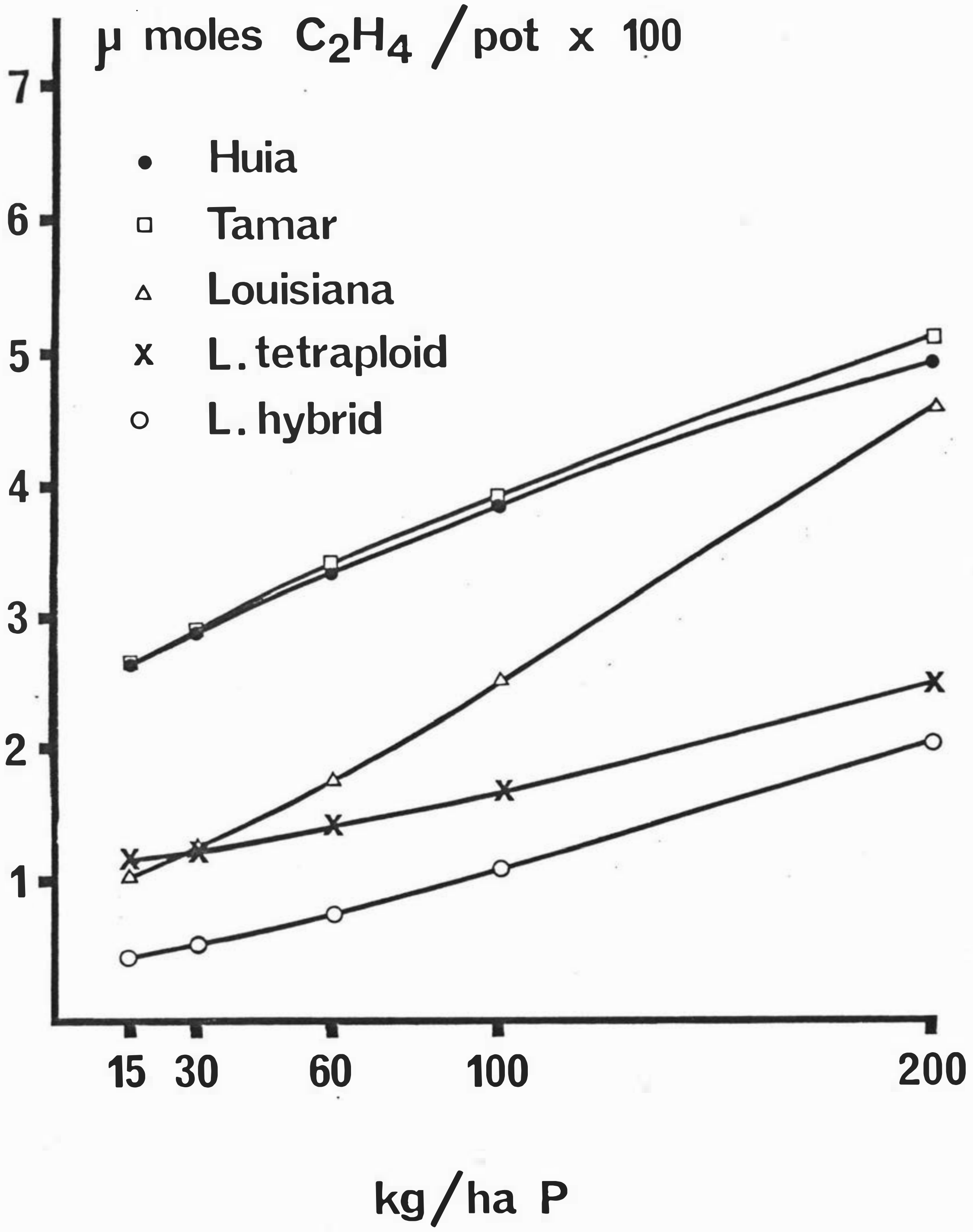

Fig. 16 Fitted values for ethylene production by white clover and Lotus in response to applied P under the Tara Hills climate.

(v) N absorption and utilization.

Results of N absorption and utilization measurements are given in Tables XVI and XVII respectively. They show highly significant interactions between species/strains and level of P under the Tara Hills and Palmerston North climates. These are shown as line drawings of fitted values in Figs. 17 and 18.

Under the Tara Hills climate the interaction occurred due to the strongly positive curvature of the P absorption curve of Tamar compared to Huia. Absorption of N by Lotus strains showed little increase with increasing P whereas absorption of N by white clovers increased markedly.

N utilization under the Tara Hills climate increased more by Lotus strains than by white clover strains, but did not differ within species according to level of P.

Under the Palmerston North climate mean N absorption was lower for Lotus strains than for white clover strains. Within species there were no differences. Utilization of N followed a pattern similar to that recorded under the Tara Hills climate.

Absorption of N under the Taieri climate did not differ between species/strains according to level of P. Utilization did not differ in any orderly manner - there was no linear or quadratic relationship.

## DISCUSSION

Before discussing results of this experiment mention needs to be made of the presence of root knot nematodes (Meloidogyne hapla) in all strains examined. No measurement was made of their relative degree of infestation. It is possible that strains may have been differentially infested and in view of the findings of Widdowson et al (1973) (that nematodes markedly increase the P requirements of white clover) it is possible that their presence may have affected relative strain performance. Accordingly these results need to be considered bearing this possibility in mind.

In considering results of this experiment the work of Brock (1973) and Scott et al (1974) is particularly relevant, as they also examined species X P interactions. In the experiment under report a significant interaction was recorded under the Tara Hills climate in which Huia exhibited Type 1 RNE and Tamar Type 2 RNE. However the differences under this climate did not reveal differences between Lotus and white clover strain although it is to be noted that under the Palmerston North and Taieri climates white clover gave yields which were higher than those from Lotus at high levels of P. This is in

Table XVI. Effect of P on N absorption in shoots of white clover and Lotus. (mg N/pot)

| | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 100 | 200 |
| **Tara Hills** | | | | | |
| Huia | 119 | 127 | 127 | 127 | 194 |
| Tamar | 105 | 126 | 150 | 175 | 174 |
| Louisiana | 111 | 104 | 122 | 137 | 185 |
| L.tetraploid | 107 | 106 | 121 | 131 | 148 |
| L.hybrid | 111 | 101 | 115 | 121 | 135 |

L.S.D. 5% 20, 1% 25, C.V.% 10.88

Sig. int. Strain X P $p<0.001$, Strain X lin. $p<0.001$, Strain X quad. $p<0.001$

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| **Palmerston North** | | | | | |
| Huia | 93 | 103 | 135 | 147 | 191 |
| Tamar | 99 | 110 | 127 | 167 | 207 |
| Louisiana | 110 | 101 | 123 | 160 | 207 |
| L.tetraploid | 75 | 87 | 98 | 93 | 139 |
| L.hybrid | 73 | 85 | 88 | 95 | 117 |

L.S.D. 5% 26, 1% 32, C.V.% 15.14

Sig. int. Strain X P $p<0.05$, Strain X lin. $p<0.001$, Strain X quad. n.s.

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| **Taieri** | | | | | |
| Huia | 61 | 68 | 86 | 83 | 90 |
| Tamar | 76 | 82 | 89 | 99 | 107 |
| Louisiana | 55 | 62 | 77 | 87 | 94 |
| L.tetraploid | 50 | 56 | 62 | 71 | 87 |
| L.hybrid | 43 | 47 | 75 | 79 | 93 |

L.S.D. 5% 17, 1% 22, C.V.% 16.27

Sig. int. Strain X P n.s., Strain X lin. n.s., Strain X quad. n.s.

Table XVII. Effect of P on utilization of N by strains of white clover and Lotus (g D.M./g N absorbed)

| | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 100 | 200 |
| <u>Tara Hills</u> | | | | | |
| Huia | 40.1 | 37.6 | 41.9 | 39.1 | 37.5 |
| Tamar | 40.4 | 42.7 | 37.5 | 40.1 | 41.6 |
| Louisiana | 40.4 | 41.0 | 46.5 | 43.7 | 43.9 |
| L.tetraploid | 42.9 | 44.4 | 49.8 | 50.4 | 54.5 |
| L.hybrid | 44.0 | 48.8 | 51.0 | 53.1 | 54.8 |

L.S.D. 5% 5.7, 1% 7.6, C.V.% 9.76

Sig.int. Strain X P n.s. Strain X lin. $p<0.05$, Strain X quad. n.s.

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| <u>Palmerston North</u> | | | | | |
| Huia | 32.1 | 34.9 | 33.1 | 33.9 | 32.1 |
| Tamar | 34.5 | 36.5 | 36.7 | 33.9 | 31.8 |
| Louisiana | 27.3 | 32.0 | 37.2 | 35.1 | 36.5 |
| L.tetraploid | 29.2 | 36.5 | 36.9 | 40.8 | 42.2 |
| L.hybrid | 32.2 | 42.7 | 40.1 | 43.4 | 45.2 |

L.S.D. 5% 5.8, 1% 7.7, C.V.% 8.93

Sig. int. Strain X P $p<0.01$, Strain X lin. $p<0.001$, Strain X quad. n.s.

| | 15 | 30 | 60 | 100 | 200 |
|---|---|---|---|---|---|
| <u>Taieri</u> | | | | | |
| Huia | 19.7 | 18.7 | 21.2 | 21.7 | 29.5 |
| Tamar | 19.9 | 20.6 | 22.6 | 26.2 | 34.2 |
| Louisiana | 17.9 | 18.3 | 19.2 | 26.2 | 34.5 |
| L.tetraploid | 21.1 | 22.2 | 23.4 | 25.6 | 29.5 |
| L.hybrid | 19.7 | 19.6 | 20.1 | 20.7 | 25.2 |

L.S.D. 5% 2.3, 1% 3.0, C.V.% 6.00

Sig.int. Strain X P $p<0.001$, Strain X lin. n.s., Strain X quad. n.s.

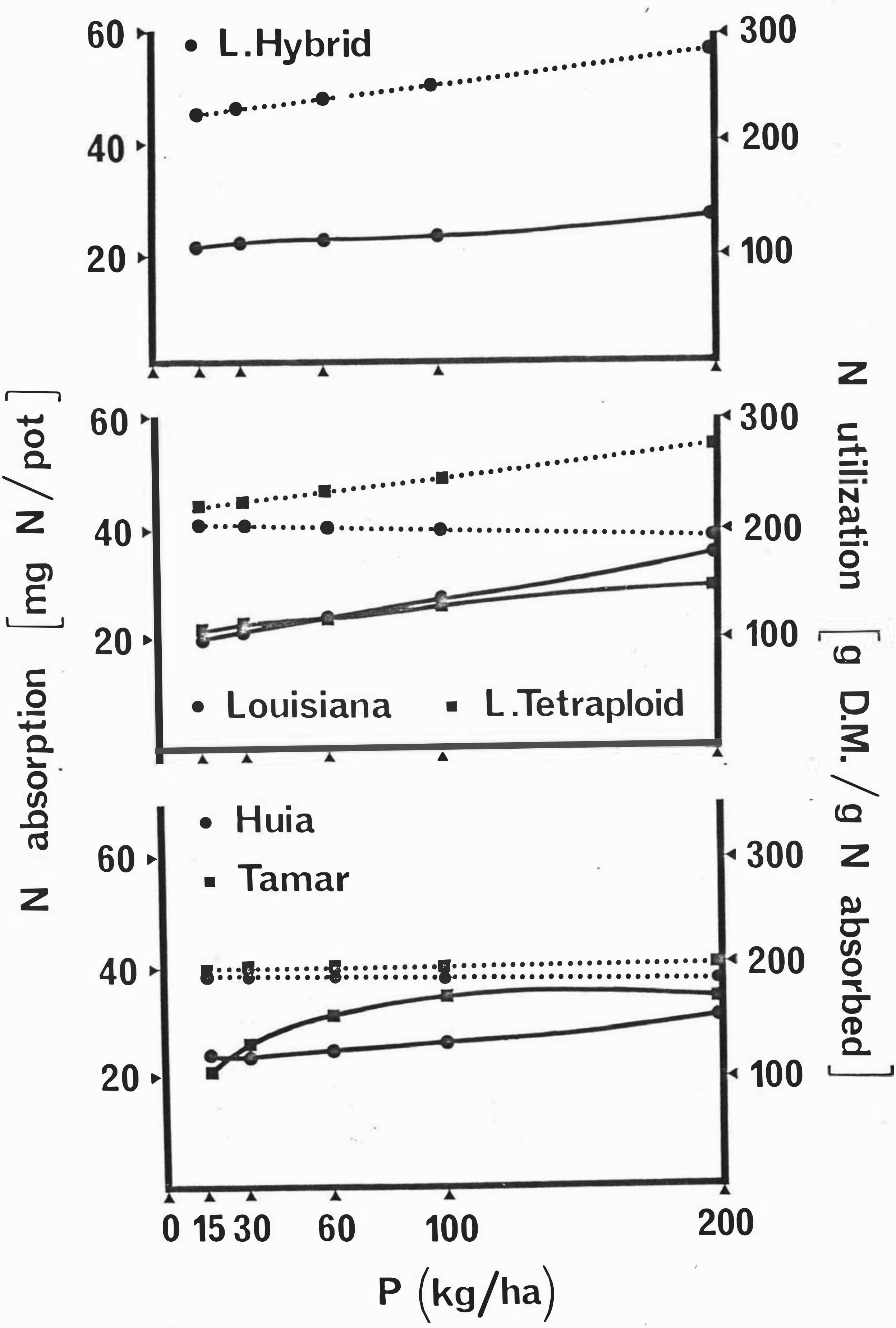

Fig. 18 N absorption ( ——— ) and utilization ( ····· ) by white clover and Lotus under the Tara Hills climate using fitted values.

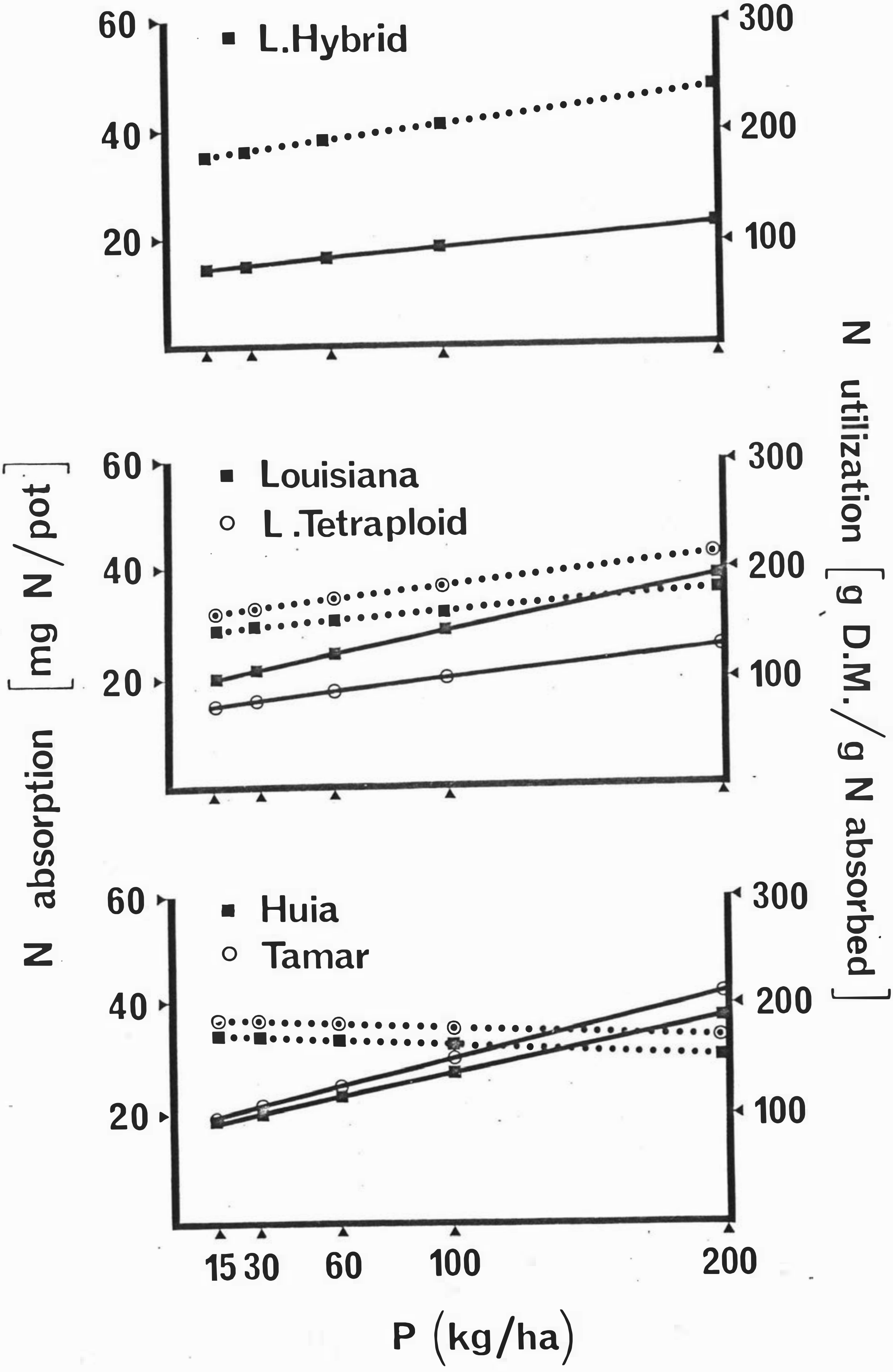

Fig. 18 N absorption ( —— ) and utilization ( ····· ) by white clover and Lotus under the Palmerston North climate using fitted values.

conformity with the findings of Brock. Scott et al on the other hand stated that 'white clover was more responsive to nil and moderate levels (production X 2), and white clover, alsike and Lotus more responsive between moderate and high levels (prodution X 1.6 - 1.9)'. The description does not enable clear assessment of what the authors actually found.

The greater RNE of white clover at high levels of P under the Palmerston North climate and Taieri climate is explained by P absorption in that absorption, like yields, was greater for white clover. Under the Palmerston North climate the low relative yields of Lotus can be attributed to lower P utilization. These findings are not completely in agreement with those of Brock as he found that Lotus had a lower P absorption than white clover at low but not high levels of P. He also found, using N:P ratios as a criterion, that P utilization was greater for Lotus than for white clover. However this is not considered to be a good criterion for assessing utilization where dry matter production is the end result being studied.

Nitrogen fixation and N absorption under the three climates agree reasonably well in that they were both lower for Lotus compared to white clover, and this became particularly apparent at high levels of applied P. A similar finding for N absorption was reported by Brock. Utilization of N tended to operate in the opposite direction to absorption in that Lotus showed a greater increase as the level of P was increased. However this effect was not sufficiently great to negate the effects of greater N fixation and absorption by white clover.

This study has shown that Lotus has a lower RNE than white clover at high levels of P, indicating that Lotus tends towards Type 2 RNE whereas white clover tends towards Type 1 RNE. Further, this difference was associated with differences in P absorption which in turn affected N fixation and absorption.

The discussion wil now be directed to the differences in yield responses between Huia and Tamar white clovers under the Tara Hills climate. Differences occurred at intermediate levels of P where Tamar showed greater RNE. This was not associated with differences in shoot:root ratios, which suggests that the size of the root absorbing systems and relative distribution of carbohydrates between shoot and root were similar for both.

Yield differences were attributable to differences in P absorption and uitlization. At intermediate levels of P Tamar absorbed more P - a finding compatable with yield data. Utilization of P declined more for Huia as the level of P was increased. Thus Tamar was more efficient as an absorber and user of P. Both these differences were reflected in N fixing activity and N absorption. Utilization of N also contributed to the yield differences as it declined more for Huia than for Tamar as the level of P was increased.

Thus it can be concluded that Tamar exhibited Type 2 RNE because of its greater ability to absorb and utilize P at intermediate levels of P and that this set in train the effects on N fixation and absorption and utilization. This finding for yields is in contrast to results of Experiment 4 where Huia was classified as Type 2 RNE and Tamar as Type 1. Precision in the present experiment was high, and much greater than in Experiment 4, and because of this results of the present experiment are accepted as representing the true behaviour of the strains.

Louisiana gave yield responses intermediate between those of Huia and Tamar. Because it gave positive curvature but was not superior to Tamar it is classified as belonging to Type 3 RNE. The relatively high utilization of P noted in Experiment 4 was clearly apparent in this experiment where precision was considerably greater. Its absorption of P did not differ from that of Tamar or Huia and in view of this it is of interest to note the low level of N fixing activity of Louisiana at low levels of P. This was not however borne out in N absorption data and because of this is considered to be of little significance.

No yield differences were obtained between the two strains of Lotus, or in absorption or utilization of P. N fixation at low levels of P was less for L.hybrid than for L. tetraploid under the Palmerston North and Taieri climates and there was a suggestion in the data that this was due to low absorption and utilization of N under the Taieri climate. These effects were not sufficiently great so as to be reflected in yields of shoots.

Clearly climate affected the RNE of species/strains in this experiment. It is interesting to note that the differences were found under the Tara Hills which differed from the Palmerston North climate in temperature. This is in contrast to results from the grass experiment where RNE differed between species/strains under the Palmerston North climate. This is attributed to temperature differences where conditions for growth of white clover and Lotus were near optimum under the Tara Hills climate and were near optimum for grasses under the Palmerston North climate (Mitchell, 1956). Thus it appears that differences in RNE of both grasses and legumes included in this study were associated with conditions permitting maximum photosynthetic activity. The explanation for the differences between the two white clovers (Huia and Tamar) is basically associated with the greater ability of Tamar to absorb and utilize P and that high temperatures permitted Tamar to better utilize this P for N fixation in view of the findings of Dart and Day (1971) that nitrogenase activity increases within the range of 10-35°C.

## CONCLUSIONS

This pot experiment compared three strains of white clover and two strains of *L. penduncula tus* under three climatic environments in the presence of five levels of applied P.

Results need to be interpreted with some reservations owing to the presence of root knot nematodes which may have differentially affected species/ strain performance. Results showed the following:-

1. The RNE of species/strains was dependent upon climate, where under the Tara Hills climate Huia showed Type 1 RNE and Tamar Type 2 RNE.
2. The greater RNE of Tamar at intermediate levels of applied P was associated with greater absorption and utilization of P and as a result in greater N fixation and absorption and utilization of N.
3. There was some evidence to suggest that Lotus strains were less responsive to high levels of P under the Palmerston North and Taieri climates and that this was associated with a lesser ability to absorb P and fix and absorb N.

# SECTION 6

## EXPERIMENT 7

## A STUDY OF THE SOURCE-SINK CONCEPT IN RELATION TO THE RELATIVE NUTRIENT EFFICIENCY OF HUIA AND TAMAR WHITE CLOVER

# INTRODUCTION

The objectives of this programme of research were to identify strains of pasture plants differing in RNE, to examine the effects of climate on RNE and to identify mechanisms causing high RNE whether it be of Type 1 or Type 2. As the programme developed research was concentrated on legume species.

In Experiment 6 two white clover strains (Tamar and Huia) were found to differ in RNE in a climate characterised by high temperatures and light intensities and high soil moisture stress. Yield patterns of Tamar were of Type 2 RNE while yield patterns of Huia were of Type 1 RNE. While yields from Huia were increasing at $X_{max}$ and those of Tamar were greater than 95 percent of their asymptote value at $X_{max}$ at no point in the response was Huia higher yielding than Tamar. There was good evidence to suggest that Tamar had higher RNE through its greater ability to absorb and utilize N and P more efficiently. It was also shown in Experiment 6 that the level of N fixation and P absorption into plant tissue was dependent upon the P nutrition of the plant. For these reasons it is considered that the basis for the higher RNE of Tamar must reside in its more efficient P nutrition.

An examination of the P nutrition of Tamar and Huia in Experiment 6 showed one outstanding relationship. This was the high relative yields and low relative concentration of P in Tamar. This suggests that Tamar either (1) re-utilized P to a greater extent by re-mobilization from older to younger tissue, or (2) required less P for photosynthesis and the production of new tissue.

The mobility of P within the plant from source to sink has already been reviewed. In line with this concept it is hypothesised that Tamar was meeting its requirements for P through a greater ability to continuously re-utilize absorbed P through re-mobilization from older tissue. This then was one mechanism selected for study in this experiment.

It was noted in Experiment 6 that Tamar showed a greater tendency than Huia to absorb P regardless of its lower P concentration. Assuming that this result can be confirmed, it raises the questions - (1) was its absorption greater because of greater root weights, length or number of apices? All of these paramaters affect the surface area of root available for P absorption, as discussed in the review of literature. Within the limits of experimental precision in Experiment 6 there was no indication that root weight was involved, suggesting (2) absorption of P per unit effective absorbing surface was greater for Tamar than for Huia. Both these possibilities were considered worthy of examination in this experiment.

The reason for the high RNE of Tamar in terms of environmental conditions under the one climate in Experiment 6 was not identified. It was clear that high temperature and/or high moisture stress was involved. One further objective of this experiment was therefore to distinguish between the effects of these two environmental variables on the RNE of the two strains.

In the discussion of Experiment 6 attention was drawn to the possibility that root knot nematode infestation may have influenced strain performance. To avoid the problem in this experiment the soil was sterilized with methyl bromide.

## EXPERIMENTAL

### A. Details

1. Strains: Huia (Grasslands No. C2548)
   Tamar ( " " C2419)

2. Environmental

| Treatments | | Day (°C) | Night (°C) | Wm$^{2}$ |
|---|---|---|---|---|
| High temp. high light | (HTHL) | 22.5 | 7.5 | 180 |
| " " low " | (HTLL) | 22.5 | 7.5 | 96 |
| Low " high " | (LTHL) | 15.0 | 7.5 | 170 |
| " " low " | (LTLL) | 15.0 | 7.5 | 98 |

Vapour pressure deficit: -8 mb (day), -3 mb (night)
Day length: 14 hours
$CO_2$: ambient

3. Phosphorus. Sodium dihydrogen phosphate at 15, 30, 60, 120 and 240kg/ha, where 15kg/ha = 0.137g/pot. The phosphate was mixed throughout the soil.

Basal nutrients. Potassium sulphate at 190kg/ha (0.531g/pot) and sodium molybdate (0.39img/pot) mixed throughout the soil.

4. Respiration: 'Pre-dark' - harvested after normal 10 hour dark.
   'Post dark' - harvested after 48 hours dark.

5. Moisture stress: 'Low' = 80 percent field capacity.
   'High' = 50 percent field capacity, cycling to 30 percent and applied only to the HTHL treatment and providing an additional 30 pots.

6. Replication: Between environments = 1
Within " = 3

7. Cultural: The soil was sterilized with methyl bromide to remove nematodes. Nine seedlings were sown per pot on 6-4-74 with additional pots sown to enable selection for uniformity. Seedlings were grown in a glasshouse and placed under controlled environmental conditions on days 29 to 32 in the order HTHL, HTLL, LTHL, LTLL, with post-dark treatments going in 24 hours after pre-dark treatments.

B. Harvesting

Pre-dark treatments harvested on day 75
Post-dark " " " " 77

Harvesting took place in the order in which treatments were placed under controlled conditions.

Stolons from all replicates were dissected into three 'positional' samples (proximal, middle and distal) as indicated in Fig. 19 and one replicate was subdivided further into 'structural' components within each position: nodes, internodes and laminae and petioles. Terminal stolon branches were sampled with 15-20 being sampled per pot.

C. Measurements.

1. 'Positional', 'structural', herbage residue and root dry weights.
2. N and P contents of above.
3. Nitrogen fixing activity by acetylene reduction assay.
4. Soluble sugar content of herbage and roots for each pot.
5. Root length, number of apices according to the method of Evans (1970).

## RESULTS

A. Factors affecting the entire plant.

1. Yield of dry matter. Shoot and root yields of strains at the different levels of P are shown in Table XVIII. While the interaction were not significant there were differences in the levels of P at which strains differed under the various environments. Under all except HTLL, Tamar gave higher shoot yields than Huia at 240kg/ha P. Under HTLL Tamar gave higher yields at 30 and 60kg/ha P. Root yields were greater for Huia only under HTHL - at 15, 30 and 120kg/ha.

High moisture stress failed to differentially affect strain yields and

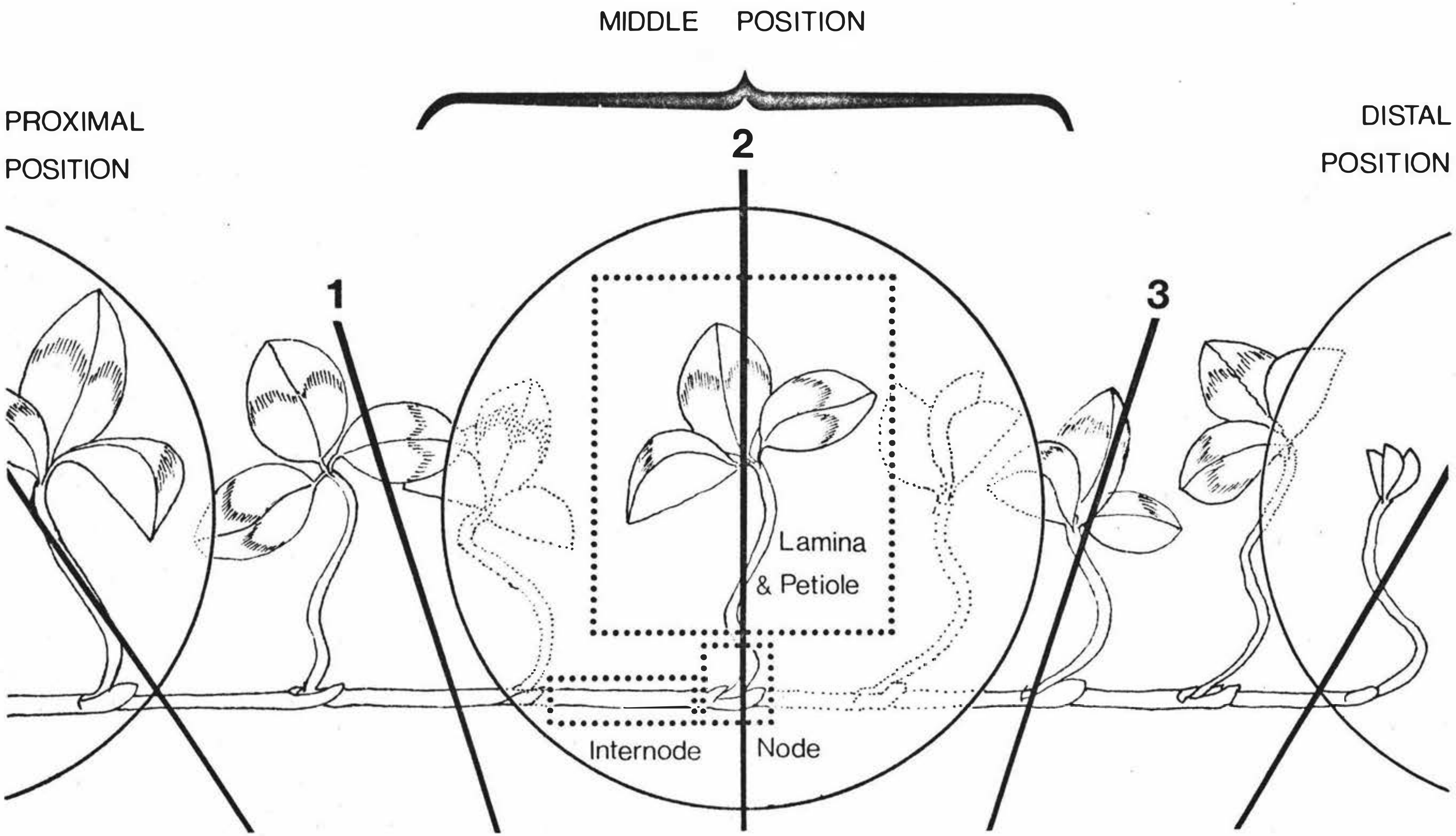

Fig. 19. Diagrammatic sketch of a stolon unit of white clover showing the method of sampling 'positions' and 'structures'.

Table XVIII. Effect of environment and the level of P on shoot and root yields of white clover (g/pot)

| Environment | Strain | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|---|
| | | 15 | 30 | 60 | 120 | 240 |
| Shoots | | | | | | |
| HTHL | Huia | 5.475 | 10.777 | 17.616 | 20.459 | 20.471 |
| | Tamar | 6.914 | 10.505 | 16.801 | 21.270 | 22.181 |
| HTLL | Huia | 5.654 | 8.888 | 15.844 | 18.393 | 20.185 |
| | Tamar | 5.660 | 11.245 | 17.515 | 19.507 | 21.221 |
| LTHL | Huia | 3.806 | 6.084 | 11.933 | 14.320 | 15.355 |
| | Tamar | 3.289 | 6.305 | 13.283 | 14.715 | 18.147 |
| LTLL | Huia | 2.341 | 5.495 | 9.732 | 12.540 | 13.049 |
| | Tamar | 3.337 | 5.529 | 11.099 | 13.257 | 15.377 |
| L.S.D. 5% 1.644, 1% 2.164, Sig. interaction p<0.19 | | | | | | |
| Roots | | | | | | |
| HTHL | Huia | 3.933 | 5.333 | 5.133 | 5.700 | 4.900 |
| | Tamar | 3.083 | 3.650 | 5.116 | 4.766 | 5.416 |
| HTLL | Huia | 2.133 | 3.133 | 4.150 | 3.666 | 3.633 |
| | Tamar | 2.100 | 3.116 | 4.583 | 3.666 | 4.433 |
| LTHL | Huia | 1.733 | 2.483 | 4.033 | 4.233 | 4.006 |
| | Tamar | 1.150 | 2.216 | 3.550 | 3.583 | 4.200 |
| LTLL | Huia | 1.166 | 1.900 | 2.866 | 3.233 | 3.234 |
| | Tamar | 1.216 | 1.616 | 2.250 | 3.000 | 2.983 |
| L.S.D. 5% 0.816. 1% 1.074, Sig. interaction p<0.661 | | | | | | |
| Mean strain yields | | | | | | |
| Shoots | Huia | 4.319 | 7.811 | 13.781 | 16.428 | 17.265 |
| | Tamar | 4.800 | 8.396 | 14.674 | 17.287 | 19.231 |
| L.S.D. 5% .822, 1% 1.082, Sig. interaction p<0.09, C.V.% 11.45 | | | | | | |
| Roots | Huia | 2.242 | 3.212 | 4.045 | 4.208 | 3.958 |
| | Tamar | 1.887 | 2.650 | 3.875 | 3.754 | 4.258 |
| L.S.D. 5% 0.408, 1% 0.537, Sig. interaction p<0.11, C.V.% 25.60 | | | | | | |

because of this absorption and utilization data will not be presented.

The shoot:root ratio of strains (Table XIX (a)) differed, with Tamar having a greater ratio at all levels of P than Huia. Shoot yields per unit root length (Table XIX (b)) were greater for Tamar at 30 and 240kg/ha P and shoot yields per root apex (Table XIX (c)) were greater for Tamar at 30kg/ha P and approaching significance at 240kg/ha P. Root lengths (Table XIX (d)) did not consistently differ between strains at the various levels of P.

2. Nitrogen fixing activity. The only treatment effect on nitrogen fixing activity was between strains, environments and dark treatements. In this interaction (Table XX) Huia showed lower activity than Tamar in all but the HTLL environment following a normal harvest (pre-dark). After a period of 48 hours dark the only significant interstrain difference occurred under HTLL, where Tamar had higher nitrogen fixing activity.

3. N absorption. Strains interacted with level of P (Table XXI), where Huia had a greater shoot content of N at 120kg/ha P and lower root content of N than Tamar at 240kg/ha P.

4. N utilization. Total dry matter produced per plant per unit N absorbed gave a highly significant interaction between strains and levels of P (Table XXII). At the three higher levels of P, Tamar exhibited greater efficiency of utilization of absorbed N than Huia. This interaction was not affected by environment or dark treatments.

5. P absorption. There was a highly significant interaction between strains and levels of P and dark treatment on root uptake which also approached significance for shoot and total uptake. Results (Table XXIII) show a markedly greater absorption of P by shoots of Huia at 240kg/ha P as a result of 48 hours dark. The same treatments lowered uptake in roots of Huia but increased it in roots of Tamar. Total uptake differed only at 120kg/ha P, where Tamar had a lower uptake in the pre-dark treatment but similar uptake to Huia in the post-dark treatment.

P uptake per unit root length, per root apex and per unit weight were calculated. Only the former showed any difference between strains (Table XXIV). This occurred in a strain X P X dark interaction in which differences of greatest relevance were the greater uptakes of P per unit root length by Tamar

Table XIX. Effect of level of P on shoot:root ratio, shoot yield/unit root length and per root apex and on root length.

| | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 120 | 240 |
| (a) Shoot:root. | | | | | |
| Huia | 2.22 | 2.63 | 3.49 | 4.12 | 4.45 |
| Tamar | 2.81 | 3.29 | 4.15 | 4.77 | 4.97 |
| L.S.D. 5% .056, 1% .073, Sig. interaction $p<0.99$, C.V.% 26.84 | | | | | |
| (b) Shoot yield/unit root length (g/m X 100/pot) | | | | | |
| Huia | .352 | .360 | .654 | .793 | .702 |
| Tamar | .377 | .543 | .693 | .741 | .885 |
| L.S.D. 5% .141, 1% .182, Sig. interaction $p<0.05$, C.V.% 40.56 | | | | | |
| (c) Shoot yield/root apex (g/apex X 10) | | | | | |
| Huia | .139 | .123 | .187 | .238 | .231 |
| Tamar | .129 | .179 | .237 | .218 | .281 |
| L.S.D. 5% .052, 1% .068, Sig. interaction $p<0.01$, C.V.% 46.48 | | | | | |
| (d) Root length (m X 10/pot) | | | | | |
| Huia | 151 | 254 | 240 | 244 | 285 |
| Tamar | 147 | 170 | 260 | 262 | 266 |
| L.S.D. 5% 55, 1% 73, Sig. interaction $p<0.91$, C.V.% 44.74 | | | | | |

Table XX. Interaction of environment, strains and dark treatment on ethylene production by white clover. (micromoles/pot).

| Environment | Strain | Pre-dark | Post-dark |
|---|---|---|---|
| HTHL | Huia | 264 | 196 |
| | Tamar | 316 | 235 |
| HTLL | Huia | 425 | 362 |
| | Tamar | 428 | 423 |
| LTHL | Huia | 256 | 269 |
| | Tamar | 271 | 257 |
| LTLL | Huia | 129 | 150 |
| | Tamar | 170 | 154 |

L.S.D. 5% 50
1% 65

Sig. interaction $p<0.04$

C.V.% 26.40

Table XXI. Interaction of strain x P on the N content of shoots, roots and shoot + roots of white clover (mg/pot)

| Strain | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 120 | 240 |
| Shoots | | | | | |
| Huia | 143 | 228 | 427 | 576 | 620 |
| Tamar | 162 | 234 | 425 | 537 | 646 |

L.S.D. 5% 31, 1% 41, Sig. interaction $p<0.05$, C.V.% 14.13

| Strain | 15 | 30 | 60 | 120 | 240 |
|---|---|---|---|---|---|
| Roots | | | | | |
| Huia | 51 | 73 | 99 | 104 | 105 |
| Tamar | 43 | 65 | 90 | 99 | 121 |

L.S.D. 5% 16, 1% 20, Sig. interaction $p<0.01$, C.V.% 24.16

| Strain | 15 | 30 | 60 | 120 | 240 |
|---|---|---|---|---|---|
| Shoots + roots | | | | | |
| Huia | 194 | 301 | 526 | 680 | 725 |
| Tamar | 205 | 299 | 515 | 636 | 767 |

L.S.D. 5% 35, 1% 46, Sig. interaction $p< 0.05$, C.V.% 12.37

Table XXII. Interaction of strain X level of P on dry matter produced per unit N absorbed. (g/g)

| | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 120 | 240 |
| Huia | 32.47 | 35.88 | 33.89 | 30.27 | 29.15 |
| Tamar | 31.50 | 36.25 | 36.07 | 33.03 | 30.61 |

L.S.D. 5% 1.44
1% 1.90
Sig. interaction $p < 0.01$
C.V.% 7.77

Table XXIII. Interaction of strain X level of P X dark on P uptake by white clover (mg/pot)

| Strain | Dark | Level of P (kg/ha) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 15 | 30 | 60 | 120 | 240 | Mean |
| Shoots | | | | | | | |
| Huia | Pre- | 9.12 | 15.71 | 27.77 | 55.00 | 83.82 | 38.29 |
| | Post | 9.03 | 16.46 | 34.00 | 55.54 | 94.68 | 41.94 |
| | Mean | 9.07 | 16.08 | 30.89 | 55.27 | 89.25 | 40.11 |
| Tamar | Pre- | 8.41 | 15.18 | 29.60 | 46.84 | 84.90 | 36.99 |
| | Post | 9.38 | 16.30 | 30.20 | 52.87 | 90.73 | 39.90 |
| | Mean | 8.90 | 15.74 | 29.90 | 49.87 | 87.82 | 38.44 |

L.S.D. Strain : 5% 1.38, 1% 1.82, Sig.int. $p<0.19$
Strain X dark: 5% 1.96, 1% 2.58, Sig.int. $p<0.59$
Strain X P : 5% 3.10, 1% 4.08, Sig.int. $p<0.12$
Strain X P X dark: 5% 4.39, 1% 5.78, Sig. int. $p<0.08$, C.V.% 14.58

| Strain | Dark | 15 | 30 | 60 | 120 | 240 | Mean |
|---|---|---|---|---|---|---|---|
| Roots | | | | | | | |
| Huia | Pre- | 4.70 | 5.79 | 9.44 | 13.41 | 23.21 | 11.31 |
| | Post | 4.47 | 7.49 | 8.98 | 12.21 | 18.65 | 10.65 |
| | Mean | 4.59 | 6.65 | 9.21 | 12.81 | 20.93 | 10.84 |
| Tamar | Pre- | 4.24 | 6.08 | 8.00 | 10.52 | 21.69 | 10.11 |
| | Post | 3.16 | 6.01 | 9.53 | 13.88 | 24.63 | 11.45 |
| | Mean | 3.70 | 6.05 | 8.76 | 12.20 | 23.16 | 10.77 |

L.S.D. Strain : 5% 0.71, 1% 0.94, Sig.int. $p<0.86$
Strain X dark: 5% 1.01, 1% 1.33, Sig.int. $p<0.02$
Strain X P : 5% 1.59, 1% 2.10, Sig.int. $p<0.60$
Strain X P X dark: 5% 2.26, 1% 2.97, Sig. int. $p<0.001$, C.V.% 26.07

| Strain | Dark | 15 | 30 | 60 | 120 | 240 | Mean |
|---|---|---|---|---|---|---|---|
| Shoots + roots | | | | | | | |
| Huia | Pre- | 13.83 | 21.50 | 37.23 | 68.41 | 107.03 | 49.60 |
| | Post | 13.50 | 23.95 | 42.98 | 67.76 | 113.34 | 52.31 |
| | Mean | 13.67 | 22.73 | 40.11 | 68.08 | 110.19 | 50.95 |
| Tamar | Pre- | 12.65 | 21.26 | 37.60 | 57.37 | 106.60 | 47.10 |
| | Post | 12.55 | 22.32 | 39.73 | 66.75 | 115.37 | 51.34 |
| | Mean | 12.60 | 21.79 | 38.67 | 62.06 | 110.99 | 49.22 |

L.S.D. Strain : 5% 1.53, 1% 2.02, Sig.int. $p<0.02$
Strain X dark: 5% 2.17, 1% 2.85, Sig.int. $p<0.32$
Strain X P : 5% 3.43, 1% 4.51, Sig.int. $p<0.08$
Strain X P X dark: 5% 4.85, 1% 6.38, Sig.int. $p<0.06$, C.V.% 13.11

Table XXIV. Interaction of strain, level of P and dark treatment on total P uptake per unit root length of white clover (mg P/m X 10/pot)

| Strain | Dark | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|---|
| | | 15 | 30 | 60 | 120 | 240 |
| Huia | Pre- | .111 | .119 | .178 | .342 | .411 |
| | Post- | .110 | .096 | .212 | .322 | .507 |
| | Mean | .110 | .107 | .195 | .332 | .459 |
| Tamar | Pre- | .095 | .135 | .191 | .262 | .562 |
| | Post- | .110 | .159 | .168 | .283 | .475 |
| | Mean | .102 | .147 | .180 | .272 | .519 |

L.S.D. Strain X P: 5% 0.056, 1% .077, Sig.int. $p<0.05$

Strain X P X dark: 5% .083, 1% .109, Sig.int. $p<0.05$

C.V.% 42.99

in the pre-dark treatment (i.e. the normal harvest).

6. P utilization. Main effects on P utilization (Table XXV) showed greater ($p<0.01$) utilization by Tamar than by Huia (408 and 378g dry matter per g P absorbed respectively). No significant interactions involving strains with other treatments were recorded.

7. Soluble sugar content. There were highly significant interactions between strains and levels of P on shoot, root and total soluble sugar contents, and interactions of these with the dark treatment. They are shown in Table XXVI, Dealing firstly with the strain X level of P interaction, results show a greater increase in soluble sugar contents in Tamar than in Huia as the level of P increased. The interaction with dark treatments showed a decline in soluble sugar contents of shoots and shoots and roots in Huia following 48 hours dark whereas that of Tamar increased as the level of applied P was increased.

Environment also affected soluble sugar contents of roots in an interaction involving strains and levels of P (Table XXVII). Results show that the interaction occurred because of the higher yields of soluble sugars by Huia compared to Tamar under HT treatments and the lower yields of soluble sugars under LT treatments.

B. Factors within the stolon.

(i) Dry matter distribution.

These results need to be interpreted with care as it was observed that Huia had a greater number of stolon branches than Tamar. As Huia gave lower yields than Tamar at higher levels of P this means that the weight of the stolon unit sampled is likely to have been lower for Huia. Thus proportionate changes in distribution of dry matter within the stolon will be considered.

Significant interactions were recorded between strains and levels of P on dry matter distribution in positions (Table XXVIII (a) and structures (Table XXVIII(b). Tamar contained a greater proportion in its laminae and petioles and nodes than Huia. These differences became greater as the level of P was increased.

A highly significant interaction between strains and dark treatment in the distribution of dry matter in structures (Table XXIX) showed that 48 hours dark brought about little proportionate change in Tamar but a decrease in the proportion of dry matter in the laminae and petioles of Huia.

Table XXV. Effect of level of P on P utilization by strains of white clover (g DM/g P absorbed)

| | Level of P | | | | | |
|---|---|---|---|---|---|---|
| | 15 | 30 | 60 | 120 | 240 | Mean |
| Huia | 476 | 476 | 444 | 305 | 192 | 378 |
| Tamar | 515 | 500 | 477 | 340 | 211 | 409 |

Strain effect: L.S.D. 5% 13, 1% 17, Sig. interaction $p<0.001$

Strain X level of P: L.S.D. 5% 29, 1% 38, Sig. interaction $p<0.87$

C.V.% 13.07

Table XXVI. Interaction of strain X P X dark treatments on yields of soluble sugars in white clover (mg/pot)

| Strain | Dark | Levels of P (kg/ha) | | | | |
|---|---|---|---|---|---|---|
| | | 15 | 30 | 60 | 120 | 240 |
| Shoots | | | | | | |
| Huia | Pre- | 294 | 477 | 894 | 1 241 | 1 347 |
| | Post | 179 | 424 | 828 | 895 | 959 |
| | Mean | 236 | 451 | 861 | 1 068 | 1 153 |
| Tamar | Pre- | 325 | 658 | 1 316 | 1 436 | 1 629 |
| | Post | 220 | 437 | 981 | 1 226 | 1 315 |
| | Mean | 273 | 547 | 1 149 | 1 331 | 1 472 |

L.S.D. Strain X P: 5% 110, 1% 145, Sig.int. $p<0.001$

Strain X P X dark: 5% 156, 1% 206, Sig.int. $p<0.064$

| | | | | | | |
|---|---|---|---|---|---|---|
| Roots | | | | | | |
| Huia | Pre- | 29 | 41 | 88 | 103 | 82 |
| | Post | 31 | 53 | 80 | 88 | 75 |
| | Mean | 30 | 47 | 84 | 95 | 78 |
| Tamar | Pre- | 27 | 42 | 68 | 83 | 98 |
| | Post | 19 | 32 | 65 | 102 | 127 |
| | Mean | 23 | 37 | 67 | 92 | 113 |

L.S.D. Strain X P: 5% 14, 1% 17, Sig.int. $p<0.001$

Strain X P X dark: 5% 19, 1% 25, Sig.int. $p<0.01$

| | | | | | | |
|---|---|---|---|---|---|---|
| Shoots + roots | | | | | | |
| Huia | Pre- | 323 | 518 | 982 | 1 344 | 1 429 |
| | Post | 211 | 478 | 909 | 983 | 1 034 |
| | Mean | 257 | 498 | 945 | 1 164 | 1 232 |
| Tamar | Pre- | 352 | 700 | 1 358 | 1 518 | 1 727 |
| | Post | 240 | 470 | 1 047 | 1 328 | 1 442 |
| | Mean | 296 | 585 | 1 216 | 1 424 | 1 585 |

L.S.D. Strain X P: 5% 114, 1% 150, Sig.int. $p<0.001$

Strain X P X dark: 5% 161, 1% 212, Sig.int. $p<0.04$,

C.V.% 21.86

Table XXVII. Interaction of environment x strain x P on yields of soluble sugars in roots of white clover (mg/pot)

| Environment | Strain | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|---|
| | | 15 | 30 | 60 | 120 | 240 |
| HTHL | Huia | 52 | 76 | 117 | 103 | 92 |
| | Tamar | 49 | 65 | 103 | 136 | 189 |
| HTLL | Huia | 16 | 37 | 52 | 44 | 46 |
| | Tamar | 17 | 33 | 62 | 83 | 81 |
| LTHL | Huia | 38 | 45 | 108 | 139 | 113 |
| | Tamar | 16 | 36 | 69 | 96 | 133 |
| LTLL | Huia | 16 | 33 | 59 | 85 | 62 |
| | Tamar | 11 | 15 | 34 | 56 | 48 |

L.S.D. 5% 27
1% 36
Sig. interaction $p<0.001$
C.V.% 35.98

(page 133 follows)

Table XXVIII. Effect of level of P on the distribution of dry matter within positions and structures of stolons. (g/pot)

(a)

| Strain | Level of P | | Position | |
|---|---|---|---|---|
| | | Proximal | Middle | Distal |
| Huia | 15 | .155 | .162 | .122 |
| | 30 | .218 | .287 | .182 |
| | 60 | .381 | .369 | .236 |
| | 120 | .408 | .411 | .312 |
| | 240 | .441 | .466 | .325 |
| Tamar | 15 | .200 | .214 | .136 |
| | 30 | .246 | .290 | .183 |
| | 60 | .401 | .486 | .316 |
| | 120 | .430 | .528 | .315 |
| | 240 | .508 | .619 | .378 |

L.S.D. 5% .041, 1% .056, Sig. interaction $p<0.02$, C.V.% 12.78

(b)

| | | | Structure | |
|---|---|---|---|---|
| | | Lamina + petiole | Node | Internode |
| Huia | 15 | .331 | .055 | .053 |
| | 30 | .543 | .107 | .099 |
| | 60 | .699 | .148 | .139 |
| | 120 | .805 | .177 | .149 |
| | 240 | .986 | .173 | .163 |
| Tamar | 15 | .428 | .070 | .052 |
| | 30 | .554 | .090 | .075 |
| | 60 | .899 | .161 | .144 |
| | 120 | .959 | .176 | .138 |
| | 240 | 1.125 | .215 | .164 |

L.S.D. 5% .041, 1% 0.056, Sig. interaction $p<0.001$, C.V.% 12.78

Table XXIX. Effect of dark treatment of the distribution of dry matter within structures (g/pot)

| Strain | Dark | Structures | | |
|---|---|---|---|---|
| | | Lamina + petioles | Nodes | Internodes |
| Huia | Pre- | .695 | .142 | .126 |
| | Post | .614 | .123 | .116 |
| Tamar | Pre- | .782 | .152 | .116 |
| | Post | .803 | .132 | .113 |

L.S.D. 5% .025
1% .033
Sig. interaction $p<0.001$
C.V.% 12.78

### (ii) Mobilization of N and P within the stolon.

The ability of strains to mobilize N and P can only be gauged by changes in the total amounts present. Content, being a product of concentration and yield, varies depending upon whether a particular tissue is importing or exporting assimilates. For this reason total amounts of N and P in stolon tissue will be considered.

1. N distribution. The effects of environment on N distribution are shown in Table XXX. The interaction of position X strain showed that Tamar contained less N in older tissue and more in photosynthetically active tissue. The interaction of strain with environment was not significant, but the data shows under conditions of most active photosynthesis (HTHL) Tamar contained a greater amount of N in the youngest tissue - at the distal end of the stolon. Thus Tamar possessed a greater ability to mobilize N from older tissue.

Distribution within positions ('structural' distribution) showed that Tamar contained a greater amount of N in the most actively growing tissue - the laminae + petioles. While not significant, there is reason to believe that this was due in part to a lesser accumulation of N in nodal and internodal tissue in Tamar.

The interaction of strain with environment on structural distribution occurred through a reversal of the above trend under HTLL, where Tamar accumulated less N in laminae + petioles.

Further evidence on the relative abilities of strains to mobilize N can be seen in the effects of the 48 hour dark treatment (Table XXXI). There was a significantly greater accumulation of N in the middle and distal positions of Tamar stolons following 48 hours dark. The data did not show this to be mobilized from older tissue. Distribution between structures showed a greater accumulation of N in laminae + petioles of Tamar following 48 hours dark. Values for nodes and internodes were too low to enable differences to be detected.

Results of a four factor interaction involving strains, levels of P, positions and structures are shown in Table XXXII. This interaction shows differences residing only in the laminae + petioles where N contents were sufficiently great to provide a reliable measure of concentrations and dry weight. The main cause of the interaction was the level of P at which N was mobilized from proximal to distal positions in the stolon. In the case of Huia, there was significantly more N in the middle and distal positions at 120 and 240 kg/ha P whereas with Tamar this difference first appeared at a lower level of

Table XXX. Effect of environment on N distribution in stolons of white clover (mg X 10)

| Environment | Strain | 'Position' | | | 'Structure' | | |
|---|---|---|---|---|---|---|---|
| | | Proximal | Middle | Distal | Lamina + petiole | Node | Internode |
| HTHL | Huia | .121 | .131 | .131 | .281 | .054 | .048 |
| | Tamar | .114 | .152 | .148 | .324 | .052 | .038 |
| HTLL | Huia | .083 | .125 | .125 | .261 | .036 | .037 |
| | Tamar | .073 | .122 | .118 | .254 | .035 | .032 |
| LTHL | Huia | .098 | .128 | .127 | .255 | .054 | .044 |
| | Tamar | .080 | .130 | .135 | .268 | .045 | .031 |
| LTLL | Huia | .084 | .106 | .116 | .231 | .041 | .135 |
| | Tamar | .070 | .129 | .116 | .259 | .029 | .028 |

L.S.D. 5% .018
1% .026
Sig. interaction p<0.6

Strain means:

| | | | | | | |
|---|---|---|---|---|---|---|
| Huia | .097 | .123 | .125 | .257 | .046 | .041 |
| Tamar | .084 | .133 | .129 | .275 | .041 | .032 |

L.S.D. 5% .009
1% .012
Sig. interaction p<0.001
C.V.% 32.7

Table XXXI. Effect of 48 hours dark on the distribution of N in stolons of white clover. (mg X 10).

| Strain | Dark | 'Position' | | | 'Structure' | | |
|---|---|---|---|---|---|---|---|
| | | Proximal | Middle | Distal | Lamina + petiole | Node | Internode |
| Huia | Pre- | .103 | .121 | .125 | .263 | .047 | .039 |
| | Post | .090 | .124 | .126 | .251 | .045 | .042 |
| Tamar | Pre- | .086 | .124 | .121 | .262 | .039 | .031 |
| | Post | .083 | .142 | .137 | .286 | .042 | .034 |

L.S.D. 5% .014

1% .018

Sig. interaction $p<0.9$

C.V.% 32.7

Table XXXII. Effect of level of P on the distribution of N in stolons of white clover (mg X 10).

| Level P | Position | Huia | | | Tamar | | |
|---|---|---|---|---|---|---|---|
| | | Lamina + petiole | Node | Inter- | Lamina + petiole | Node | Internode |
| $P_1$ | P* | .125 | .023 | .021 | .141 | .022 | .010 |
| | M | .151 | .021 | .021 | .157 | .018 | .014 |
| | D | .158 | .022 | .020 | .134 | .020 | .017 |
| $P_2$ | P | .175 | .033 | .022 | .134 | .023 | .016 |
| | M | .201 | .034 | .030 | .207 | .024 | .020 |
| | D | .205 | .035 | .036 | .219 | .034 | .030 |
| $P_3$ | P | .214 | .047 | .036 | .189 | .041 | .025 |
| | M | .265 | .050 | .047 | .339 | .064 | .039 |
| | D | .269 | .050 | .049 | .365 | .046 | .048 |
| $P_4$ | P | .244 | .056 | .040 | .216 | .037 | .029 |
| | M | .332 | .071 | .060 | .420 | .045 | .059 |
| | D | .371 | .060 | .57 | .338 | .047 | .044 |
| $P_5$ | P | .312 | .059 | .045 | .293 | .059 | .033 |
| | M | .424 | .071 | .064 | .476 | .064 | .051 |
| | D | .411 | .-65 | .066 | .482 | .063 | .052 |

L.S.D. 5% .074

1% .123

Sig. interaction $p<0.05$

C.V.% 32.7

P viz. 30kg/ha P. With both strains this was due in part to remobilization of N from older tissue (or to absorption and remobilization), as levels of N in younger tissue increased in greater proportion as the level of P was increased. In Tamar the proportion of N in younger compared to older tissue was greater than the proportion in Huia.

2. P distribution.

The effects of environment on P distribution within positions and structures of the stolons of strains are shown in Table XXXIII. Within positions the relative amount of P in the middle and distal positions of Tamar was greater than for Huia. Environment did not interact with strains in positional distribution. There was a relatively greater accumulation of P within structures in laminae + petioles of Tamar and the interaction with environment showed this to occur (at highly significant levels) in all except the HTLL treatment - where differences barely reached significance. Levels of P in nodal and inter-nodal tissue were too low to enable differences to be detected.

The effects of 48 hours dark on P distribution within the stolon are presented in Tabel XXXIV. Results show increased accumulation in middle and distal positions of the stolon of Tamar, but no such change in the distribution in Huia. Within structures, Tamar accumulated more P in its laminae + petioles and nodes following 48 hours dark whereas Huia showed no such change.

The level of P applied did not interact with strains in the P content of positions or structures. This indicates that both followed the same trend of increasing P content as the level of P was increased. Differences were present in the level of P at which accumulation of P in laminae + petioles differed between strains (Table XXXV); Tamar showed a proportionately greater increase in the P content of this tissue at levels of applied P greater than 60kg/ha. There was some evidence (approaching significance) to indicate that part of this additional P was mobilized from internodal tissue.

## DISCUSSION

This experiment was designed to examine possible mechanisms to account for the higher RNE of Tamar compared to Huia white clover at intermediate levels of applied P. Before considering these factors it must firstly be established whether or not the yield differences recorded in Experiment 6 were produced in this experiment.

In Experiment 6 Tamar differed from Huia in one environment - HTHL with

Table XXXIII. Effect of environment on P distribution in stolons of white clover (mg X 100).

| Environment | Strain | Position | | | Structure | | |
|---|---|---|---|---|---|---|---|
| | | Proximal | Middle | Distal | Lamina + petiole | Node | Internode |
| HTHL | Huia | .098 | .121 | .148 | .262 | .055 | .050 |
| | Tamar | .097 | .136 | .157 | .297 | .057 | .036 |
| HTLL | Huia | .075 | .129 | .144 | .268 | .043 | .038 |
| | Tamar | .061 | .119 | .129 | .239 | .039 | .032 |
| LTHL | Huia | .079 | .130 | .149 | .257 | .056 | .045 |
| | Tamar | .097 | .161 | .193 | .321 | .079 | .051 |
| LTLL | Huia | .082 | .122 | .155 | .264 | .054 | .041 |
| | Tamar | .072 | .157 | .161 | .316 | .042 | .032 |

L.S.D. 5% .026

1% .035

Sig. interaction $p<0.60$

Strain means;

| | Proximal | Middle | Distal | Lamina + petiole | Node | Internode |
|---|---|---|---|---|---|---|
| Huia | .083 | .126 | .149 | .263 | .052 | .044 |
| Tamar | .081 | .143 | .160 | .293 | .054 | .038 |

L.S.D. 5% .012

1% .016

Sig. interaction $p<0.09$

C.V.% 13.26

Table XXXIV. Effect of 48 hours dark on the distribution of P in stolons of white clover. (mg X 100)

| Strain | Dark | 'Position' | | | 'Structure' | | |
|---|---|---|---|---|---|---|---|
| | | Proximal | Middle | Distal | Lamina + petiole | Node | Internode |
| Huia | Pre- | .089 | .119 | .143 | .262 | .050 | .039 |
| | Post | .078 | .132 | .156 | .264 | .053 | .048 |
| Tamar | Pre- | .076 | .125 | .142 | .266 | .045 | .031 |
| | Post | .087 | .161 | .178 | .320 | .063 | .044 |

L.S.D. 5% .018
1% .024
Sig. interaction p>0.9
C.V. 13.26

Table XXXV. Effect of level of P on the distribution of P within 'structures' of white clover stolons (mg X 100)

| | | 'Structure' | | |
|---|---|---|---|---|
| Strain | Level of P | Lamina + petiole | Node | Internode |
| Huia | $P_1$ | .111 | .017 | .013 |
| | $P_2$ | .153 | .027 | .020 |
| | $P_3$ | .217 | .042 | .032 |
| | $P_4$ | .335 | .065 | .050 |
| | $P_5$ | .496 | .106 | .101 |
| Tamar | $P_1$ | .124 | .019 | .012 |
| | P2 | .153 | .020 | .013 |
| | $P_3$ | .256 | .043 | .027 |
| | $P_4$ | .376 | .084 | .057 |
| | $P_5$ | .554 | .104 | .077 |

L.S.D. 5% .029
1% .039
Sig. interaction $p>0.3$
C.V.% 13.26

high moisture stress. The response curve to applied P had positive curvature for Tamar and negative curvature for Huia. In the present experiment the two again differed in RNE at intermediate levels of P under HT but with LL and no moisture stress. Under conditions comparable to those of Experiment 6 the two differed only at the highest level of P - as they did under LTHL and LTLL.

The fact that strains did not reproduce results of Experiment 6 under identical environmental conditions may be due to biological variability between experiments and to lack of experimental precision. It may also be due to the presence of root knot nematodes in Experiment 6 and their absence in the present experiment. For arguments sake, assume that Huia carried a greater infestation of nematodes than Tamar. If this were the case then yields of Huia would be depressed relative to yields of Tamar in Experiment 6. This is in fact what occurred. The findings of Widdowson et al (1973) have shown that infestation of white clover plants increases the P requirements five times to give equivalent yields to non-infested plants. In light of this, the suggestion that Huia contained a greater infestation of nematodes would account for its lack of response to low levels of P. Further, Widdowson et al noted that nematode infestation became less at higher levels of applied P. Hence it can be argued that this provides an explanation for the rapid recovery in yields of Huia relative to Tamar at high levels of P in Experiment 6.

The question arises as to the reason for the superiority of Tamar only under the one environment in Experiment 6. A feasible explanation is that it was under the HTHL environment that conditions were most favourable for growth, and if Huia was more greatly infested with nematodes (particularly at low levels of P) then it follows that its ability to absorb and translocate water under moisture stress would be less owing to lesser root development and to disruption of the translocation process in roots. Yields would then be depressed relative to Tamar, as was the case. Thus there is good evidence from which to believe that the marked differences in RNE found in Experiment 6 may, to a large extent, have been an artifact of differential infestation of strains by root knot nematodes.

Yield results from the present experiment need to be placed in perspective. Differences in RNE between strains were present and differed at the level of P at which they attained significance under different environments. Examination of yield data (Table XVIII) shows the superiority of Tamar commencing at about 30 to 60kg/ha P and continuing to increase under three environments until significance was reached at the highest level of applied P. While yields under

HTLL were not significantly different at the highest level of P, it should be noted that values were higher for Tamar. Both strains gave more than 95 percent of their asymptote value at 240kg/ha P. However it was pointed out earlier that $X_{max}$ must necessarily be an arbitary level of P at which relative performances of strains can be compared within an experiment. If $X_{max}$ were taken as 120kg/ha P then Tamar could be classified as Type 1 RNE as it would not have reached 95 percent of its asymptote value, whereas Huia had reached this value and would, because it was inferior to Tamar at all points in the range of P applications, be classified as Type 3 RNE.

Differences in patterns of response of strains between environments in the present experiment were not great and would need replication of environments to justify their recognition as distinct populations. Thus it is concluded from data available that Tamar was more efficient in responding to P at 240kg/ha. The discussion relating yield responses to mechanisms associated with the higher RNE of Tamar will therefore pay particular attention to results at 240kg/ha P.

The relationship between shoot yields and root yields, length and number of apices (Tables XIX (a) to (c)) do not provide sufficiently consistent data to enable a relationship to be established with the higher RNE of Tamar at 240kg/ha.

Total absorption of P was generally similar for both strains (Table XXIII). Thus the greater P absorption per unit root length by Tamar at 240kg/ha P following a normal harvest (pre-dark) must have been offset by the lower, but non-significant, root length (Table XIX (d)) at this level of P. Utilization of P was greater for Tamar irrespective of the amount of P applied. Hence neither absorption or utilization provide explanation for the higher RNE of Tamar at 240kg/ha P.

More light is shed on this question by examination of the effects of 48 hour dark. By subtracting post from pre-dark P absorption values at the 240kg/ha level of applied P, the following differences (Table XXXVI) were recorded:-

Table XXXVI.

P absorbed (post - pre-dark)
(mg/pot)

| | Total P | Shoot P | Root P |
|---|---|---|---|
| Huia | 6.31 | 10.86 | -4.56 |
| Tamar | 8.77 | 5.83 | 2.94 |

Of the P gained by Huia during the 48 hour dark, there was a net transportation of absorbed P to shoots and an additional amount drawn from root reserves. Tamar on the other hand translocated two-thirds to its own shoots and retained one-third in its roots. Two interpretations can be placed on these differences. (1) It suggests that Tamar had a greater propensity to supply P to its roots and/or retain P in its roots. This would ensure that P requirements for root metabolism would be more adequately met, thereby providing a better supply of assimilates and P to growing tissue. (2) Russell and Martin (1953), Williams (loc.cit.) and Wilkinson and Gross (1965a) have shown that roots of a P deficient plant usually retain more P and transport less which, when applied to results of this experiment, suggest that Tamar may have been more P deficient than Huia in spite of the high level of P at which differences occurred. The yield response curve of Tamar was upwards between 120 and 240kg/ha P whereas that of Huia had reached its asymptote.

Both the above possibilities are equally accountable in terms of the supply of assimilates. Examination of the movement of soluble sugars following 48 hours dark at 240kg/ha P (Table XXXVII) showed the following post - pre-dark differences:-

Table XXXVII. Soluble sugars (post - pre-dark) (mg/pot)

| | Total | Shoot | Root |
|---|---|---|---|
| Huia | -395 | -388 | -7 |
| Tamar | -285 | -314 | 28 |

During dark respiration Huia utilized a much greater amount of soluble sugar reserves than Tamar and utilized both shoot and root reserves. Tamar on the other hand utilized only shoot reserves and showed a net gain in root reserves. This better supply of assimilates in the roots of Tamar are in line with hypothesis (1) and (2) above; that Tamar was better able to not only supply P but also assimilates to its roots to maintain metabolic processes and continued growth at high levels of P. As will be discussed later, this greater supply of metabolites in roots and in the plant as a whole would meet the energy requirements of Tamar for greater mobilization of N and P within the stolon.

In relating differences in dry matter yields between strains to their N status, consideration will firstly be given to their nitrogen fixing ability. The only treatment effect involving strains occurred where Tamar gave greater

N fixation than Huia following a normal harvest and smaller differences following 48 hours dark (Table XX). Virtanen (1955), Gibson (1966), Wheeler (1971) and Lawn et al (1974) have shown that symbiotic N fixation is dependent upon the supply of assimilates to nodules. Results from Experiment 4 (details of which have not been given) showed that it is also dependent upon the supply of P - yield of shoots apart. Thus the lower N fixing activity of Huia can be attributed to its lower levels of soluble sugars in its roots and to its lower P status where a net transportation of P occurred from roots to shoots when photosynthesis was inhibited. In the case of Tamar, P was retained in its roots. This would ensure that metabolizable energy and ATP necessary for the maintenance of high levels of N fixation (Bergersen, 1971; Moustafa et al, 1971) were present to a greater extent in Tamar than in Huia. However it must be recognised that N fixation is likely to alter as seedlings become older and that acetylene reduction assay was made at the end of the experiment. It does not reflect the situation throughout the course of the experiment as would N absorption and utilization measurements. Total absorption of N was greater for Tamar than for Huia at 240 kg/ha P; a finding which is in accordance with its greater N fixation. The greater utilization of absorbed N provides another explanation for its greater RNE in yield. It was not complicated by environmental or dark treatment effects.

Distribution of dry matter and N and P within the stolon will now be discussed. The first point to note is that their distribution followed the pattern recorded by many workers cited in the review of literature and little point would be served in re-iterating their findings.

Strains differed in their distribution of dry matter along the stolon. Tamar contained a greater proportion in the mid-region and a relatively greater proportion in its laminae + petioles and nodes (Table XXVIII). These differences were most marked at the highest level of P. They are consistent with the high RNE of Tamar at 240 kg/ha P, as the mid-region contained fully mature laminae - those most active in photosynthesis and the formation of metabolites (Thrower, 1962). Cessation of active photosynthesis by the imposition of 48 hours dark brought about little change in the distribution of dry matter in Huia, but it caused an increase in the dry weight of laminae + petioles of Tamar at the expense of nodal tissue. This can be accounted for by the fact that the soluble sugar status in shoots of Tamar was greater than in shoots of Huia, and may have been sufficent to meet the needs for growth. Huia on the other hand, with its lower levels of soluble sugars needed to provide the requirements for growth and respiration in laminae + petioles by export from nodal tissue. Further justification for this view is provided by the fact that Tamar was able to export P from its roots following 48 hours dark, whereas Huia needed to import P into its shoots - again to make good its inadequate shoot supply. Thus the greater RNE of Tamar at high levels of P can be related to its better shoot reserves of soluble sugars.

At the outset of this experiment it was hypothesised that Tamar had a greater RNE than Huia because of its ability to remobolize N and P from older to younger tissue, and thereby enable it to make more active growth irrespective of its lower N and P concentrations. This hypothesis will now be examined in light of results obtained. Data show that Tamar was in fact better able to remobolize N (Table XXX) and P (Table XXXIV) into its younger tissue - the mid to distal region of the stolon and to the laminae + petioles. There was evidence to suggest this ability was greater under conditions most favourable for maximum growth (HTHL) and that Tamar lost some of this advantage over Huia in being able to remobolize N and P under conditions of low light. Examination of the soluble sugar content of roots (Table XXVII) indicates that the supply of assimilates was less in Tamar under LTLL. Hence it can be concluded that the greater ability of Tamar to remobolize N and P is dependent upon conditions of high light and maximum photosynthetic activity (as set by temperature). Where activity is restricted by light and the supply of energy in the form of soluble sugars is reduced, Tamar looses some of its superiority over Huia in remobolizing N and P.

Altering the photosynthetic/respiration balance by the imposition of 48 hours dark gave further evidence to confirm the greater ability of Tamar to remobolize N (Table XXXI) and P (Table XXXIV). Again this greater ability can be associated with a higher level of soluble sugars in Tamar compared to Huia.

In relating this greater ability of Tamar to remobolize N and P it must be borne in mind that the yield superiority of Tamar commenced at a lower level than the 240 kg/ha P at which statistical significance was recorded. Tamar was classified as Type 1 RNE and Huia as Type 3 so that the differences resided at the higher levels of applied P. It was at these higher levels of applied P that Tamar showed its greatest superiority over Huia in remobolizing N (Table XXXII) and P (XXXV).

Thus the data confirm the hypothesis that Tamar, with its lower concentration of N and P (greater utilization) was able to remobolize N and P to a greater extent than Huia and that this would account for its higher RNE.

## CONCLUSIONS

This experiment, comparing the RNE of Huia and Tamar white clovers in response to applied P, identified Tamar as the more efficient strain. It continued to respond to high levels of P and was classified as possessing Type 1 RNE. Huia reached its yield asymptote at a lower level of applied P and was at no point superior to Tamar. It was classified as possessing Type 3 RNE. These results differ from those obtained in Experiment 6 where differences between strains were greater. It is suggested that the different patterns of response between the two experiments was due to greater infestation of Huia with root knot nematodes.

The hypothesis examined in this experiment was confirmed. Tamar contained a lower concentration of N and P than Huia (confirming results of Experiment 6) and hence greater utilization. With N, this was related to the high RNE of Tamar at high levels of P. With P, greater utilization was recorded at all levels of applied P.

The greater utilization of N and P was associated with a greater ability of Tamar to remobilize N and P to the mid-region of the stolon (that most active in photosynthesis) and to the distal end of the stolon and the laminae and petioles where meristematic tissue was present. These differences between strains were most evident at high levels of applied P and are considered to account for the greater RNE of Tamar.

Tamar contained a greater content of soluble sugars than Huia, and at high levels of P it was better able to mobilize soluble sugars and P to its roots to meet the energy requirements and ATP production for greater N fixation. Also the greater content of soluble sugars in Tamar provided the energy necessary for mobolization of N and P within the stolon.

There was an indication that the ability of Tamar to mobilize N and P and to produce soluble sugars was light limited at high temperatures, and this may account for the apparent lack of response of Tamar relative to Huia under high temperatures and low light at high levels of applied P.

No relationship was established between the RNE in yield or P absorption of strains and root weight, length or number of apices.

# SECTION 7

# GENERAL DISCUSSION AND CONCLUSIONS

In this series of experiments the RNE of strains of Yorkshire fog, perennial ryegrass, L. penduncu1atus and white clover was studied. Experiments 1 and 2 examined the RNE of the grass species in response to N and P while Experiments 3 and 4 examined the RNE of the legume species in response to P. These four experiments were designed as screening experiments to give a basis for the selection of promising material with which to work in more detailed experiments. Because of this a large number of strains were examined and as a result experimental precision was not at times high, and results of these experiments were not in some instances reproduced in subsequent experiments.

In Experiments 3 and 4 the RNE of Yorkshire fog and perennial ryegrass strains were studied in response to N, and the effects of climate on RNE was also compared. Experiments 5 and 6 studied the RNE of Lotus and white clover in response to applied P and also the effects of climate. These experiments measured shoot yield,shoot:root ratios and N absorption and utilization and in addition with legumes N fixing activity and P absorption and utilization were measured. In Experiment 7 a study was made of possible mechanisms controlling RNE in white clover. The same paramaters were measured as well as the ability of strains to re-mobolize and re-utilize P within the plant.

Literature cited in the review of literature indicates that RNE is under genetic control. In these experiments interspecific and interstrain differences in RNE were recorded and it was shown that RNE was dependent upon climate. Results will now be discussed in relation to interstrain and interspecific differences.

## Yorkshire fog.

Results of Experiment 1 did not enable the clear identification of strains according to the definition provided in the introductory section to this thesis. Massey Basyn was identified as Type 1 RNE in which its yields at less than the maximum level of applied N were lower than for other strains. The others generally showed a Type 1 RNE but because of their lower overall yields relative to Massey Basyn were classified as Type 3. From among these Tara Hills strain was selected for further study as it had the greatest contrast in relative yields at less than the maximum level of applied N relative to Massey Basy.

In Experiment 5, where the RNE of Massey Basyn and Tara Hills strains was compared under differing climates, the RNE of the two strains was found to be dependent upon climate. Under a climate of high temperatures and high light and high moisture stress they did not differ whereas under conditions of lower temperature and or low light (Palmerston North and Taieri climates) they differed. Under the Palmerston North climate, which differed from the Tara Hills climate in having lower temperatures and no moisture stress, Massey Basyn gave Type 2 RNE through being responsive at low levels of N, while Tara Hills gave Type 1 RNE. Yields from the Tara Hills strain were extremely low at low levels of applied N but at high levels were equal to those from Massey Basyn. This difference in RNE was attributed to the inability of Tara Hills to absorb N at lower temperatures due to poor root development and in addition to a lack of ability to utilize absorbed N at a high level for the production of dry matter.

Under the Taieri climate, which featured low temperatures and low light without moisture stress, yields from Tara Hills declined as the level of N was increased while yields from Massey Basyn did not alter. It is suggested that the decline in yields of Tara Hills were attributable to high protein synthesis at high levels of N at the expense of carbohydrate reserves and the possibility of ammonium toxicity.

Thus the more detailed study of two strains of Yorkshire fog showed that genetic variability exists within the species and that its expression is dependent upon climate. Of the two strains Massey Basyn had the desirable attribute of high responsiveness to less than maximum levels of N applied (60 kg/ha N applied four times in 52 days). No previous studies into the RNE of strains of Yorkshire fog have been reported in the literature.

## Perennial ryegrass.

Efficiency in response to N and P among strains of perennial ryegrass have been reported by a number of workers cited in the review of literature in Experiment 2. Also high heritability for yields at low and moderate levels of N and P have been reported. While these studies compared different strains to those in the experiments under report, differences in RNE were also found.

Results of Experiment 2 showed that two strains of significance to New Zealand agriculture - Ruanui and Mangere, possessed Type 1 RNE, as they continued to respond more than other strains to high levels of applied N. Two other strains, R.v.P. and W.W.Virus were classified as possessing Type 2 RNE as they gave high yield responses relative to Ruanui and Mangere at low levels of applied N. These four strains were therefore studied in Experiment 5.

Experiment 5 showed differences in RNE according to climate. Under the

Tara Hills climate no differences were found. Under the Palmerston North climate Mangere was again classified as Type 1 RNE whereas the others, including Ruanui were classified as Type 2 RNE and did not differ among themselves. The classification of Mangere as Type 1, was due to its very low yields at low levels of applied N, and the same explanation is given for this strain as for the Tara Hills strain in the Yorkshire fog experiment viz. an inability to absorb N at low levels of applied N due to restricted root development.

Yorkshire fog versus perennial ryegrass.

In the literature review of Experiment 1 it was pointed out that Yorkshire fog is generally regarded as a low fertility species in comparison with perennial ryegrass. This classification is based on field studies on low fertility soils where environmental limitations to plant growth were also present. Thus it is difficult to distinguish between the greater ability of Yorkshire fog to survive under these conditions due to adaptability to low fertility or to adverse climate. Further, most experiments used unspecified strains of Yorkshire fog.

From the experiments in this present study in which a comparison of the most efficient strains of each species can be made (i.e. Type 2), it is concluded that their RNE does not differ. The comparison between Massey Basyn and Ruanui, R.v.P. and W.W. Virus failed to show any differences. Thus it appears likely that the classification of Yorkshire fog as a 'low' fertility species as based on field experiments must be a reflection of the adaptability of Yorkshire fog to adverse climatic conditions rather than to any greater RNE compared to perennial ryegrass. If however the strains used in the field experiments were similar in RNE to the Tara Hills strain used in this experiment (and results of Experiment 1 would suggest they were) then the conclusions that Yorkshire fog is a lower fertility demanding species than perennial ryegrass would be correct - in Experiment 5 under the Palmerston North climate it was lower yielding than Ruanui under low levels of N. In other words an inefficient example of one species was being compared with an efficient example of the other.

The fact that the bred strain Massey Basyn showed greater RNE at low levels of N than other regional strains indicates that genetic variability exists and that considerable progress should be possible in further raising its RNE through breeding. Among the perennial ryegrass examined (all of which were bred strains) differences in RNE were not demonstrated - with the exception of Mangere which reacted adversely to one climate. Thus if differences in RNE exist then they cannot be of a large order and will need extremely precise experiments to identify them.

Lotus.

In Experiment 3 two strains of Lotus, L.hybrid and Timaru were identified as possessing Type 1 RNE in response to applied P. The remaining strains were Type 2 as they gave greater responses to low levels of P. Among these L.tetraploid gave highest yields and was therefore classified as Type 2 and the remainder, because of lower overall yields were classified as Type 3 RNE. L.tetraploid obtained its greater RNE at low levels of P through its greater ability to utilize absorbed P.

In Experiment 6 L.hybrid and L.tetraploid were compared under different climates. No differences in RNE were detected. There are no reports in the literature of studies into the RNE of strains of Lotus with which to compare these findings.

White Clover.

Snaydon and Bradshaw (1962) have reported differences among strains of white clover in RNE as the level of P was varied. Using different strains the present experiments of this study have produced a similar finding. In Experiment 4, four strains (Tamar, Spanish, S.184 and Kentish wild white) were classified as possessing Type 1 RNE. From among these Tamar was selected for further study as it exhibited a greater ability to utilize absorbed P than did other strains of this Type. Of the remaining strains Huia was identified as possessing Type 2 RNE and because of its importance to New Zealand agriculture was selected as representative of its Type for further study. Louisiana was also Type 2 and was included in the subsequent experiment because it showed unusually high utilization of P.

Experiment 6 compared the RNE of the above strains under three climates. Results differed from those of Experiment 4 in that Tamar was identified as Type 2 RNE and Huia as Type 1. Louisiana fell between these two in its yield response. Differences between Tamar and Huia occurred at intermediate levels of P (where Tamar was more efficient) under only the one climate - Tara Hills. This was attributed to greater P absorption and utilization, greater N fixing activity and absorption and utilization of N by Tamar.

In Experiment 7 the two strains Huia and Tamar were examined in more detail to provide confirmatory evidence of earlier findings where the presence of root knot nematodes could have been a contributing factor to differences recorded. Also it was designed to examine possible mechanisms controlling RNE. From earlier results the greater utilization of P by Tamar appeared to be a factor worthy of examination. To this end the relative abilities of Huia and Tamar to re-mobolize and re-utilize P within the stolon was examined.

Results of Experiment 7 confirmed that Tamar was the more efficient strain. However in this experiment there was a shift in the level of P at which its greater efficiency was expressed. In three of the four climates (HTHL, LTHL, LTLL) it was superior at 240 kg/ha P, whereas under HTLL it was superior at 30 and 60 kg/ha P. Thus as was the case with the other experiments the expression of RNE was dependent upon climate. According to the definition of RNE given at the outset of this study Tamar exhibited Type 1 RNE and Huia Type 2. This is the opposite classification to that given these strains in Experiment 6 - even though Tamar gave greater yields at the level of P at which differences occurred in both experiments. It highlights the problem of classifying Types of RNE and arises to some extent through $X_{max}$ not reaching the yield asymptote. It also highlights the need for experiments to include more points on the response surface to enable more precise curve fitting.

Experiment 7 confirmed the hypothesis that Tamar gained its greater RNE through a greater utilization of absorbed N and P, and that this occurred through its greater ability to remobolize N and P from senescing to meristematic tissue. In addition it was more able to mobolize soluble sugars to its roots and to better meet energy requirements for N fixation and growth. In these factors lie one mechanism to account for the greater RNE of Tamar. Absorption of P was not found to be related to the greater RNE of Tamar although there was a suggestion in the data that it may have contributed - as also recorded in Experiment 6.

### White clover versus Lotus.

Experiment 6 was the only one including this comparison and results showed that Lotus strains were less responsive to high levels of P under the Palmerston North and Taieri climates compared to white clover strains. Attention has already been drawn to the only other documented comparison of the RNE of these two species (Brock, 1973). He found that it was at low levels of P that Lotus had greater RNE than white clover. The disparity between experimental results could well lie in the levels of P defined as 'low' and 'high'. Also in Brock's study plants were able to develop a normal rooting system with the result that they were able to explore a greater volume of soil for P. Thus the effective amount of P available to plants may have been greater in Brock's experiment and more comparable to the intermediate levels of P in the pot experiments. Nevertheless both experiments confirm the commonly held belief that white clover is a 'high' fertility species compared to Lotus.

Throughout this study there has been repeated reference to absorption and utilization of N and P being related to RNE. At the outset, attention was drawn to the difficulty of determining which of these factors was cause and which effect or whether they were unrelated. It was considered that subjective assessment of their response curves provided the only means of differentiation. Studies of the absorption mechanisms and the way in which plant species differ in their absorption of different nutrient ions have been reviewed earlier. These studies frequently suffered from the limitation that excised roots were studied or where intact plants have been used (as for example in the work of Powell (1974) with Carex coriacea) absorption has been considered with little reference to utilization as a complicating factor. It is possible that absorption is controlled by utilization - in other words an extension of the source-sink concept where absorption by roots is limited by the degree to which nutrients already absorbed are utilized. There is some evidence in a recent report of a comparison of the phophate response of Wimmera ryegrass and subterranean clover by Barrow (1975b) to show this is the case. He showed that the former has a greater ability to absorb P due to a greater volume of root hairs and to a greater ability to reduce the concentration at the root surface to a lower value. The grass was also able to transfer more P to its tops which utilized it more efficiently. Further the ryegrass was better able to divert photosynthates to its roots for greater absorption and growth. It is also possible that absorption and utilization act independently and that the findings of Barrow are fortuitous. Foot and Howell (1964) in a reciprocal grafting experiment between shoots and roots of a P tolerant and P sensitive strain of soybean found that toxicity symptoms and P accummulation was determined by the genotype of the root. However it is to be noted that the P sensitive strain, which gave greater P absorption, also contained greater P concentrations (i.e. its utilization of P was lower). This suggests that high P absorption alone may not have been the sole determinant of the P sensitivity; it could equally have been due to the inability of the strain to utilize absorbed P. If utilization had been higher absorption could well have been reduced. This was tested by the grafting of the shoots of the P tolerant strain (which had high utilization) onto the roots of the P sensitive strain (which had low utilization). Absorption was still high. Thus it is concluded that absorption and utilization must be independently operating characterisitcs of the plant - both exerting an influence on RNE.

In this study climate was found in many instances to affect the expression of RNE. While evidence is very limited in this series of experiments there is reason to believe that the climate which is optimum for the growth of a particular species/strain is the one which best enables the expression of maximum nutrient efficiency. This is thought to be due to the plant being in a physiological state in which its supply of assimilates is sufficiently great to meet the energy demands for high levels of absorption and utilization of nutrients.

This programme of research was designed to determine whether it is possible to fit the plant to the soil by the use of species/strains which have higher nutrient efficiency. This objective has been met in this study in that strains of Yorkshire fog, perennial ryegrass and white clover have been identified which differ in RNE. At times experimental precision for adequate curve fitting was not as great as required and more detailed experiments are needed to better characterize the shape of the response surfaces of these species/strains. Nevertheless the research programme has provided sound evidence that further research on the selection and breeding of grasses and clovers, which are more efficient in their use of fertiliser and require less fertiliser to give equal yields to other presently used species/strains, offers considerable opportunities to bring about economies in the use of fertiliser.

APPENDIX I. Shoot yields of strains of Yorkshire fog in response to applied N and P. (g D.M./pot)

| Strain | N level (kg/ha) | P level (kg/ha) | | | N mean |
|---|---|---|---|---|---|
| | | 10 | 60 | 180 | |
| Massey Basyn | 0 | 4.240 | 3.703 | 7.474 | 5.139 |
| | 50 | 6.607 | 9.060 | 9.029 | 8.232 |
| | 100 | 9.048 | 12.036 | 11.954 | 11.012 |
| | P mean | 6.631 | 8.266 | 9.486 | 8.128 |
| Dipton | 0 | 3.216 | 3.636 | 5.062 | 3.971 |
| | 50 | 5.969 | 8.823 | 8.046 | 7.613 |
| | 100 | 8.407 | 9.858 | 10.494 | 9.586 |
| | P mean | 5.864 | 7.439 | 7.867 | 7.057 |
| Abbotsford | 0 | 3.193 | 4.511 | 4.947 | 4.217 |
| | 50 | 6.246 | 8.843 | 9.063 | 8.051 |
| | 100 | 6.788 | 10.393 | 10.120 | 9.100 |
| | P mean | 5.409 | 7.916 | 8.043 | 7.123 |
| Rotorua | 0 | 3.126 | 4.785 | 4.852 | 4.254 |
| | 50 | 7.557 | 8.560 | 9.836 | 8.651 |
| | 100 | 6.189 | 10.260 | 11.985 | 9.478 |
| | P mean | 5.624 | 7.868 | 8.891 | 7.461 |
| Glen Innes | 0 | 3.269 | 3.699 | 5.006 | 3.991 |
| | 50 | 6.450 | 8.917 | 8.793 | 8.053 |
| | 100 | 6.078 | 10.374 | 11.991 | 9.481 |
| | P mean | 5.265 | 7.663 | 8.596 | 7.175 |
| Almeida de Sayago | 0 | 3.375 | 3.789 | 4.199 | 3.788 |
| | 50 | 5.702 | 8.672 | 8.375 | 7.583 |
| | 100 | 4.186 | 9.741 | 9.844 | 7.924 |
| | P mean | 4.421 | 7.401 | 7.472 | 6.431 |
| Stratford | 0 | 3.482 | 4.564 | 3.688 | 3.911 |
| | 50 | 6.879 | 7.431 | 9.228 | 7.846 |
| | 100 | 4.561 | 9.828 | 11.241 | 8.543 |
| | P mean | 4.974 | 7.274 | 8.052 | 6.767 |
| Tara Hills | 0 | 3.435 | 3.307 | 3.661 | 3.468 |
| | 50 | 7.030 | 7.879 | 8.198 | 7.702 |
| | 100 | 7.008 | 8.051 | 11.212 | 8.757 |
| | P mean | 5.824 | 6.412 | 7.690 | 6.642 |

| | L.S.D. 5% | L.S.D. 1% | Sig. int. |
|---|---|---|---|
| Strains: | 0.701 | 0.928 | *** |
| Strains X N and P: | 1.214 | 1.608 | N.S. |
| Strains X N X P: | 2.103 | 2.786 | * |

C.V. % 14.81

APPENDIX II. Absorption of N by shoots of Yorkshire fog in response to applied N and P (mg/pot)

| Strain | N level (kg/ha) | P level (kg/ha) 10 | 60 | 180 | N mean |
|---|---|---|---|---|---|
| Massey Basyn | 0 | 119 | 84 | 161 | 121 |
| | 50 | 274 | 291 | 278 | 281 |
| | 100 | 441 | 510 | 479 | 477 |
| | P mean | 278 | 295 | 306 | 293 |
| Dipton | 0 | 101 | 87 | 119 | 102 |
| | 50 | 275 | 294 | 276 | 282 |
| | 100 | 433 | 429 | 498 | 453 |
| | P mean | 269 | 270 | 298 | 279 |
| Abbotsford | 0 | 97 | 105 | 110 | 104 |
| | 50 | 219 | 308 | 304 | 277 |
| | 100 | 343 | 443 | 479 | 421 |
| | P mean | 220 | 285 | 298 | 268 |
| Rotorua | 0 | 99 | 109 | 121 | 110 |
| | 50 | 332 | 297 | 347 | 325 |
| | 100 | 331 | 503 | 519 | 451 |
| | P mean | 254 | 303 | 329 | 295 |
| Glen Innes | 0 | 96 | 111 | 140 | 116 |
| | 50 | 307 | 348 | 353 | 336 |
| | 100 | 322 | 477 | 564 | 454 |
| | P mean | 242 | 312 | 352 | 302 |
| Almeida de Sayago | 0 | 78 | 86 | 109 | 91 |
| | 50 | 166 | 267 | 229 | 221 |
| | 100 | 192 | 362 | 329 | 294 |
| | P mean | 145 | 238 | 222 | 202 |
| Stratford | 0 | 97 | 112 | 88 | 99 |
| | 50 | 288 | 264 | 306 | 286 |
| | 100 | 248 | 457 | 481 | 395 |
| | P mean | 211 | 278 | 292 | 260 |
| Tara Hills | 0 | 65 | 66 | 70 | 68 |
| | 50 | 222 | 201 | 217 | 213 |
| | 100 | 305 | 252 | 331 | 296 |
| | P mean | 197 | 173 | 206 | 192 |

| | L.S.D. 5% | L.S.D. 1% | Sig.int. |
|---|---|---|---|
| Strains: | 27 | 36 | *** |
| Strains X N and P: | 47 | 63 | N***, P* |
| Strains X N X P: | 88 | 117 | N.S. |

C.V.% 20.74

APPENDIX III. Absorption of P by shoots of Yorkshire fog in response to applied N and P. (mg/pot)

| Strain | N level (kg/ha) | P level (kg/ha) 10 | 60 | 180 | N mean |
|---|---|---|---|---|---|
| Massey Basyn | 0 | 14.3 | 21.1 | 50.5 | 28.6 |
| | 50 | 13.9 | 31.1 | 42.5 | 29.2 |
| | 100 | 16.6 | 28.0 | 44.2 | 29.6 |
| | P mean | 14.9 | 26.7 | 45.7 | 29.1 |
| Dipton | 0 | 10.9 | 21.0 | 32.6 | 21.5 |
| | 50 | 10.3 | 29.4 | 45.1 | 28.3 |
| | 100 | 16.9 | 23.3 | 45.8 | 28.7 |
| | P mean | 12.7 | 24.6 | 41.2 | 26.1 |
| Abbotsford | 0 | 10.0 | 23.7 | 35.2 | 23.0 |
| | 50 | 13.0 | 34.8 | 53.3 | 34.0 |
| | 100 | 12.8 | 25.9 | 44.6 | 27.8 |
| | P mean | 12.3 | 28.1 | 44.4 | 28.2 |
| Rotorua | 0 | 9.0 | 21.9 | 32.2 | 21.0 |
| | 50 | 15.3 | 29.8 | 62.3 | 35.8 |
| | 100 | 14.0 | 28.3 | 59.1 | 33.8 |
| | P mean | 12.7 | 26.6 | 51.2 | 30.2 |
| Glen Innes | 0 | 10.5 | 17.0 | 32.2 | 19.9 |
| | 50 | 13.7 | 28.7 | 38.7 | 27.0 |
| | 100 | 11.3 | 29.7 | 42.4 | 27.8 |
| | P mean | 11.8 | 25.1 | 37.7 | 24.9 |
| Almeida de Sayago | 0 | 11.5 | 18.8 | 22.5 | 17.6 |
| | 50 | 13.0 | 26.8 | 40.8 | 26.8 |
| | 100 | 7.5 | 20.6 | 41.4 | 23.2 |
| | P mean | 10.7 | 22.1 | 34.9 | 22.5 |
| Stratford | 0 | 12.5 | 25.0 | 26.9 | 21.4 |
| | 50 | 13.3 | 17.6 | 48.8 | 26.6 |
| | 100 | 9.2 | 22.6 | 39.5 | 23.8 |
| | P mean | 11.7 | 21.7 | 38.4 | 23.9 |
| Tara Hills | 0 | 10.9 | 13.7 | 21.2 | 15.3 |
| | 50 | 13.5 | 26.6 | 43.7 | 27.9 |
| | 100 | 14.2 | 19.1 | 46.2 | 16.5 |
| | P mean | 12.8 | 19.8 | 37.1 | 23.2 |

| | L.S.D. 5% | L.S.D. 1% | Sig. int. |
|---|---|---|---|
| Strains: | 3.6 | 4.8 | *** |
| Strains X N and P: | 6.2 | 8.3 | N = N.S., P = N.S. |
| Strains X N X P: | 8.8 | 11.7 | N.S. |

C.V.% 20.74

APPENDIX IV. Utilization of N by shoots of Yorkshire fog in response to applied N and P (g D.M./g N absorbed)

| Strain | N level (kg/ha | P level (kg/ha) 10 | 60 | 180 | N mean |
|---|---|---|---|---|---|
| Massey Basyn | 0 | 35.5 | 43.6 | 45.8 | 41.6 |
| | 50 | 23.7 | 31.0 | 32.4 | 29.1 |
| | 100 | 20.5 | 23.6 | 24.8 | 22.9 |
| | P mean | 26.6 | 32.7 | 34.3 | 31.2 |
| Dipton | 0 | 31.8 | 41.5 | 42.0 | 38.4 |
| | 50 | 21.7 | 29.9 | 29.0 | 26.8 |
| | 100 | 19.3 | 22.8 | 21.1 | 21.1 |
| | P mean | 24.3 | 31.4 | 30.7 | 28.8 |
| Abbotsford | 0 | 32.6 | 42.8 | 44.6 | 40.0 |
| | 50 | 31.3 | 28.6 | 29.7 | 29.9 |
| | 100 | 19.6 | 23.4 | 21.1 | 21.4 |
| | P mean | 27.8 | 31.6 | 31.8 | 30.4 |
| Rotorua | 0 | 31.6 | 43.6 | 40.1 | 38.4 |
| | 50 | 22.9 | 28.7 | 28.2 | 26.6 |
| | 100 | 18.6 | 20.3 | 23.3 | 20.7 |
| | P mean | 24.4 | 30.9 | 30.5 | 28.6 |
| Glen Innes | 0 | 34.8 | 33.5 | 35.6 | 34.6 |
| | 50 | 20.8 | 25.6 | 24.9 | 23.8 |
| | 100 | 18.7 | 21.9 | 21.2 | 20.6 |
| | P mean | 24.8 | 27.0 | 27.2 | 26.3 |
| Almeida de Sayago | 0 | 42.7 | 44.3 | 41.2 | 42.7 |
| | 50 | 33.5 | 32.3 | 36.4 | 34.1 |
| | 100 | 21.6 | 27.0 | 30.5 | 26.4 |
| | P mean | 32.6 | 34.6 | 36.0 | 34.4 |
| Stratford | 0 | 35.1 | 41.2 | 42.2 | 39.5 |
| | 50 | 23.5 | 28.1 | 30.2 | 27.3 |
| | 100 | 18.3 | 21.5 | 23.3 | 21.0 |
| | P mean | 25.6 | 30.3 | 31.9 | 29.3 |
| Tara Hills | 0 | 54.3 | 50.3 | 51.5 | 52.1 |
| | 50 | 31.6 | 39.0 | 38.0 | 36.2 |
| | 100 | 22.9 | 31.7 | 34.0 | 29.6 |
| | P mean | 36.3 | 40.4 | 41.2 | 39.3 |

| | L.S.D. 5% | L.S.D. 1% | Sig. int. |
|---|---|---|---|
| Strains: | 2.4 | 3.4 | *** |
| Strains X N and P: | 4.2 | 5.5 | N = N.S., P = N.S. |
| Strains X N X P: | 6.0 | 7.9 | N.S. |

C.V.% 11.68

APPENDIX V. Utilization of P by shoots of Yorkshire fog in response to applied N and P (D.M./g P absorbed)

| Strain | N level (kg/ha) | P level (kg/ha) 10 | 60 | 180 | N mean |
|---|---|---|---|---|---|
| Massey Basyn | 0 | 299 | 175 | 147 | 207 |
| | 50 | 477 | 292 | 212 | 327 |
| | 100 | 543 | 429 | 270 | 414 |
| | P mean | 440 | 298 | 210 | 316 |
| Dipton | 0 | 321 | 172 | 153 | 216 |
| | 50 | 576 | 299 | 178 | 351 |
| | 100 | 496 | 419 | 230 | 382 |
| | P mean | 464 | 297 | 187 | 316 |
| Abbotsford | 0 | 323 | 196 | 140 | 220 |
| | 50 | 464 | 256 | 169 | 297 |
| | 100 | 541 | 400 | 228 | 390 |
| | P mean | 443 | 284 | 179 | 302 |
| Rotorua | 0 | 354 | 216 | 151 | 240 |
| | 50 | 511 | 287 | 162 | 320 |
| | 100 | 446 | 365 | 208 | 339 |
| | P mean | 437 | 289 | 174 | 300 |
| Glen Innes | 0 | 315 | 216 | 155 | 229 |
| | 50 | 496 | 311 | 227 | 345 |
| | 100 | 530 | 369 | 283 | 394 |
| | P mean | 447 | 299 | 221 | 322 |
| Almeida de Sayago | 0 | 291 | 210 | 190 | 230 |
| | 50 | 463 | 323 | 204 | 330 |
| | 100 | 560 | 471 | 239 | 423 |
| | P mean | 438 | 335 | 211 | 328 |
| Stratford | 0 | 319 | 179 | 137 | 212 |
| | 50 | 523 | 422 | 189 | 378 |
| | 100 | 487 | 437 | 287 | 403 |
| | P mean | 443 | 346 | 204 | 331 |
| Tara Hills | 0 | 312 | 241 | 172 | 242 |
| | 50 | 519 | 300 | 188 | 336 |
| | 100 | 494 | 435 | 243 | 390 |
| | P mean | 442 | 325 | 201 | 322 |

| | L.S.D. 5% | L.S.D. 1% | Sig. int. |
|---|---|---|---|
| Strains: | 32 | 43 | N.S. |
| Strains X N and P: | 34 | 45 | N = N.S., P = N.S. |
| Strain X N X P: | 59 | 79 | N.S. |

APPENDIX VI. Shoot yields of strains of perennial ryegrass in response to applied N and P (g D.M./pot)

| Strain | N level (kg/ha) | P level (kg/ha) 0 | 60 | 180 | N mean |
|---|---|---|---|---|---|
| R.v.P. | 0 | 1.913 | 2.467 | 2.868 | 2.416 |
| | 50 | 2.054 | 5.530 | 7.311 | 4.965 |
| | 100 | 0.984 | 5.111 | 7.110 | 4.402 |
| | P mean | 1.650 | 4.369 | 5.763 | 3.927 |
| W.W. Virus | 0 | 1.971 | 2.249 | 2.598 | 2.273 |
| | 50 | 1.070 | 3.960 | 5.684 | 3.571 |
| | 100 | 1.656 | 4.784 | 5.658 | 4.032 |
| | P mean | 1.565 | 3.664 | 4.647 | 3.292 |
| Manhattan | 0 | 1.454 | 2.456 | 2.507 | 2.139 |
| | 50 | 1.396 | 4.390 | 6.779 | 4.188 |
| | 100 | 1.714 | 4.826 | 6.635 | 4.392 |
| | P mean | 1.521 | 3.890 | 5.307 | 3.573 |
| Medea | 0 | 1.107 | 2.248 | 2.844 | 2.066 |
| | 50 | 1.619 | 6.238 | 6.608 | 4.821 |
| | 100 | 1.602 | 4.990 | 6.241 | 4.278 |
| | P mean | 1.443 | 4.492 | 5.231 | 3.722 |
| S.23 | 0 | 1.300 | 2.778 | 3.397 | 2.492 |
| | 50 | 1.638 | 6.234 | 5.786 | 4.552 |
| | 100 | 1.513 | 5.534 | 8.006 | 5.017 |
| | P mean | 1.483 | 4.848 | 5.730 | 4.020 |
| Ruanui | 0 | 1.741 | 2.307 | 2.802 | 2.283 |
| | 50 | 1.845 | 5.156 | 6.914 | 4.638 |
| | 100 | 1.749 | 6.249 | 8.313 | 5.437 |
| | P mean | 1.778 | 4.571 | 6.009 | 4.119 |
| Mangere | 0 | 1.628 | 2.580 | 3.340 | 2.516 |
| | 50 | 2.135 | 5.790 | 7.710 | 5.212 |
| | 100 | 1.463 | 5.137 | 8.949 | 5.183 |
| | P mean | 1.742 | 4.502 | 6.666 | 4.303 |

| | L.S.D. 5% | L.S.D. 1% | Sig. int. |
|---|---|---|---|
| Strains: | 0.492 | 0.653 | *** |
| Strains X N and P: | 0.853 | 1.131 | N***, P = N.S. |
| Strain X N X P: | 1.478 | 1.959 | N.S. |

C.V.% 17.67

## APPENDIX VII. Absorption of N by shoots of perennial ryegrass in response to applied N and P. (mg/pot)

| Strain | N level (kg/ha) | P level (kg/ha) 0 | 60 | 180 | N mean |
|---|---|---|---|---|---|
| R.v.P. | 0 | 88 | 72 | 90 | 84 |
| | 50 | 100 | 246 | 330 | 232 |
| | 100 | 51 | 261 | 346 | 219 |
| | P mean | 80 | 199 | 255 | 178 |
| W.W. Virus | 0 | 87 | 68 | 74 | 77 |
| | 50 | 52 | 187 | 278 | 172 |
| | 100 | 85 | 243 | 297 | 208 |
| | P mean | 75 | 166 | 217 | 152 |
| Manhattan | 0 | 61 | 78 | 77 | 72 |
| | 50 | 67 | 196 | 308 | 190 |
| | 100 | 84 | 243 | 340 | 228 |
| | P mean | 71 | 172 | 242 | 161 |
| Medea | 0 | 53 | 74 | 92 | 73 |
| | 50 | 81 | 313 | 327 | 240 |
| | 100 | 83 | 260 | 336 | 226 |
| | P mean | 72 | 216 | 251 | 180 |
| S.23 | 0 | 55 | 80 | 85 | 73 |
| | 50 | 77 | 267 | 258 | 201 |
| | 100 | 75 | 279 | 385 | 247 |
| | P mean | 69 | 209 | 242 | 174 |
| Ruanui | 0 | 76 | 62 | 81 | 73 |
| | 50 | 90 | 238 | 297 | 208 |
| | 100 | 85 | 305 | 429 | 273 |
| | P mean | 83 | 202 | 269 | 185 |
| Mangere | 0 | 77 | 72 | 87 | 78 |
| | 50 | 104 | 279 | 343 | 242 |
| | 100 | 70 | 274 | 445 | 263 |
| | P mean | 84 | 208 | 292 | 195 |

| | L.S.D. 5% | L.S.D. 1% | Sig. int. |
|---|---|---|---|
| Strains: | 25 | 33 | *** |
| Strains X N and P: | 43 | 57 | N = ***, P = N.S. |
| Strain X N X P: | 61 | 81 | N.S. |

C.V. % 20.23

APPENDIX VIII. Absorption of P by shoots of perennial ryegrass in response to applied N and P. (mg/pot)

| Strain | N level (kg/ha) | P level (kg/ha) 0 | 60 | 180 | N mean |
|---|---|---|---|---|---|
| R.v.P. | 0 | 4.2 | 8.3 | 12.2 | 8.2 |
| | 50 | 4.1 | 13.1 | 20.5 | 12.6 |
| | 100 | 2.2 | 12.4 | 20.1 | 11.6 |
| | P mean | 3.5 | 11.3 | 17.6 | 10.8 |
| W.W. Virus | 0 | 4.6 | 7.9 | 11.4 | 7.8 |
| | 50 | 2.4 | 8.3 | 17.7 | 9.5 |
| | 100 | 3.6 | 11.5 | 18.6 | 11.2 |
| | P mean | 3.5 | 9.2 | 15.8 | 9.5 |
| Manhattan | 0 | 3.4 | 8.3 | 10.5 | 7.4 |
| | 50 | 2.8 | 9.3 | 18.1 | 10.1 |
| | 100 | 3.5 | 11.5 | 19.2 | 11.4 |
| | P mean | 3.2 | 9.7 | 16.0 | 9.6 |
| Medea | 0 | 2.9 | 8.3 | 15.3 | 8.8 |
| | 50 | 3.9 | 15.8 | 20.7 | 13.5 |
| | 100 | 3.3 | 11.0 | 18.1 | 10.8 |
| | P mean | 3.4 | 11.7 | 18.1 | 11.1 |
| S.23 | 0 | 3.2 | 9.4 | 13.4 | 8.6 |
| | 50 | 3.1 | 13.6 | 17.0 | 11.2 |
| | 100 | 3.5 | 12.9 | 22.2 | 12.9 |
| | P mean | 3.3 | 11.9 | 17.5 | 10.9 |
| Ruanui | 0 | 4.5 | 8.7 | 12.4 | 8.5 |
| | 50 | 4.2 | 11.1 | 20.4 | 11.9 |
| | 100 | 2.9 | 13.5 | 25.0 | 13.8 |
| | P mean | 3.9 | 11.1 | 19.2 | 11.4 |
| Mangere | 0 | 4.3 | 9.5 | 14.1 | 9.3 |
| | 50 | 4.9 | 14.4 | 21.4 | 13.6 |
| | 100 | 2.6 | 14.4 | 24.8 | 13.9 |
| | P mean | 3.9 | 12.8 | 20.1 | 12.3 |

| | L.S.D. 5% | L.S.D. 1% | Sig. int. |
|---|---|---|---|
| Strains: | 1.4 | 1.9 | *** |
| Strains X N and P: | 2.5 | 3.3 | N = *, P = N.S. |
| Strains X N X P: | 3.5 | 4.6 | N.S. |

C.V. % 18.96

APPENDIX IX. Utilization of N by shoots of perennial ryegrass in response to applied N and P (g D.M./g N absorbed)

| Strain | N level (kg/ha) | P level (kg/ha) | | | N mean |
|---|---|---|---|---|---|
| | | 0 | 60 | 180 | |
| R.v.P. | 0 | 21.8 | 34.1 | 31.7 | 29.2 |
| | 50 | 20.4 | 20.9 | 22.0 | 21.1 |
| | 100 | 19.3 | 19.5 | 20.5 | 19.8 |
| | P mean | 20.5 | 24.8 | 24.7 | 23.4 |
| W.W. Virus | 0 | 22.3 | 32.9 | 34.9 | 30.0 |
| | 50 | 19.6 | 21.1 | 20.4 | 20.3 |
| | 100 | 19.4 | 19.6 | 18.9 | 19.3 |
| | P mean | 20.4 | 24.5 | 24.7 | 23.2 |
| Manhattan | 0 | 23.5 | 31.6 | 32.5 | 29.2 |
| | 50 | 20.6 | 22.3 | 21.9 | 21.6 |
| | 100 | 20.1 | 19.8 | 19.4 | 19.8 |
| | P mean | 21.4 | 24.6 | 24.6 | 23.5 |
| Medea | 0 | 20.4 | 30.0 | 21.0 | 27.1 |
| | 50 | 20.1 | 20.4 | 20.2 | 20.2 |
| | 100 | 19.0 | 19.1 | 18.5 | 18.9 |
| | P mean | 19.8 | 23.2 | 23.2 | 22.1 |
| S.23 | 0 | 23.2 | 34.3 | 40.1 | 32.6 |
| | 50 | 21.3 | 23.7 | 22.6 | 22.5 |
| | 100 | 19.9 | 19.8 | 20.7 | 20.1 |
| | P mean | 21.5 | 25.9 | 27.8 | 25.1 |
| Ruanui | 0 | 22.9 | 36.8 | 34.2 | 31.3 |
| | 50 | 20.2 | 21.7 | 23.2 | 21.7 |
| | 100 | 20.6 | 20.4 | 19.3 | 20.1 |
| | P mean | 21.2 | 26.3 | 25.6 | 24.4 |
| Mangere | 0 | 21.0 | 35.6 | 38.2 | 31.6 |
| | 50 | 20.3 | 20.7 | 22.4 | 21.1 |
| | 100 | 20.6 | 18.6 | 20.1 | 19.7 |
| | P mean | 20.6 | 24.9 | 26.9 | 24.2 |

| | L.S.D. 5% | L.S.D. 1% | Sig. int. |
|---|---|---|---|
| Strains: | 0.9 | 1.2 | *** |
| Strains X N and P: | 1.6 | 2.2 | N = ***, P = *** |
| Strain X N X P: | 2.8 | 3.7 | *** |

C.V.% 7.82

APPENDIX X. Utilization of absorbed P by shoots of perennial ryegrass in response to applied N and P. (g D.M./g P absorbed)

| Strain | N level | P level (kg/ha) | | | N level |
|---|---|---|---|---|---|
| | (kg/ha) | 0 | 60 | 180 | |
| R.v.P. | 0 | 456 | 297 | 234 | 329 |
| | 50 | 522 | 426 | 356 | 435 |
| | 100 | 444 | 409 | 352 | 402 |
| | P mean | 474 | 377 | 314 | 388 |
| W.W. Virus | 0 | 421 | 284 | 235 | 313 |
| | 50 | 411 | 475 | 319 | 401 |
| | 100 | 463 | 415 | 301 | 393 |
| | P mean | 432 | 391 | 285 | 369 |
| Manhattan | 0 | 440 | 294 | 237 | 324 |
| | 50 | 498 | 470 | 372 | 447 |
| | 100 | 417 | 426 | 344 | 417 |
| | P mean | 473 | 397 | 317 | 396 |
| Medea | 0 | 373 | 269 | 185 | 276 |
| | 50 | 413 | 409 | 320 | 381 |
| | 100 | 476 | 451 | 342 | 423 |
| | P mean | 421 | 376 | 282 | 360 |
| S.23 | 0 | 401 | 295 | 254 | 317 |
| | 50 | 521 | 455 | 339 | 439 |
| | 100 | 417 | 429 | 359 | 402 |
| | P mean | 447 | 393 | 318 | 386 |
| Ruanui | 0 | 382 | 263 | 226 | 290 |
| | 50 | 433 | 463 | 337 | 411 |
| | 100 | 595 | 468 | 334 | 466 |
| | P mean | 470 | 398 | 299 | 389 |
| Mangere | 0 | 380 | 271 | 236 | 296 |
| | 50 | 431 | 401 | 359 | 397 |
| | 100 | 543 | 356 | 361 | 420 |
| | P mean | 452 | 343 | 319 | 371 |

| | L.S.D. 5% | L.S.D. 1% | Sig. int. |
|---|---|---|---|
| Strains: | 20 | 27 | *** |
| Strains X N and P: | 36 | 47 | N**, P N.S. |
| Strains X N X P: | 62 | 82 | ** |

C.V. % 7.82

APPENDIX XI. Dry matter yields, absorption and utilization of P by strains of Lotus (g D.M./pot, mg P/pot and g D.M./g P absorbed).

| | Level of P | | | | |
|---|---|---|---|---|---|
| Yield | 15 | 30 | 60 | 100 | 200 |
| L. hybrid | 4.258 | 5.897 | 7.106 | 7.716 | 8.980 |
| Marshfield | 5.522 | 5.935 | 6.896 | 7.515 | 8.663 |
| L. tetraploid | 5.621 | 6.858 | 7.656 | 8.851 | 10.322 |
| Dunedin | 4.799 | 5.717 | 7.442 | 7.447 | 7.401 |
| Log Lagos | 4.868 | 5.940 | 6.950 | 7.339 | 8.420 |
| Chile | 5.173 | 6.433 | 6.740 | 7.370 | 8.914 |
| Wairoa | 4.826 | 6.003 | 6.835 | 8.154 | 8.543 |
| Timaru | 4.097 | 4.503 | 6.177 | 6.745 | 7.886 |

| | | | | |
|---|---|---|---|---|
| Strains: | L.S.D. 5%, 0.444, | L.S.D. 1% 0.588, | Sig. int. | $p<0.001$ |
| P: | " " 0.351, | " " 0.465, | " " | $p<0.001$ |
| Strains X P: | " " 0.994, | " " 1.315, | " " | $p>0.05$ |

C.V.% 10.35

Absorption

| | | | | | |
|---|---|---|---|---|---|
| L.hybrid | 13.4 | 18.9 | 23.2 | 27.4 | 32.9 |
| Marshfield | 16.1 | 18.2 | 23.2 | 23.6 | 33.9 |
| L.tetraploid | 13.9 | 18.9 | 23.5 | 28.3 | 40.7 |
| Dunedin | 14.8 | 17.6 | 24.7 | 25.5 | 28.6 |
| Los Lagos | 13.6 | 18.8 | 22.1 | 24.5 | 31.8 |
| Chile | 14.7 | 19.0 | 21.9 | 27.2 | 35.5 |
| Wairoa | 13.1 | 18.3 | 21.9 | 27.2 | 34.2 |
| Timaru | 14.1 | 16.2 | 21.8 | 25.3 | 31.7 |

| | | | | |
|---|---|---|---|---|
| Strains: | L.S.D. 5% 1.3, | L.S.D. 1% 1.7, | Sig. int. | $p<0.05$ |
| P: | " " 1.1, | " " 1.4, | " " | $p<0.001$ |
| Strains X P: | " " 3.0, | " " 4.0, | " " | $p>0.05$ |

C.V.% 14.09

Utilization

| | | | | | |
|---|---|---|---|---|---|
| L.hybrid | 317 | 312 | 306 | 281 | 272 |
| Marshfield | 343 | 325 | 296 | 318 | 255 |
| L.tetraploid | 403 | 361 | 324 | 312 | 253 |
| Dunedin | 323 | 324 | 300 | 291 | 258 |
| Los Lagos | 356 | 315 | 314 | 298 | 264 |
| Chile | 349 | 338 | 307 | 303 | 251 |
| Wairoa | 365 | 328 | 311 | 299 | 249 |
| Timaru | 290 | 277 | 283 | 266 | 248 |

| | | | | |
|---|---|---|---|---|
| Strains: | L.S.D. 5% 41, | L.S.D. 1% 55, | Sig. int. | $p<0.001$ |
| P: | " " 32, | " " 43, | " " | $p<0.001$ |
| Strains X P: | " " 92, | " " 122, | " " | $p>0.05$ |

C.V.% 14.14

APPENDIX XII. Dry matter yields of strains of white clover.
(g D.M./pot)

|  | Level of P (kg/ha) |  |  |  |  |
|---|---|---|---|---|---|
|  | 15 | 30 | 60 | 100 | 200 |
| Huia | 2.611 | 3.537 | 4.804 | 5.503 | 5.658 |
| Pitau | 3.069 | 3.690 | 4.774 | 5.843 | 5.249 |
| Tekapo | 2.076 | 2.242 | 3.639 | 3.075 | 4.087 |
| Bituanai | 2.456 | 3.595 | 4.007 | 5.079 | 5.894 |
| S.100 | 2.905 | 3.347 | 4.826 | 5.704 | 5.825 |
| S.184 | 2.975 | 2.517 | 3.951 | 5.523 | 6.641 |
| Tamar | 2.604 | 3.275 | 4.934 | 5.387 | 6.608 |
| Spanish | 2.487 | 3.387 | 3.520 | 4.577 | 6.253 |
| K.W.W. | 2.302 | 2.403 | 3.835 | 4.362 | 5.729 |
| Louisiana | 2.813 | 3.830 | 4.127 | 5.270 | 6.164 |

|  | L.S.D. 5% | L.S.D. 1% | Sig. int. |
|---|---|---|---|
| Strains: | 0.540 | 0.698 | $p<0.001$ |
| P: | 0.381 | 0.492 | $p<0.001$ |
| Strains X P: | 1.205 | 1.558 | $p>0.05$ |

C.V.% 17.84

APPENDIX XIII. Absorption of P by strains of white clover. (mg/pot)

| Strains | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 100 | 200 |
| Huia | 7.48 | 20.20 | 14.97 | 18.28 | 20.40 |
| Pitau | 6.74 | 10.75 | 14.02 | 18.56 | 19.46 |
| Tekapo | 6.23 | 7.56 | 12.11 | 12.12 | 14.19 |
| Bituanai | 6.67 | 10.82 | 13.09 | 14.42 | 22.07 |
| S.100 | 8.61 | 7.85 | 16.43 | 18.04 | 20.80 |
| S.184 | 9.45 | 8.41 | 13.31 | 19.61 | 22.50 |
| Tamar | 7.14 | 8.39 | 13.77 | 16.46 | 20.97 |
| Spanish | 7.35 | 10.47 | 12.15 | 15.03 | 20.90 |
| K.W.W. | 6.95 | 8.17 | 13.33 | 16.77 | 21.32 |
| Louisiana | 6.89 | 10.11 | 11.84 | 14.09 | 17.75 |

| | L.S.D. 5% | L.S.D. 1% | Sig. int. |
|---|---|---|---|
| Strains: | 1.74 | 2.31 | $p<0.001$ |
| P: | 0.54 | 1.63 | $p<0.001$ |
| Strains X P: | 3.91 | 5.16 | $p>0.05$ |

C.V.% 18.17

Appendix XIV. Utilization of P by strains of white clover (g D.M./g P absorbed)

| Strains | Level of P (kg/ha) | | | | |
|---|---|---|---|---|---|
| | 15 | 30 | 60 | 100 | 200 |
| Huia | 348 | 345 | 321 | 301 | 281 |
| Pitau | 415 | 347 | 339 | 318 | 271 |
| Tekapo | 305 | 297 | 298 | 251 | 287 |
| Bituanai | 368 | 331 | 304 | 366 | 233 |
| S.100 | 337 | 380 | 293 | 317 | 278 |
| S.184 | 312 | 299 | 298 | 283 | 292 |
| Tamar | 366 | 390 | 315 | 335 | 238 |
| Spanish | 344 | 322 | 292 | 304 | 280 |
| K.W.W. | 332 | 293 | 287 | 257 | 279 |
| Louisiana | 415 | 375 | 344 | 375 | 356 |

| | L.S.D. 5% | L.S.D. 1% | Sig.int. |
|---|---|---|---|
| Strains: | 39 | 43 | $p<0.001$ |
| P: | 19 | 31 | $p<0.001$ |
| Strain X P: | 74 | 98 | $p<0.05$ |

C.V.% 14.35

APPENDIX XV. Allocation of degrees of freedom in the analysis of variance.

| Source | df |
|---|---|
| Species/strain | 5 |
| N treatment | 4 |
| N linear | 1 |
| N quadratic | 1 |
| N residual | 2 |
| Species/strain X N | 20 |
| Species/strain X linear | 5 |
| Species/strain X quadratic | 5 |
| Species/strain X residual | 10 |
| Error | 90 |
| Total | 143 |

Appendix XVI. Allocation of degrees of freedom in the analysis of variance of Experiment 6.

| Source | df |
|---|---|
| Species/strains | 4 |
| P treatment | 4 |
| P linear | 1 |
| P quadratic | 1 |
| P residual | 2 |
| Species/strains X P | 16 |
| Species/strains X linear | 4 |
| Species/strains X quadratic | 4 |
| Species/strains X residual | 8 |
| Error | 75 |
| Total | 119 |

# BIBLIOGRAPHY

Alcock, M.B. 1964. The physiological significance of defoliation on subsequent regrowth of grass clover mixtures and cereals. pp 25-41. In British Ecological Society. Grazing in terrestrial and marine environment; a symposium, Bangor, 11-14 April, 1962, edited by Crisp, D.J., Blackwell, Oxford.

Allos, H.F., Bartholomew, W.V. 1959. Replacement of symbiotic fixation by available nitrogen. Soil Sci. 87, 61-66.

Anderson, W.P. 1972. Ion transport in the cells of higher plants. Annu.Rev. Plant Physiol. 23, 51-72.

Andrews, C.S. 1966. A kinetic study of phosphate absorption by excised roots of Stylosanthas humilis, Phaseolas lathyroides, Desmodium unicatum, Medicago ratiba and Hordeum vulgare. Aust. J. agric. Res. 17, 611-624.

Asia, T. 1944. Uber die Mykorrhizen bildung der Leguminosen-Pflazen. Jap.J.Bot. 13, 463-468.

Asher, C.J., Loneragan, J.F. 1967. Response of plants to phosphate concentration in solution culture. I Growth and phosphorus content. Soil. Sci. 103, 225-233.

Barclay, P.C. 1957. Improvement of Lotus uliginosus. Proc. N.Z. Grassl.Ass. 19, 75-80.

________________, 1969. Some aspects of the development and performances of 'Grasslands 4700' white clover. Proc. N.Z.Grassl.Ass. 31, 127-150.

________________, Lambert, J.P. 1970. The breeding and testing of Lotus pendunculatus Cav. in New Zealand. Proc. 11th Int. Grassl.Congr., Surfers Paradise, 278-281.

Barber, D.A. 1974. The absorption of ions by micro-organisms and excised roots. New Phytol. 73, 91-96.

________________, Frankenburg, U.C. 1971. The contribution of micro-organisms to the apparent absorption of ions by roots grown under non-sterile conditions. New Phytol, 70, 1027-1034.

Barberr, S.A. 1962. A diffusion and mass flow concept of soil nutrient availability. Soil Sci. 93, 39-49.

Barrow, N.J. 1975a. The response to phosphate of two annual pasture species. I. Effects of the soil's ability to absorb phosphate on comparative phosphate requirement. Aust.J.Agric.Res. 26, 137-143.

________________, 1975b. The response to phosphate of two annual pasture species. II. The specific rate of uptake of phosphate, its distribution and use for growth. Aust.J.Agric.Res., 26, 145-156.

Barton, C.J. 1948. Photometric analysis of phosphate rock. *Anal.Chem.* *20*, 1068-1076.

Baylis, G.T.S. 1970. Root hair and phycomycetous mycorrhixaz in phosphorus deficient soil. *Pl. Soil.* *33*, 713-16.

____________, 1971. Endogenous mycorrhizas synthesised in *Leptospermum* (Myrtacwae), *N.Z.J.Bot.*, *9*, 293-296.

____________, 1972. Minimum levels of available phosphorus for non-mycorrhizal plants. *Pl. Soil*, *36*, 233-234.

Beard, D.F., Hollowell, E.A. 1952. The effect on performance when seed of forage crop varieties is grown under different climatic and environmental conditions. *Proc. 16th Int.Grassl.Congr.*, Reading, 860-966.

Beevers, H. 1969. Metabolic sinks. p. 169-184. *In* Dinauer, R.C. ed. Physiological aspects of crop yield. American Society of Agronomy,Crop Science Society of America, Madison.

Beevers, L., Hageman, R.H. 1969. Nitrate reduction in higher plants. *Annu Rev. Plant Physiol.* *20*, 495-522.

Bergesen, F.J. 1971. The central reaction of nitrogen fixation. pp 511-524. *In* Lie, T.E., Mulder, E.G. eds. Biological nitrogen fixation in natural and agricultural habitats. *Pl. Soil*, Special Volume. Martinus Nijhoff, The Hague.

Biddulph, O. 1941. Diurnal migration of injected radiophosphorus from bean leaves. *Am.J.Bot.* *28*, 348-52.

____________, 1969. Mechanisms of translocation of plant metabolites, pp 143-164. *In* Dinauer, R.C. ed. Physiological aspects of crop yield. American Society of Agronomy, Crop Science Society of America, Madison.

____________, Cory, R. 1960. Demonstration of two translocation mechanisms in studies of bidirectional movement. *Plant Physiol.* *35*, 689-695.

____________, _______. 1965. Translocation of $C^{14}$ metabolites in the phloem of the bean plant. *Plant Physiol.* *40*, 119-129.

____________, Biddulph, S., Cory, R., Koontz, H. 1958. Circulation patterns for phosphorus, sulfur and calcium in the bean plant. *Plant Physiol.* *33*, 293-300.

____________, Markle, J. 1944. Translocation of radiophosphorus in the phleum of the cotton plant. *Am.J.Bot.* *31*, 65-70.

Bieleski, R.L. 1973. Phosphate pools, phosphate transport, and phosphate availability. *Annu. Rev. Plant Physiol.* *24*, 225-252.

Black, C.A. 1969. Soil-plant relationships. 2nd ed. John Wiley, New York, 792 p.

Black, J.N. 1957. The influence of varying light intensity on the growth of herbage plants. *Herb.Abstr.* *27*, 89-97.

Blackman, G.E., Black, J.N. 1959. Physiological and ecological studies in the analysis of plant environment. II. A further assessment of the influence of shading on the growth of different species in the vegetative phase. Ann.Bot. (Lond.) N.S., 23, 51-63.

Blackman, G.E., Templeman, W.G. 1938. The interaction of light intensity and nitrogen supply in the growth and metabolism of grasses and clovers (Trifolium repens.). 2. The influence of light intensity and nitrogen supply on the leaf production of frequently defoliated plants. Ann.Bot. 2, 765-791.

______________, Wilson, G.L. 1951a. Physiological and ecological studies in the analysis of plant environment. 6. The constancy of different species of a logarithmic relationship between net assimilation rate and light intensity and its ecological significance. Ann.Bot.(Lond.)N.S. 15, 63-94.

______________, ____________ 1951b. Physiological and ecological studies in the analysis of plant environment. 7. An analysis of the differential effects of light intensity on the net assimilation rate, leaf area, ratio and relative growth rate of different species. Ann.Bot.(Lond.) N.S. 15, 373-408.

Blackmore, L.C., Searle, P.L., Daly, P.K. 1972. Methods for chemical analysis of soils. In New Zealand. Soil Bureau, Laboratory Methods. (Soil Bureau Scientific Report 10, New Zealand.)

Brewster, J.L., Tinker, P.B.H. Nutrient flow-rates in roots. Soils Fertil. 35, 355-359.

Briggs, G.E., Hope, A.B., Pitman, M.G. 1958. Exchangeable ions in beet disks at low temperature. J.Exp.Bot. 9, 128-41.

Brock, J.L. 1973. Growth and nitrogen fixation of pure stands of three pasture legumes with high/low phosphate. N.Z.J.agric.Res. 16, 483-491.

______________, Hoglund, J.H. 1974. The growth of 'Grasslands 4700' white clover. 2. Effects of nitrogen and phosphorus. N.Z.J.agric.Res. 17, 47-63.

Chowdbury, M.S., Marshall, K.C., Parker, C.S. 1968. Growth rates of Rhizobium trifolli and Rhizobium lupini in sterilized soil. Aust.J.agric.Res. 19, 919-925.

Clarkson, D.T., Sanderson, J. 1971. Relationship between the anatomy of cereal roots and the absorption of nutrients and water, pp 16-25. (Agricultural Research Council. Letcombe Laboratory, Report 1970).

Cooper, J.P., Tainton, N.M. 1968. Light and temperature requirements for the growth of tropical and temperate grasses. Herb.Abstr. 38, 167-176.

Conway, E.J., Downey, M. 1950. An outer metabolic region of the yeast cell. Biochem.J. 47, 347-355.

Cowan, I.R. 1965. Transport of water in the soil-plant atmosphere system. J.appl.Ecol. 2, 221-238.

Craft, A.S., Crisp, C.E. 1971. Phloem transport in plants. W.H. Freeman, San Fransisco.

Crossley, G.K., Bradshaw, A.D. 9168. Differences in response to mineral nutrition of populations of ryegrass, Lolium perenne L. and Orchardgrass, Dactylis glomerata L. Crop Sci. 8, 383-87.

Crush, J.R. 1973. The effects of Rhizophagus tenuis mycorrhiza on ryegrass, cocksfoot and sweet vernal. New Phytol. 72, 965-973.

__________, 1974. Plant growth response to vesicular-arbuscular mycorrhiza. 7. Growth and nodulation of some herbage legumes. New Phytol. 73, 743-49.

Cullen, N.A. Personal communication. Ruakura Agricultural Research Centre, Hamilton.

Dart, P.J., Day, J.M. 9171. Effect of incubation temperature and oxygen tension on nitrogenase activity of legume root nodules. pp 167-184. In Lie, T.E., Mulder, E.G. eds. Biological nitrogen fixation in natural habitats. Pl. Soil Special Volume. Martinus Nijhoff, The Hague.

__________, __________, Harris, D. 1972. Assay of nitrogenase activity by acetylene reduction. pp 85-100. In FAO/IAEA, Use of isotopes for study of fertiliser utilization by legume crops. Rome. (Technical Report No. 149).

Davies, W. 1940. The yield and other characteristics of grasses and clovers when sown in simple mixtures on exposed hills at an elevation of 1300 ft. Welsh,J.Agric. 16, 175-205.

Davies, W.E. 1958. The yield of pure sown plots of eight white clover strains under cutting. J.Br.Grassl.Soc. 13, 34-38.

__________, Evans, D.R. 1968. Improving competition ability in white clover. Rep. Welsh Pl. Breed. Stn for 1967, 43-44.

Drew, M.C., Ashley, T.W. 1971. Effect of variation in the supply of nitrate on root development. Rep. agric. Res. Coun. Letcombe Lab. 1970, 7-8.

Dunbar, G.A. 1970. Grasses and high altitude revegetation. Soil and Water. (Wellington) 6, 20-23.

__________. 1971. The effectiveness of some herbaceous species for montane sub-alpine revegetation. Proc.N.Z.Ecol.Soc. 18, 48-57.

__________, Adams, S.N. 1972. Nutrient responses on exposed mountain subsoils. Proc.N.Z.Grassl.Ass. 34, 129-138.

During,C. 1972. Fertilisers and Soils in New Zealand farming. 2nd ed. A.R. Shearer, Wellington. 312p.

__________, Cullen N.A., Bennett, G.M. 1962. The establishment of pastures on yellow-brown loams near Te Anau. N.Z.J.agric.Res. 5, 278-293.

Edwards, D.G. 1968. The mechanisms of phosphate absorption by plant roots. pp 183-189. In Proc. 9th Int. Congr.Soil Sci. Trans.

Elliot, I.L. 1968. Making full use of fertiliser. Proc.Ruakura Farmers' Conf., 84-90.

Engin, M., Spront, J.I. 1973. Effect of water stress on growth and nitrogen fixation of Trifolium repens. New Phytol. 72, 117-126.

Epstein, E. 1955. Passive permeation and active transport of ions in plant roots. Plant Physiol. 30, 529-535.

__________, 1963. Selective ion transport in plants and its genetic control. pp 284-298. In Desalination Research Conference, Natl.Acad.Sci.Natl.Counc. Publ. 942.

__________, 1972. Mineral Nutrition of plants: principals and perspectives. John Wiley, New York. 412 p.

__________, Leggett, J.E. 1954. The absorption of alkaline earth cations by by barley roots. Am.J.Bot. 41, 785-791.

__________, Rains, D.W., Elzam, O.E. 1963. Resolution of dual mechanism of potassium absorption by barley roots. Proc.Natl.Acad.Sci. (U.S.A.) 49, 684-692.

Esau, K. 1969. The Phloem. Gebruder Borntraeger, Berlin. ix + 505p.

Evans, P.S. 1970. Root growth of Lolium perenne L. 1. Effect of plant age, seed weight and nutrient concentration on root weight, length and number of apices. N.Z.J.Bot. 8, 344-356.

Fageria, N.K. 1974. Kinetics of phosphate absorption by intact rice plant . Aust.J.agric.Res. 25, 395-400.

Finn, R.F. 1942. Mycorrhizal inoculation of soil of low fertility. Black Rock Forest Papers 1, 116-117.

Foote, B.D., Howell, R.W. 1964. Phosphorus tolerance and sensitity of soybeans as related to uptake and translocation. Plant Physiol. 39, 610-613.

Forde, B.J. 1966. Effect of various environments on the anatomy and growth of perennial ryegrass and cocksfoot. 1. Leaf growth. N.Z.J.Bot. 4, 455-468.

Gardner, A.L., Hunt, I.V. 1961. Inter-varietal competition in perennial ryegrass swards. J.Br.Grassl.Soc. 17, 285-291.

Gauch, H.G. 1972. Inorganic plant nutrition. Dowden, Hutchison and Ross, Stroudsburg, Pa. 488p.

Gerloff, G.C. 1963. Comparative mineral nutrition of plants. Annu.Rev.Plant Physiol. 14, 107-124.

Gibson, A.H. 1966. The carbohydrate requirements for symbiotic nitrogen fixation, a "Whole-plant" growth analysis approach. Aust.J.biol.Sci. 19, 499-451.

__________, 1967. Physical environment and symbiotic nitrogen fixation. 4. Factors affecting the early stages of nodulation. Aust.J.biol.Sci. 20, 1087-1104.

__________, 1967. Physical environment and symbiotic nitrogen fixation. 5. Effect of time of exposure to unfavourable root temperature. Aust.J.biol.Sci. 20, 1105-1117.

Gibson, A.H. 1973. The control of dinitrogen assimilation by nodulated legumes. Unpublished paper delivered to N.Z.R. Soc. Conf., Massey University.

Goodman, P.J. Personal communication. Welsh Plant Breeding Station, Aberystwyth

__________, Hughes, D.M. 1972. Ryegrass-plant nutrition. Rpt Welsh Pl. Breed. Stn for 1971, 12-14.

__________, __________. 1973. Variation in plant nutrition. (a) Nutrient response in the glasshouse, (b) Nutrient response in the field. Rpt Welsh Pl. Breed. Stn for 1972, 23-24.

__________, Shurety, J. 1971. Plant nutrition. (i) Nitrogen, (ii) Phosphorus. Rpt Welsh Pl. Breed. Stn for 1970, 11-12.

Grobbelaar, N., Clark, B., Hough, M.C. 1971. The nodulation and nitrogen fixation of isolated roots of Phaseolus vulgaris L. 2. The influence of light on nodulation. pp 203-214. In Lie, T.A., Mulder, E.G. eds. Biological nitrogen fixation in natural and agricultural habitats. Pl.Soil, Special Volume. Martinus Nijhoff, The Hague.

Hagen, C.E. Hopkins, H.T. 1955. Ionic species in orthophosphate absorption by barley roots. Plant Physiol. 30, 193-299.

Hardy, R.F.W., Holsten, R.D., Jackson, E.K., Burns, R.C. 1968. The acetylene-ethylene assay for $N_2$ fixation: Laboratory and field evaluation. Plant Physiol. 43, 1185-1207.

Harkness, R.D., Hunt, I.V., Frame, J. 1970. The effect of variety and companion grass on productivity of white clover. pp 175-180. In Lowe, J. ed. White clover research. (Br.Grassl.Soc. Occasional Symposium No. 6.)

Harlan, J.R. 1956. Theory and dynamics of grassland agriculture. Van Nostrand, Princeton. 291p.

Harley, J.L. 1959. Mycorrhiza in plants. New Sci. 6, (156), 930-931.

Haunold, E., Drinneburg, O., Fiser, I. 1968. $NH_4$ and $NO_3$ - Aufnahme durch a geschnittene gerstenwurzeln. Int.Congr.Soil Sci.Trans. 9(2), 203-207.

Healy, W.B., Widdowson, J.P., Yeates, G.W. 1973. Effect of root nematodes on the growth of seedling and established white clover on a yellow-brown loam. N.Z.J.agric.Res. 16, 70-76.

Hiatt, A.J. 1968. Electrostatic association and Donnan phenomena as mechanisms of ion accumulation. Plant Physiol. 34, 645-650.

Higinbotham, N. 1973. The mineral absorption process in plants. Bot.Rev. 39, 15-69.

Hoglund, J.H., Brock, J.L. 1974. Growth of 'Grasslans Huia' and 'Grasslands 4700' white clover. 1. Effects of temperature and nitrogen. N.Z.J.agric. Res. 17, 41-45.

Holmes, D.P. 1967. Genetic control of nitrogen uptake. 1967. Rep. Welsh Pl. Breed. Stn for 1966, 30-31.

Hopkinson, J.M. 1964. Studies on the expansion of the leaf surface. 4. The carbon and phosphorus economy of a leaf. J.exp.Bot. 15, 125-137.

Hughes, R., Nicholson, I.A. 1961. Comparison of grass varieties for surfaces seeding upland pasture types. 1. Molinia and Nardus pastures. J.Br.Grassl. Soc. 16, 210-221.

__________, ______________. 1961. Comparison of grass varieties for surface seeding pasture types. 2. Deep peat. J.Br.Grassl.Soc. 16, 314-322.

Hunt, I.V. 1964. Comparative productivity of herbage varieties on upland peat. J.Br.Grassl.Soc. 19, 55-61.

__________, Frame, J., Harkness, R.D. 1972. Classification of herbage varieties for West of Scotland conditions. Auchincruive, Grassland Husbandry Dept, West of Scotland Agricultural College, 1972. (West of Scotland Agricultural College, Grassland Husbandry Department Publication No. 320).

Jackman, R.H. 1971a. Grass clover pasture and symbiotic nitrogen-an analysis. 1. Main Factors. N.Z.Agric.Sci. 6(1), 3-7.

____________, 1971b. Grass clover pasture and symbiotic nitrogen-an analysis. 2. Interactions and evaluation. N.Z.Agric.Sci. 6(2), 9-12.

____________, Mouat, M.C.H. 1972. Competition between grass and clover for phosphate. 2. Effect of root activity, efficiency of response to phosphate and soil moisture. N.Z.J.agric.Res. 15, 667-675.

Jacques, W.A. 1962. Yorkshire fog as a pasture grass. Proc.N.Z.Grassl.Ass. 24, 139-150.

____________, 1974. Yorkshire fog (Holcus lanatus): Its potential as a pasture species. Proc.N.Z. Grassl.Ass. 35, 249-257.

Jenkin , T.J. 1930. Perennial ryegrass at Aberystwyth. Welsh J.Agric. 6, 140-165.

Jenny, H., Overstreet, R. 1939. Cation interchange between plant roots and soil colloids. Soil Sci. 47, 257-272.

Jones, R.K. 1974. A study of the phosphorus response of a wide range of accessions from genus Stylosanthes. Aust.J.agric.Res. 25, 847-962.

Karlovsky, J. 1966. Assessing fertiliser maintenance requirements. N.Z.Agric.Sci. 2, 15-18.

Keay, J., Manage, P.M.A. 1969. Automated distillation procedure for the determination of nitrogen. Analyst (Lond). 94, 895-899.

Koontz, H., Biddulph, D. 1957. Factors regulating absorption and translocation of foliar applied phosphorus. Plant Physiol. 32, 463-470.

Kramer, P.J. 1969. Plant and soil water relationships - a modern synthesis. McGraw-Hill, New York.

Lambert, J.P., Boyd, A.F. 1974. An evaluation of five varieties of Lotus pendunculatus Cav. compared with 'Grasslands Huia' white clover under grazing at Kaikohe. N.Z.J.exp.Agric. 2, 359-363.

____________, Vartha, E.W., Harris, A.J. 1969. Progress report on 'Grasslands 4700' white clover. Proc.N.Z.Grassl.Ass. 31, 135-142.

Langer, R.H.M. 1973. Growth of grasses and clovers. pp 41-63. In Langer, R.H.M. ed. Pastures and pasture plants. Dai Nippon, Hong Kong.

Lawn, R.J., Brun, W.A. 1974. Symbiotic nitrogen fixation in soybeans. 1. Effect of photosynthetic source-sink manipulation. Crop Sci. 14, 11-16.

____________, Fischer, K.S. Brun, W.J. Symbiotic nitrogen fixation in soybeans. 2. Interrelationship between carbon and nitrogen assimilation. Crop Sci. 14, 17-22.

Legget, J.E., Galloway, R.A., Gauch, H.G. 1965. Calcium activation of orthophosphate absorption by barley roots. Plant Physiol. 40, 639-641.

Levy, E.G. 1925. The grasslands of New Zealand. Preliminary ecological classification of species. N.Z.J.Agric. 28, 357-374.

__________, 1933. Strain testing and strain building. pp 6-16. In Imp.Bur. Pl. Genet., Hurley. Techniques employed in grassland research in New Zealand. (Imp.Bur.Pl.Genet. Herb. Plts) Bull. 11.

Ling, G.N. Cope, F.W. 1963. Potassium ion: Is the bulk of intracellular $K^+$ absorbed? Science (N.Y.), 1335-1336.

Loneragan, J.F., Asher, C.J. 1967. Response of plants to phosphate concentration in solution culture. 2. Ratio of phosphate absorption and its relation to growth. Soil Sci. 103, 311-313.

Ludecke, T.E. 1965. Further aspects of sulphur nutrition of legumes. Proc. N.Z.Grassl.Ass. 27, 128-137.

Luxmore, R.J., Millington, R.J. 1971. Growth of perennial ryegrass (Lolium perenne L.) in relation to water, nitrogen and light intensity. 1. Effect of leaf growth and dry weight. Plant Soil 34, 269-281.

Lycklama, J.C. 1963. The absorption of ammonium and nitrate by perennial ryegrass. Acta bot.neerl. 12, 361-423.

Mason, T.G., Maskell, E.J. 1928. Studies on the transport of carbohydrates in the cotton plant. 2. The factors determining the rate and the direction of movement of sugars. Ann.Bot. 42, 571-636.

___________, ____________. 1931. Further studies on transport in the cotton plant. 1. Preliminary observations of transport of phosphorus, potassium and calcium. Ann.Bot. 45, 129-173.

___________, Phillis, E. 1937. The migration of solutes. Bot.Rev. 3, 47-

Maw, G.A., Kempton, R.J. Methyl bromide as a soil fumigant. Soils Fertil. (Harpenden). 36(2) 41-47.

May, L.H. 1960. The utilization of carbohydrate reserves in pasture plants after defoliation. Herb.Abstr. 30, 239-245.

________, Chapman, F.H., Aspinal, D. 1965. Quantitative studies of root development. 1. The influence of nutrient concentration. Aust.J.biol.Sci. 18, 23-53.

McMeekan, C.P., Walshe, M.J. 1963. The inter-relationship of grazing method and stocking rate in the efficiency of pasture utilization by dairy cattle. J.agric.Sci. (Camb.) 61, 147-166.

Milos, J., Miles, A.M. 1970. Consequences of species poverty in the uplands. pp 28-30. In Nature Conservancy. The Nature Conservancy Research in Scotland. Report for 1968-1970. Edinburgh.

Milthorpe, F.L., Moorby, J. 1969. Vascular transport and its significance in plant growth. Ann.Rev. Plant Physiol. 20, 117-138.

Mitchell, K.J. 1953. Influence of light and temperature on the growth of ryegrass (Lolium spp.) 2. The control of lateral bud development. Physiologia Pl. 6, 425-442.

____________, 1956. Growth of pasture species under controlled environment. 1. Growth at various levels of constant temperature. N.Z.J.Sci.Tech., 38A, 203-216.

____________, Renate, L. 1960. Growth of pasture species in controlled environment. 2. Growth at low temperatures. N.Z.J.agric.Res. 3, 647-655.

____________, Soper, K. 1958. Effects of differences in light intensity and temperature on the anatomy and development of leaves of Lolium perenne and Paspalam dilatatum. N.Z.J.agric.Res. 1, 1-16.

Mosse, B. 1973a. Plant growth responses to vesicular arbuscular mycorrhiza. 4. In soil given additional phosphate. New Phytol. 72, 127-136.

________, Hayman, D.S., Arnold, D.J. 1973. Plant growth responses to vesicular orbuscular mycorrhiza. 5. Phosphate uptake by three plant species from P-deficient soils labelled with $^{32}$P. New Phytol. 72, 809-813.

Mountier, N.S., Grigg, J.L., Oomen, G.A.C. 1966. Sources of error in advisory soil tests. 1. Laboratory sources. N.Z.J.agric.Res. 9, 328-338.

Moustafa, E., Boland, M., Greenwood, R.M. 1971. Transport of phosphate from leaves to leguminous root nodules. Plant Soil 35, 651-653.

Murata, Y. 1961. Studies on the photosynthesis of rice plants and its culture significance. Bull.Nat.Inst.Agric.Sci., Japan Ser. D,9: 1-169.

__________, 1969. Physiological responses to nitrogen in plants. pp 235-256. In Dinauer, R.C. ed. Physiological aspects of crop yields. American Society of Agronomy, Crop Science Society of America, Madison.

Murphy, J. Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica chim. Acta 27, 31-36.

Munch, E. 1930. Die stoffbewegungen in der pflanze. In Gustav Fisher, Jana, 234p.

Nelder, J.A. 1966. Inverse polynomials, a useful group of multi-factor response functions. Biometrics, 22, 128-141.

New Zealand Soil Bureau,1954. General survey of the soils of North Island, New Zealand. New Zealand Soil Bureau Bull. (n.s.), Govt. Print, Wellington.

Newbould, P., Taylor, R., Howse, K.R. 1971. The absorption of phosphate and calcium from different depths in soil by swards of perennial ryegrass. J.Br.Grassl.Ass. 26, 201-208.

O'Connor, K.F. 1961. Nitrogen and grassland production in the mid-altitude zone of Canterbury, New Zealand. 3. The effects of nitrogenous and other fertiliser materials on uncultivated pasture. N.Z.J.agric.Res., 4, 709-721.

Pate, J.S. 1962. Nodulation studies in legumes. 5. The effects of temperature on symbiotic performance of bacterial associations of Medicago tribuloides Desr. and Vicia atropurpurea Desf. Phyton (Buenos Aires) 18, 65-74.

Petrie, A.H.K. 1937. Physiological ontogeny in plants and its relation to nutrition. 3. The effect of nitrogen supply on the drifting composition of the leaves. Aust.J.exp.Biol.med.Sci. 15, 385-404.

Rovira, A.D., Bowen, G.D. 1968. Anion uptake by plant roots: distribution of anions and effects of micro-organisms. Int.Congr. Soil Sci.Trans. 9(2), 209-217.

Rumball, W. 1969. Pasture species and their performance in the Wairarapa. Proc.N.Z.Grassl.Ass. 31, 35-42.

__________, Armstrong, C.S. 1974. The performance of overseas cultivars in New Zealand. Proc.N.Z.Grassl.Assoc. 36, 94-104.

Russell, R.S., Martin, R.P. 1953. A study of the absorption and utilization of phosphate by young barley plants. 1. The effect of external concentration on the distribution of absorbed phosphate between roots and shoots. J.exp.Bot. 4, 108-127.

Saunders, F.E., Tinker, P.B. 1971. Mechanism of absorption of phosphate from soil by Endogene Mycorrhiza. Nature (Lond.) 223-278.

Saunders, W.M.H. 1963. Residual effects of phosphate on a yellow-brown loam from andesitic ash. N.Z.J.agric.Res. 6, 485-507.

Sansing, N.G. 1963. Translocation of $P^{32}$, $N^{15}$, and $C^{14}$ in plants. Diss.Absts, 23, 3610.

Saxby, S.H. 1956. Pasture production in New Zealand. New Zealand Dept of Agric, Wellington, (New Zealand Dept. of Agric. Bull. 250).

Scott, D., Stringer, G.C., O'Connor, K.F., Clifford, P.T. 1974. Growth of legume varieties on a yellow-brown high country soil. N.Z.J.exp.Agric. 2, 251-9.

Scott, R.S. 1968. Animal and pasture production as indices of fertiliser maintenance. Proc.N.Z.Soc.Anim.Prod. 28, 53-64.

__________, 1971. Pasture ageing in relation to method of yield estimation. Proc. N.Z.Grassl.Ass. 33, 105-113.

__________, Cullen, N.A. 1965. Some residual effects of phosphatic fertilisers. N.Z.J.agric.Res. 8, 652-666.

Sears, P.D. 1953. Pasture growth and soil fertility. 7. General discussion of the experimental results and of their application to farming practice in New Zealand. N.Z.J.Sci.Techn. 35A, 221-236.

__________, Evans, 1953. Pasture growth and soil fertility. 3. The influence of red and white clovers, superphosphate, lime and dung and urine on soil compaction and on earthworm and grass-grub populations. N.Z.J.Sci. Tech. 35A, Suppl. 1, 42-52.

__________, Goodall, V.C., Jackman, R.H., Robinson, C.S. 1965. Pasture growth and soil fertility. 8. The influence of grasses, white clover, fertiliser, and the return of herbage clippings on pasture production of an impoverished soil. N.Z.J.agric.Res. 8, 270-283.

Sinclair, A.G. 1973. Non-destructive acetylene reduction assay of nitrogen fixation applied to white clover plants growing in soil. N.Z.J.agric.Res. 16, 263-270.

Smith, A. 1972. Other grasses. In Spedding, C.R.W., Diekmahns, E.C. eds. Grasses and legumes in British agriculture, pp 255-279. Commonw Bur. Pastures and Field crops, Hurley (Commonw.Bur.Pastures and Field Crops Bull. 49).

Smith, B.A.J. 1964. Potash plus high stocking pays. N.Z.J.Agric. 109, 378-381.

Smith, D. 1970. Influence of temperature and chemical composition of five forage legume species. Agron.J. 62, 520-523.

Snaydon, R.W., Bradshaw, A.D. 1962. Differences between natural populations of Trifolium repens L. in response to mineral nutrients. 1. Phosphate. J.exp.Bot. 13, 422-434.

Soil Conservation Authority, Victoria 1971. Agronomical studies: stabilization of eroded areas. pp 30-32. In Soil Conservation Authority, 22nd Annual Report for year ended 30 June, 1971, Victoria.

Sprent, J.I. 1971. The effects of water stress on nitrogen-fixing root nodules. 1. Effects on the physiology of detached soybean nodules. New Phytol. 70, 9-17.

Stocking, C.R., Ongun, A. 1962. The intracellular distribution of some metallic elements in leaves. Am.J.Bot. 49, 284-289.

Stoy, V. 1969. Interrelationships among photosynthesis, respiration and movement of carbon in developing crops. pp 185-202. In Dinauer, R.C. ed. Physiological aspects of crop yield. American Society of Agronomy, Crop Science Society of America, Madison.

Suckling, F.E.T. 1960. Productivity of pasture species on hill country. N.Z.J.agric.Res. 3, 579-591.

Syers, J.K. 1974. Effect of phosphate fertiliser on agriculture and the environment. N.Z.Agric.Sci. 8, 149-164.

Thorne, J.H., Koller, H.R. 1974. Influence of assimilate demand on photosynthesis diffusive resistances, translocation, and carbohydrate levels of soybean leaves. Plant Physiol. 54, 201-207.

Thrower, S.L. 1962. Translocation of labelled assimilates in soybean. 2. The pattern of translocation in intact and defoliated plants. Aust.J.biol.Sci. 15, 629-649.

Tiver, N.S. 1942. Studies of the flax plant: physiology of growth, stem anatomy and fibre development in fibre flax. Aust.J.exp.Biol. med. Sci. 20, 149-16.

__________, Williams, R.F. 1943. Studies of the flax plant. 2. The effects of artificial drought on growth and oil production in a linseed variety. Aust.J.exp.Biol.med.Sci. 21, 201-209.

Trip, P., Gorham, P.R. 1968. Bidirectional translocation of sugars in sieve tubes of squash plants. Plant Physiol. 43, 877-882.

Troughton, A. 1957. The underground organs of herbage grasses. Commw.Bur. of Pastures and Field Crops, Reading. (Commonw.Bur. of Pastures and Field Crops. Bull. 44).

__________, 1959. Report on the work carried out during the tenure of a National Research Fellowship, New Zealand, July 1958 - July, 1959 (unpub.)

__________, 1960. Uptake of $^{32}$P by the roots of Lolium perenne Nature, (Lond.) 188, 593.

__________, Whittington, W.J. Significance of genetic variation in root systems. pp 296-313. In Whittington, W.J. ed. Root Growth. Proc. 15th Easter School in agricultural science, Univ. Nottingham, 1968. Butterworth, Lond.

University of Otago. Computer Centre. 1974. Teddybear statistical program. (Otago University, Computer Centre Rpt. T5).

Vincent, J.M. 1970. A manual for the practical study of the root-nodule bacteria. In International Biological Programme Handbook No. 15, Blackwell, Oxford. 164p.

Virtanen, A.I., Moisio, T., Burrio, R.H. 1955. Fixation of nitrogen by nodules excised from illuminated and darkened pea plants. Acta chem.scand. 9, 184-186.

Van Den Honert, 1937. Over eigenschappen van platonwortals, welks vee rol spekan bij de opname van voadingszouten. Natuurk. Tijdschr.Ned.Indie, 97, 150-162.

Van Fleet, D.S. 1961. Histochemistry and function of the epidermis. Bot.Rev. 27, 165-220.

Vartha, E.W. 1960. Grass nutrition: sulphur pp 44-61. Thesis M.Agr.Sc. Canterbury Agricultural College, 98p.

__________, Clifford, P.T. 1973. Improvement of mid-altitude tussock grassland for grazing by topdressing and oversowing, N.Z.J.exp.Agric. 1, 181-186.

Vose, P.B. 1963. Varietal differences in plant nutrition. Herb.Abstr. 33, 1-13.

Walker, T.W. 1956. The nitrogen cycle in grassland soils. J.Sci.Fd Agric. 7, 66-72.

__________, 1956. The sulphur cycle in grassland soils. J.Br.Grassl.Soc. 12, 10-18.

__________, Orchiston, H.D., Adams, A.F.R. 1954. The nitrogen economy of grass legume associations. J.Br.Grassl.Soc. 9, 249-274.

Watkin, B.R., Robinson, G.S. 1974. Dry matter production of 'Massey Basyn', Yorkshire fog, (Holcus lanatus). Proc.N.Z.Grassl.Ass. 35, 278-283.

Watson, D.J. 1971. Size, structure and activity of the productive system of crops. pp 76-88. In Wareing, P.F., Cooper, J.P. eds. Potential Crop Production. Heinemann, London.

Watson, R., Petrie, A.H.K. 1940. Physiological ontogeny in the tobacco plant. 4. The drift in nitrogen content of the parts in relation to phosphorus supply and topping, with an analysis of the determination of ontogenetic changes. Aust.J.exp.Biol.med.Sci. 18, 313-340.

Wardlaw, I.F. 1968. The control and pattern of movement of carbohydrates in plants. Bot.Rev. 34, 79-105.

Weeda, W.C., Cullen, N.A., Douglas, J.A., During, C. Progress report on 'Grasslands 4700' white clover. Proc.N.Z.Grassl.Ass. 31, 143-151.

Went, F.W., Sheps, L.O. 1969. Environmental factors in regulation of growth and development: ecological factors. In Steward, F.C. Analysis of Growth. Behaviour of plants and their organs. (Plant Physiol 5A), Academic Press, New York. 454p.

Wheeler, C.T. 1971. The causation of the diurnal changes in nitrogen fixation in the nodules of Alnus glutinosa. New Phytol. 70, 487-495.

White, J.G.H., Meijer, G., Langer, R.H.M. 1972. Oversowing grasses on sunny and shady faces. Proc.N.Z.Grassl.Ass. 34, 139-146.

Whittet, J.N. 1964. Pastures. Department of Agriculture, N.S.W. (The Farmers' Handbook Series) 632 p.

Widdowson, J.P., Yeates, G.W., Healy, W.B. 1973. The effect of root nematodes on the utilization of phosphorus by white clover on a yellow-brown loam. N.Z.J.agric.Res. 16, 77-80.

Wiersum, L.K. 1973. Some experiments on supply of ions to the absorbing root surface. Neth.J.agric.Sci. 21, 263-268.

Wilkinson, S.R. 1961. Influence of nitrogen and phosphorus fertilisers on the morphology and physiology of soybean roots. Thesis Ph.D. Purdue Univ.

______________, Gross, C.F. 1965. Response of Ladino clover to phosphorus withdrawal from nutrient solution. Agron.J. 57, 357-60.

______________, ___________, 1967. Macro and micronutrient distribution within Ladino clover (Trifolium repens L.). Agron.J. 59, 372-374.

Williams, R.F. 1948. The effect of phosphorus supply on the ratios of intake of phosphorus and nitrogen and upon certain aspects of phosphorus metabolism in gramineous plants. Aust.J.Sci.Res. (B) 1: 333-361.

Wilson, B. 1973. New Grasslands breeding lines promise boost to pasture production. N.Z.Farmer 94, 14-17.

Wilson, D., Cooper, J.P. 1969a. Effect of light intensity and $CO_2$ on apparent photosynthesis and its relationship with leaf anatomy in genotypes of Lolium perenne L. New Phytol 68, 627-644.

__________, ___________, 1969b. Effect of light intensity during growth on leaf anatomy and subsequent light saturated photosynthesis among contrasting Lolium genotypes. New Phytol. 68: 1125-1135.

__________, ___________, 1969c. Diallel analysis of photosynthetic rate and related leaf characters among contrasting genotypes of Lolium perenne L. Heredity 24, 633-649.

__________, ___________, 1969d. Effect of temperature during growth on leaf anatomy and subsequent light-saturated photosynthesis among contrasting Lolium genotypes. New Phytol. 68, 1115-1123.

Wilson, J.W. 1972. Control of crop processes. pp 7-30. In Rees, A.R. et al, eds. Crop processes in controlled environments. Academic Press, London. (Applied Botany Series.)

Wit, C.T. de., Brouwer, R., Penning de Vries, F.W.T. 1970. The simulation of photosynthetic systems. pp 47-70. In Setlik, I. ed. Measurement of photosynthetic productivity. Proc. IBP/PP technical meeting 1969. Centre of Agricultural Publishing and Documentation, Wageningen.

Zimmerman, M.H. 1969. Translocation of nutrients. pp 381-417. In Wilkins, M.B. ed. The physiology of plant growth and development. McGraw-Hill, Maidenhead.