THE INFLUENCE OF PHOSPHORUS SUPPLY ON BELOW GROUND INTERFERENCES BETWEEN BROWNTOP AND WHITE CLOVER

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ABSTRACT

A low occurrence of white clover in pastures contributes to soil nitrogen (N) deficiency and a low quality feed for stock. There is evidence to suggest that competition for soil phosphorus (P) from roots of browntop plays an important role in determining the distribution of white clover in hill country swards. However, competition for soil P between roots of browntop and white clover has not been studied separately from other factors known to affect the growth and persistence of white clover (e.g., soil moisture, grazing management, shoot interferences (shading and physical impedance), and non-competitive root interferences).

In hill country pastures, P level (phosphorus fertilisers), and defoliation (grazing management), are the main factors that can be changed by farmers to alter pasture botanical composition. The high cost of superphosphate has limited the potential of farmers to manipulate pastures using fertiliser applications. Therefore, it is important to know whether roots of browntop compete with roots of white clover for soil P, and whether a low supply of soil P will contribute to more severe competition from browntop roots. The possibility of non-competitive interferences occurring between roots of browntop and white clover must also be considered. How defoliation alters the balance of P acquisition between roots of browntop and white clover needs to be determined.

Three techniques were employed to examine the nature of root interferences occurring between browntop and white clover: plant strategies; dual P isotope labelling; and a more traditional competitive settings trial using aerial partitions. Plant growth and root interferences were studied at a range of levels of soil P supply. Responses of growth and phosphorus uptake of browntop and white clover to increasing soil P supply were examined first, in the glasshouse, by growing monocultures of browntop and white clover in pots.

Two mini-sward trials (one at deficient soil P supply, the other at adequate to luxury soil P supply) were carried out in the glasshouse to allow examination of root interactions (without shoot interactions). The basis of the experimental design was to determine the relative amounts of phosphorus-32 and -33 absorbed by a central row of plants (either browntop or white clover) from two adjacent soil spaces, one dominated by white clover roots, the other by browntop roots. ^{32}P was injected into the soil on one side of the central row of plants, and ^{33}P into the other side. ^{32}P and ^{33}P uptake was assessed by harvesting the shoots of the central plants, and counting the two isotopes.

The competitive settings type trial compared the growth and P uptake of a single central plant in a small pot (no interference with other plants) with a central plant in a larger pot grown with roots associated with roots of plants of the same species (intraspecific association), or of the other species (interspecific association). Shoots of the central plant was separated from the shoots of outer plants by an aerial partition.

The growth of browntop and white clover, and the nature of root interferences occurring within and between the two species was dependent on the level of soil P supply. However, the higher root density and specific root length (SRL) of browntop compared with white clover appeared to be the most important factor determining the success of browntop at all levels of soil P supply, regardless of whether or not browntop was grown with white clover.

According to the plant strategy theory of Grime, browntop was found to be a stress tolerant plant. At low levels of P supply, the lower growth rate of browntop compared with white clover would be an important factor contributing to the dominance of browntop in hill country pastures. At adequate to luxury levels of soil P supply, shoot growth of browntop was more responsive than white clover, and browntop was capable of luxury consumption of P. The high growth rate and large demand for P contributed to the competitiveness of browntop at high P supply. However, the lower demand for P by white clover, and the high P supply may have enabled white clover to avoid competition with browntop.

On unamended subsoil, browntop reduced P acquisition by white clover roots, and had a greater P uptake in the presence of roots of white clover than with roots of other browntop plants. Therefore, evidence of root competition for soil P from browntop with white clover was found. The competitive effect of browntop appeared to be due to browntop decreasing the availability of P in the soil, explained by browntop's ability to acquire more radioactive P from the soil than white clover.

At low P supply (subsoil), P application, but not defoliation of browntop, reduced the competitiveness of browntop. At adequate P supply, the ability of browntop to acquire P was reduced by defoliation. The effect of defoliation was rapid (four days), and browntop was able to acquire P isotope to higher concentrations in the shoots than when undefoliated. Possibly the reduction of root competitiveness of browntop may be short-lived.

Some interference, other than root competition, was occurring at intermediate to luxury levels of soil P supply, and may have masked the competitive effects of browntop. White clover appeared to benefit for P acquisition from growing with browntop, due to greater local root density compared with when growing with other white clover plants. Therefore, browntop and white clover appeared to gain mutual benefit for P acquisition from the presence of roots of the other species, and the competitive effects of browntop were not of overriding importance. The possibility of autotoxicity of white clover on its own root growth was discussed in relation to rhizosphere acidity effects on the toxicity of phenolics.

At adequate to luxury levels of soil P supply, neither undefoliated browntop nor undefoliated white clover benefited from defoliation of adjacently growing white clover plants. However, at lower P supply, defoliation of white clover led to an increased P isotope acquisition by nearby browntop plants. Therefore, defoliation reduced the demand for soil P by white clover. Roots of browntop were not as tolerant of defoliation as white clover.

In the field, the mat forming behaviour of browntop, physically impeding the growth of white clover and shading white clover stolons, would reduce the severity of competition for soil P between roots of browntop and white clover. Overall, root competition for P from browntop with white clover was found not to be as important as previously thought.

The use of several experimental techniques allowed a clearer picture of the interferences that occur between browntop and white clover to be obtained. The nature of root interference changed with increasing P supply. The responses of browntop and white clover to increasing P supply was found to be enlightening when the plant strategy theory of Grime was used to compare browntop and white clover. However, the dual P isotope technique found plant interferences that were not detected by the other methods used (P response and competitive settings trial), and allowed interferences that were occurring simultaneously to be elucidated.

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CHAPTER ONE

RESEARCH OBJECTIVES AND REVIEW OF LITERATURE

1.0 Introduction and research objectives

Hill country pastures are generally nitrogen (N) deficient (Luscombe 1981). Contributing to this N deficiency is the low occurrence of white clover in hill country swards. It is desirable to maintain white clover in pastures because it provides high quality feed and fixed nitrogen.

White clover commonly contributes a proportion of less than five percent to hill country swards (Suckling 1964; Brougham *et al.* 1974; Lambert *et al.* 1983). The persistence and productivity of white clover in hill country is affected by many interacting factors, including soil fertility (Edmeades *et al.* 1984), other species present (Mouat and Walker 1959a), grazing pressure (Lambert *et al.* 1983; Sheath and Boom 1985a), low pH/aluminium toxicity (Edmeades *et al.* 1984), and moisture stress (Sheath and Boom 1985b).

Hill country farmers rely on management strategies to maintain white clover in their pastures to provide fixed N because N fertiliser application is expensive, and inefficient. White clovers oversown into hill country pastures in New Zealand, such as Grasslands Huia white clover, have been bred to perform well in low-land pastures (White 1990), under conditions of high fertility (Lambert *et al.* 1986) and high utilization with optimum high stocking rates (Suckling 1964), although Grasslands Tahora was bred for moist hill country (Langer 1990). A problem faced by hill country farmers is that oversown white clovers tend to be replaced by smaller leaved resident white clovers that are better adapted than Grasslands Huia to hill country conditions (Macfarlane and Sheath 1984). These resident clovers tend to be of lower productivity, but are stimulated by applied fertilisers (Macfarlane and Sheath 1984).

The low percentage of white clover in hill country swards tends to be correlated with the presence of browntop and other low fertility tolerant grasses (Harris 1974). However, correlation of the ecological distribution of white clover with soil factors, and associations between species, does not provide information about the exact mechanisms of interference occurring between plant growth factors and between plant roots.

There is some evidence to suggest that competition for soil P from browntop may play an important role in the distribution of white clover (Jackman and Mouat 1970, 1972a). Jackman and Mouat (1972a) found that white clovers grown with browntop, required more fertiliser P to attain the same dry matter yield as white clover grown alone. However, other factors known to affect the growth and persistence of white clover, and to interact with root competition for soil P, were not separated from the effects of direct root competition. Also, the intra- and interspecific interferences of browntop and white clover have not been fully examined concurrently, making previous studies on competition for soil P between browntop and white clover difficult to interpret.

Harris (1974) argued that factors other than root competition for soil P are of greater importance in the interference between browntop and white clover. In particular, soil moisture supply and grazing management are known to be of significance. Also worthy of note is the ability of browntop to physically impede the spread of white clover, and to shade white clover stolons through the formation of a thick mat of live and dead shoot material (Jackman and Mouat 1970; Harris 1974).

Defoliation (grazing) is known to affect the nature of interference occurring between plant roots. The preferential defoliation of one plant may lead to a greater competitive advantage for uptake of P from a limited supply by a neighbouring plant (Caldwell *et al.* 1987). A greater proportion of white clover is grazed than is found in the pasture (Brougham 1966). The significance of this in relation to competition for soil P between roots of browntop and white clover is not known.

The main objective of this investigation was to explain the effects that various management strategies (i.e., fertiliser application and grazing management) are known to have on the productivity and persistence of white clover in hill country pastures, which are usually browntop dominant. The growth of white clover and browntop was examined in relation to P level and defoliation. White clover and browntop are major constituents of hill country pastures and P level (through the use of superphosphate), and defoliation (through grazing and management), are usually the factors able to be manipulated by farmers to bring about changes in the botanical composition of pastures. The decrease in superphosphate applied in recent years due to higher costs and lower returns

(Langer 1990) has further limited farmers' potential capacity to manipulate their pastures.

The specific aims of this study were to test the hypotheses that:
[1] browntop and white clover roots compete directly for soil P at limited supply,
[2] applications of fertiliser P to the soil alleviate competition for P between browntop and white clover,
[3] interference, other than competition for soil P, plays a role in determining the success of browntop and white clover when grown in mixtures, and
[4] defoliation alters the balance of interferences occurring between roots of browntop and white clover.

1.1 Hill country pastures in New Zealand

Nearly 5 million ha of the 14 million ha of pasture in New Zealand is on unploughable hill country (Langer 1990). Hill country farming makes a significant contribution to the New Zealand economy; of around $1000 million a year in export earnings (Shimmins 1982). In most cases production from these pastures could be greatly improved. The major limitations to steep hill country production are soil moisture, soil fertility, temperature, and grazing management (Chapman and Macfarlane 1985). White clover provides high quality feed and, more importantly, fixed nitrogen (N). Yet, the proportion of the sward composed of white clover is commonly less than five percent (Suckling 1964; Brougham *et al.* 1974; Lambert *et al.* 1983). Fertiliser programmes are aimed at ensuring vigorous clover growth to encourage increased dry matter production from high producing companion grasses (e.g., perennial ryegrass) as a result of nitrogen fixation by the clovers (Shimmins 1982).

Phosphorus (P) and nitrogen (N) deficiencies are common on most North Island hill country soils (Suckling 1975; Chapman and Macfarlane 1985). However, in recent years much less superphosphate has been applied to pastures, especially to low producing hill country. The average annual application of fertiliser on sheep and beef farms has decreased from 21 kg applied/stock unit for the 1975-1980 period to only 9 kg applied/stock unit for the 1985-1988 period (New Zealand Meat and Wool Board 1988). This was due to lower prices for meat and wool, higher costs and removal of subsidies on fertiliser (Langer 1990). Some farmers are turning to rock phosphate as a cheaper source of P. There is concern that the quality of pastures will deteriorate with low fertiliser P inputs, with an increase in the low producing, low fertility-tolerant grasses component and a decrease in high producing grasses and legumes (Luscombe *et al.* 1981). Fertiliser programmes should aim at maintaining a good species composition once the pasture has been established by oversowing.

Soil moisture deficits affect pasture production on all brown-grey and yellow-grey earths, and sometimes on yellow brown earth soils (Langer 1990). This is related to the climatic zones in which these soil types are located. The severity of this deficit depends on the aspect, with steep northerly faces being much drier than southerly faces, due to prevailing north-west winds (Langer 1990).

This effect of aspect is also accentuated by the greater amount of solar radiation on north-facing slopes.

Browntop, a low fertility-tolerant grass (Lambrechtsen 1986), has been recognised as an important component of New Zealand hill country pastures for many years (Hilgendorf 1918). A high frequency of occurrence of browntop on hill country has been recorded in the Manawatu (81% and 87% mean frequency of occurrence in early spring and summer, respectively), (Brougham *et al.* 1974), with a large variation of occurrences between farms depending on management practices, soil fertility, aspect and moisture conditions. Browntop is essentially a dryland species, but it is also tolerant of moist soils (Lambrechtsen 1986; Grime *et al.* 1988). White clover tends to occur where soil fertility is high and soils are moist (Scott *et al.* 1985) and surveys have shown that a high component of browntop and other low fertility-tolerant grasses in pastures is associated with a low occurrence of white clover (Suckling 1964; Brougham *et al.* 1974). Oversowing and top dressing have been used to improve browntop-dominated pastures, with a corresponding increase in ryegrass and white clover content and pasture production (Suckling 1964).

Many researchers have found evidence to suggest that competition for soil P from browntop partly explains the low frequency of white clover in hill country pastures, because browntop has a greater capacity than white clover to reduce soil solution P concentrations (Mouat 1983b). However, other factors (shading, soil moisture supply, and defoliation) which affect the acquisition of soil P by plants have also been involved, making interpretation of these experiments difficult. The mechanisms of interference occurring between roots of browntop and white clover are poorly understood, and further research is required to elucidate the true nature of the mechanisms involved, as distinct from the plant responses to root interference.

1.1.1 Effects of management practices and environment on the botanical composition of hill pastures

Differences in botanical composition between pastures are a reflection of the environment and management practices.

There are three main environmental factors known to influence botanical composition of pastures: interference between plants (e.g., shading), stress (e.g., moisture, nutrient supply), and disturbance (e.g., defoliation, treading) (Grime 1979; Lambert *et al.* 1986).

Besides the seasonal and annual changes in pasture botanical composition, brought about by differences in growth patterns of species in response to seasonal conditions of temperature and rainfall, longer term changes in pasture composition occur (Brougham *et al.* 1978). As pastures deteriorate and the legume component decreases, the component of low fertility-tolerant grasses increases, causing a decrease in total production under the same conditions of management practices, and an increase in the phosphorus fertiliser requirements to achieve the same level of herbage production (Edmeades *et al.* 1984). It is desirable to have a greater proportion of ryegrass than browntop in hill country swards, as pasture production is usually greater in ryegrass swards (Grant *et al.* 1981). However, fertiliser applications required to maintain ryegrass are rarely applied. It may be more desirable to aim at introducing and maintaining pastures based on more productive, bred lines of plants already well adapted to conditions found on low fertility hill country, such as browntop (Rumball and Claydon 1990).

Applications of phosphorus fertilisers to hill country pastures generally lead to initial increases in the proportion of legume in a pasture (Edmeades *et al.* 1984; Lambert *et al.* 1986). Then a decrease in the legume content occurs as a result of increased competition from high-fertility grasses growing under conditions of increased soil N availability due to cycling of nitrogen fixed by the legumes (Lambert *et al.* 1982; Lambert *et al.* 1986). Initial applications of phosphorus fertilisers also increase the ryegrass content of pastures, and decrease the content of low fertility tolerant grasses (e.g., browntop, sweet vernal, crested dogstail) (Suckling 1975; Lambert *et al.* 1986).

Grazing management effects on botanical composition interact with the effects of fertiliser applications (Lambert *et al.* 1986). One notable effect of grazing management is that a change from sheep to cattle grazing encourages ryegrass and clover dominance, particularly where severe treading damage occurs (Lambert *et al.* 1986). This was considered to be due to the ability of white clover to quickly colonise bare ground, and the ability of ryegrass to tolerate severe treading damage.

Set stocking produces more weedy pastures than rotational grazing (Harris 1974; Lambert *et al.* 1986). Browntop is known to invade hill country pastures under close continuous grazing more rapidly than under lax rotational grazing, because browntop is suppressed by interspecific shading under infrequent grazing (Harris 1974). Thus, the rate of ingress of browntop into pastures can be partly controlled by increasing the intervals between defoliations (Harris and Thomas 1972).

1.2 Biology of browntop and white clover

In order to understand the ways in which browntop and white clover interact with each other in mixtures, a knowledge of the biology of these two species is required.

1.2.1 Biology of browntop

Browntop (*Agrostis capillaris* L.) is a perennial grass that is commonly found in New Zealand hill country pastures. It was originally sown as a pasture species, but has long been recognised as hindering the production of stock grazed on hill country pastures because of its low palatability (Hilgendorf 1918). Despite this, browntop is well adapted to the harsh conditions that exist in hill country, such as low fertility, and is tolerant of both wet and dry soil conditions (Lambrechtsen 1986; Grime *et al.* 1988). In many cases, browntop reduces erosion on steep slopes.

The leaves are short, narrow, glabrous, a dark bluish-green colour, and have a dull surface. Browntop is self-propagating and spreads by rhizomes (Lambrechtsen 1986). Often the rhizomes occur on the soil surface and are mistaken for stolons. The rhizomatous and prostrate growth habit (Langer 1990) enables this grass to form a dense mat. Occurring at the nodes of the rhizomes are small scale leaves from which tillers arise in axillary buds (Langer 1990). Adventitious roots also arise from the nodes. These roots are fine and fibrous. A complete new plant with leaves and roots can be produced from a portion of the stem containing a node. Browntop's tolerance to grazing is due to this method of propagation and the fact that the stem apex is close to, or under the soil surface. This enables the plant to escape serious damage even under conditions of severe grazing. The thick mat also tends to shade out more productive species. In particular, the stolons of white clover plants are shaded (Jackman and Mouat 1972a).

1.2.2 Biology of white clover

White clover (*Trifolium repens* L.) is a perennial legume. It provides high quality feed and fixes atmospheric N, which is ultimately available to grasses. White clover is the most commonly used pasture legume in New Zealand (Horn and Hill 1985). Like browntop, white clover has a prostrate growth habit.

The primary tap root is lost once plants are established and have spread through horizontal creeping stems or stolons. The stolons grow out from buds in the axil of crown leaves, and node leaves and adventitious roots arise from nodes of the stolons (Langer 1990). Well rooted stolons are not easily pulled out from the soil and enable white clover plants to survive serious damage from grazing. The small leaved "wild" types of white clover are particularly prostrate (Caradus 1981a), but are relatively low yielding compared with introduced varieties (Langer 1990). Yet these small leaved populations collected from New Zealand hill country have been shown to persist and grow better than larger leaved white clovers (e.g., Pitau, Ladino, and Huia) in hill country pastures (Caradus 1981a). Caradus (1981a) attributed the superior performance of hill country white clover types to the more prostrate growth habit and the less tap-rooted, shallower, more fibrous root system that would be better adapted to soils of low fertility.

1.2.3 Root properties of browntop and white clover

The roots of browntop and white clover are quite different. Browntop roots are fine, with few roots greater than 1 mm in diameter, while white clover roots are coarse. Caradus (1980) found that browntop roots were longer than white clover roots and that the weights of roots of browntop and white clover were similar when harvested 24 weeks from sowing. Therefore browntop roots had a greater surface area than white clover roots, as was substantiated by the findings of Evans (1977) (Table 1.1). Similarly, root hair lengths of browntop and white clover have been found to be around 0.5 and 0.25 mm, respectively (Caradus 1980). Evans (1977) found similar root hair lengths to those measured by Caradus (1980). Details of many root properties of browntop and white clover are given in Table 1.1. These data indicate that the exploratory capacity of browntop roots is far greater than that of white clover roots (Evans 1977). The total P uptake per unit length of root of white clover has been found to be around four times that of browntop when grown in monocultures in pots in a glasshouse for 24 weeks at soil Truog P levels of 8.8 and 16.0 ppm, because white clover retained more P in the roots (Caradus 1980).

Table 1.1 Root properties of browntop and white clover plants grown on sand in pots in the glasshouse, with basal nutrients supplied (Evans 1977).

Root property	**White Clover**	**Browntop**
Length of root per unit root dry weight (cm/mg)	27.6	49.8
Mean root diameter (mm)	0.26	0.16
Surface area per unit root dry weight (mm^2/mg)	227	240
Apices per unit length of root (mm^{-1})	0.51	0.73
Root hair length (mm)	0.23	0.68
Percentage of roots with root hairs	68	95
Surface area of root hair cylinder (mm^2/mg root dry weight)	490	2190
Volume within root hair cylinder (mm^3/mg root dry weight)	68	824

1.3 Factors affecting P uptake by plants when grown in association with other plants

1.3.1 Soil properties

The rate of phosphate uptake by plant roots is affected by the concentration of phosphate in the soil solution (Russell 1973), and in particular, in the rhizosphere. The concentration of phosphate in the soil solution, and the rate at which it diffuses towards root surfaces is affected by the properties of the soil (Nye and Tinker 1977). Mass flow of phosphate towards roots is minimal due to the extremely low concentrations of P in soil solution (Nye and Tinker 1977; Marschner 1986). Soil properties that affect the concentration of P in the soil solution include phosphate adsorption capacity, soil pH, and the soil moisture content.

1.3.1.1 *Phosphate adsorption and buffer capacity of soil*

Adsorption is the process of removal of phosphate ions from the soil solution by retention at the soil surface. A soils' ability to sorb P affects the concentration of P in the soil solution, and therefore the diffusion coefficient of P, which in turn determines the supply of P at the root surface (Nye and Tinker 1977; Nye 1979). The phosphate buffer capacity helps to explain differing P uptake patterns from different soils containing similar amounts of labile P (Bache 1988). The phosphate buffer capacity ($\Delta C/\Delta C_l$) of a soil can be determined from its phosphate adsorption/desorption isotherm (Vaidyanathan and Nye 1970).

The phosphate buffer capacity affects the radius of the depletion zone that exists around P-absorbing roots by influencing the rate of diffusion of P in the soil solution (Nye and Tinker 1977). The average spread of the depletion zone around roots (not including root hairs) [$\sqrt{(D.t)}$], for a given length of time in seconds, can be calculated using the equation $D = D_l . \o . f_l . (\Delta C_l/\Delta C)$ (refer to Section 3.2.8 for details) to calculate the effective diffusion coefficient, D (Brewster *et al.* 1976; Grinsted *et al.* 1982).

The phosphate buffer capacity of the soil also affects the likelihood of competition for soil P occurring between individual roots. However, it is not enough to demonstrate that an ion limits growth to prove that competition for that ion exists. Firstly, the supply of the ion at the root surface must not arrive by mass flow at a rate faster than required by the plant, and secondly, depletion zones around roots must be wide enough to overlap (Grinsted *et al.* 1982; Fitter and Hay 1987). Mass flow of phosphate in soil is negligible (Nye and Tinker 1977) and diffusion of phosphate in soils is slow, so that in a hill country pasture in New Zealand, the supply of P at the root surface is unlikely to exceed demand. Whether or not root P depletion zones overlap depends on root density. The likelihood of this happening can be estimated for roots (excluding root hairs) if the root densities in the soil are known, by comparing half the average distance between roots (assuming completely random distribution), with the spread of the depletion zones around each root.

1.3.1.2 *Soil pH*

The effects of soil pH on the availability of phosphate are complex, and difficult to predict. Whether acidification of the rhizosphere will increase the availability of P to the plant will depend on the mineralogy of the particular soil on which the plant is growing. Some researchers have found that increasing the soil pH, diminishes the level of P sorption in soil (White 1987), while others have found that acidification (decreasing soil pH) shifts the sorption equilibria of phosphate so as to increase the concentration of phosphate ions in the soil solution (Haynes 1982; Robinson 1991).

The effect of increasing soil pH in decreasing P sorption has been explained in terms of soil surfaces becoming more negatively charged, and therefore less likely to sorb negatively charged phosphate ions (Hingston *et al.* 1968, 1972). However, it seems that the effects of soil pH on phosphate sorption depend on the properties of the particular soil in question. For example, in calcareous and alkaline soils high in P, the solubility of insoluble calcium phosphates increases as soil acidity increases (pH falls) (White 1987). In contrast, in soils with significant amounts of free aluminium and iron oxides, or fertilised acid soils with $FePO_4.nH_2O$ and $AlPO_4.nH_2O$ present, P solubility increases as soils become less acid (pH rises) (White 1987).

There is good evidence that increasing soil acidity leads to an increase in the concentration of solubilised acid-soluble and residual P in the soil solution, but not exchangeable forms of P (Hedley *et al.* 1982b; Moorby *et al.* 1988).

1.3.1.3 *Soil moisture content*

As soil moisture is depleted from a soil, the diffusion path for phosphate to root surfaces becomes more tortuous, slowing down the rate of replenishment of phosphate in the depletion zone (Russell 1973; Nye and Tinker 1977). Low and Piper (1960) found on a clay loam soil with a wilting point and field capacity of 15 and 33 g H_2O/100 g oven dry soil, respectively, that the P concentration in both white clover and perennial ryegrass were affected by soil moisture. In ryegrass, an increase in %P occurred with increasing soil moisture from 21 to 67 g water/100 g soil at the same level of soil P supply (Low and Piper 1960). In contrast, the P concentration of white clover increased with increasing soil moisture up to 54 g water/100 g soil, and then decreased at higher levels of soil moisture (Low and Piper 1960).

The findings of Jackman and Mouat (1972b) suggest that browntop may have the ability to indirectly reduce the availability of P to white clover by increasing the rate of soil drying. Soil moisture contents in a field trial have been found to be lower in the top 25 mm of soil when white clover plants were grown with browntop compared with when white clover monocultures were grown, suggesting that browntop accelerates the rate of moisture loss from soil (Jackman and Mouat 1972b). Whether this is a property specific to browntop or if it would have occurred with other grasses is not known. In any case, the validity of this finding can be questioned, as the herbage dry matter production of mixtures of browntop and white clover appeared to be greater than for monocultures of white clover (Jackman and Mouat 1972a), and the growth of browntop had been stimulated by the application of fertiliser N. However, exact details of dry matter production for the mixtures were not given.

If browntop does accelerate the rate of soil drying in the field, this will have serious consequences for white clover plants growing in association with browntop. In a field study, about 80% of the total activity of roots of browntop and white clover for ^{32}P uptake was measured in the top 25 mm (Jackman and Mouat 1972b), so this drying out of surface soil may have an important bearing on P uptake (Nye and Tinker 1977). This would affect white clover plants growing in association with browntop plants in two ways. Firstly, white clover plants are not well adapted to low soil moisture conditions, and secondly, the lower soil moisture content would decrease soil P availability, which would have a more damaging effect on white clover than browntop as browntop can grow at lower soil solution P concentrations than can white clover (Jackman and Mouat 1972b). Water use efficiency (WUE) or transpiration ratio (defined as g H_2O transpired/g dry matter produced) of the two species is another factor that may influence P uptake. It is known that species that are tolerant of low soil moisture supply tend to transpire less water to produce the same amount of dry matter (Johnson and Bassett 1991), however data on the water use efficiencies of browntop and white clover is apparently unavailable in the literature.

Soil moisture content can also affect P uptake by plant roots indirectly. There is evidence to suggest that morphological responses of plants to soil water deficits are more sensitive than physiological responses (Turner and Begg 1978). Dry soil conditions have been known to reduce nodal root production by white clover plants by 50% (Ueno and Yoshihara 1968). Detailed effects of soil moisture on root production by browntop are not known. Usually, temperate grasses are less affected by dry soil conditions than temperate pasture legumes (Haynes 1980) possibly due to the less ramified root system of legumes compared with grasses (Evans 1977, 1978). Not surprisingly, roots of white clover and perennial ryegrass only penetrate soil to the depth of wet soil, and do not penetrate into zones of air dry soil (Cullen *et al.* 1972). It is not known whether the same occurs for browntop. One could envisage that if browntop roots dry out soil at a faster rate than white clover, then this may have a deleterious effect on the spread of white clover roots into patches of soil where browntop is growing during times of low rainfall.

1.3.2 Plant properties

Differential uptake of P between plant species can be due to morphological and/or physiological properties of roots, and to the metabolic activity of the plant as a whole. These properties affect how plant roots interfere with each other.

Plant properties which influence P uptake by plants are also important for root competitive ability (Tilman 1990), such as the ability to take up P at very low concentrations of P at the root surface (Fitter and Hay 1987). The way that these plant properties affect the acquisition of P by a single plant species is relatively simple in comparison to their operation in mixtures of plants, which can be extremely complex (Newman 1973).

1.3.2.1 *Absorption from solution*

Plant species vary greatly in their ability to acquire phosphate from the soil solution (Barber 1980). White clover requires a higher soil solution P concentration than browntop to attain similar relative yield and maximum growth (Mouat 1983b). Mouat (1983b) found that white clover plants grown in a solution culture achieved maximum growth at 5 µM P, while browntop required a solution P concentration of only 3µM P. In addition to requiring a greater soil solution P concentration to achieve maximum growth, white clover was not capable of reducing the P concentration in the soil solution to the same level as browntop.

In general, grasses are known to have a lower threshold P concentration (C_{min}, below which roots cannot absorb P) than legumes. The apparent minimum concentration required for P absorption (C_{min}) of several pasture species was measured by Mouat (1983a) using a modified solution culture method in which the solution was percolated through a column of Ramiha soil (a soil with high water stable aggregation and high phosphate adsorption capacity (Cowie 1976)). The C_{min} values measured for browntop, ryegrass, and white clover were 0.04, 0.08, and 0.54 µM P, respectively.

An attribute commonly found in low-fertility tolerant plant species, is the ability to acquire P in excess of their requirements, i.e., luxury uptake. Plants which tolerate stress tend to be unresponsive, in terms of dry matter production, to luxury levels of P supply, reflected by the low solution concentration required by browntop to achieve maximum growth (Mouat 1983b). However stress tolerators generally do not repress P uptake and may have high tissue concentrations at luxury levels of P supply (Clarkson 1985). The ability of a plants' roots to take up P in excess of its requirements, depends on its ability to continue to respond to increasing soil solution P concentration by increasing P uptake. For example, Fox *et al.* (1986) found the root saturation value for white clover was only 156 µM P, compared with 313 µM P for ryegrass. Luxury absorption of P in browntop has been noted (Mouat and Walker 1959a).

1.3.2.2 Physical exploration and rate of extension of roots

The extent of soil exploration by roots determines the volume of soil from which plants can obtain water and nutrients (Ozanne *et al.* 1965; Asher and Ozanne 1966). Soil exploration by roots is most important for access to relatively immobile nutrients, such as P (Nye and Tinker 1977; Fitter and Hay 1987). An increase in local root density has been shown to increase uptake of phosphate by roots of browntop and perennial ryegrass (Fitter 1976). Thus, the more fibrous root system of browntop compared with white clover (Caradus 1980) would give browntop access to a larger pool of soil P than white clover, especially under conditions of low soil solution P concentrations and in high phosphate fixing soils.

The level of interference occurring between plant roots will depend on their density in the soil, and their pattern of distribution (Haynes 1980; Clarkson 1985; Fitter and Hay 1987). Plant roots compete for mobile nutrients, such as nitrate (NO_3^-), at much lower root densities than for immobile nutrients such as P, because the zone of soil around the roots from which ions are extracted is much smaller for P (Nye and Tinker 1977; Caldwell 1987). In terms of competition for soil P, the significance of greater root exploration by browntop compared with white clover will depend on root density, that is, whether or not roots are in close enough proximity for depletion zones to overlap (Andrews and Newman 1970), and on root distribution in the soil profile. In the field, it is in the top 25 mm of soil that competition for P between browntop and white clover would most likely occur if root densities were high enough as this is where most root P uptake activity has been measured (Jackman and Mouat 1972b (see Section 1.3.1.3)). It has been suggested that because grass roots are longer and more frequent than white clover roots, that a greater proportion of all white clover roots would likely be in competition with grass roots than grass roots in competition with white clover (Evans 1977).

Root growth rate (root extension) is also important for access to P in soil because it is a measure of the ability of a plant to exploit soil that has not yet been depleted of P (Clarkson 1985). Some species of plants are able to respond to localised patches of nutrient supply, so that plant root densities will be greatest in these soil zones (Fitter and Hay 1987). The ability of roots to change patterns of growth, and physiologically relocate zones of active absorption (Jackson *et al.* 1990) may be of significant advantage to a plant growing with other plants under conditions of low soil nutrient supply (Caldwell 1987). Competition between roots may be alleviated if plant roots are able to make physiological and physical adjustments in their zones of P uptake.

The number of root tips per unit volume of soil has been used to measure root exploratory ability, as this gives an indication of the number of root growing points exploring new sources of P (Jackman and Mouat 1972b). In a pot trial, 49 days after sowing, the distance between root tips in the top 25 mm of soil was 6.3 and 2.0 mm for white clover and ryegrass, respectively, suggesting that at that stage of growth ryegrass explored the soil more effectively than white clover (Jackman and Mouat 1972b). In a mature sward the distances between root tips would probably be smaller, and competition would be more likely under conditions of low P supply.

Root hairs increase the volume of soil exploited by roots. They are therefore most important in promoting uptake of nutrients where diffusive supply rather than mass flow is the rate limiting process, such as in the uptake of phosphate (Fitter and Hay 1987). Increasing the length of root hairs has been found to increase P absorption in white clover plants if they are not mycorrhizal (Caradus 1981b). The root hair zone has been shown by the use of autoradiographs to be largely depleted of nutrients (Bhat and Nye 1973). Whether or not root hairs play a significant role in competition for soil P is still under debate as the root hair depletion zones are extremely localised, however if the zones of depletion do overlap, competition for P would occur. Root hair lengths and the volume of the root hair cylinder of roots of browntop are far greater than for white clover (Caradus 1980), and would play an important role in the ability of browntop to fully exploit a soils P supply in localised regions.

Root architecture may also affect the ability of roots to explore soil. A “herring bone” system was considered by Fitter (1987) to be more efficient in terms of soil volume explored for a given construction cost than a diffuse branched system, and he suggested that under conditions of a limited nutrient supply that a herring bone system is more commonly found.

1.3.2.3 *Modification of the rhizosphere*

The properties of the soil directly adjacent to plant roots are different from the bulk soil. This region of the soil is called the "rhizosphere". Due to the effects of roots on soil properties, the supply of P to roots cannot be accurately predicted from the diffusion of ions from the labile pool only (Clarkson 1985).

1.3.2.3.1 *Polysaccharide secretions*

There is evidence that soil-root contact may be improved by fibrillar outgrowths of polysaccharide chains projecting into the soil from roots, and the presence of granular mucilage in the rhizosphere (Clarkson 1985). Clarkson (1985) suggested that a greater soil-root contact will give plant roots greater access to immobile nutrients, such as P. However, this may not be important because P is absorbed by plant roots from the soil solution.

1.3.2.3.2 *Acid secretion*

The rhizosphere pH may be as much as two units greater or less than the surrounding bulk soil (Marschner *et al.* 1986). In many species, the pH in the rhizosphere is affected by the balance of cation and anion uptake, leading to the release of either OH^- or H^+ from roots (Smiley 1974; Mengel and Kirkby 1982; De Swart and Van Diest 1987). Some plant species that are known to be effective in obtaining phosphate from P deficient soils, acidify their rhizospheres by proton extrusion (Clarkson 1985).

Some researchers have claimed that organic acids, such as citrate, are capable of forming chelates which could compete with P for sorption sites on soils (Parfitt 1979), and solubilise some soil P (Barber 1968). However, Hedley *et al.* (1982a) found that the release of organic acids by roots was insufficient to explain the solubilisation of P in the rhizosphere of rape seedlings.

The form of nitrogen supplied to the plant has a major influence on acid secretion in many species (Marschner *et al.* 1986; Kirlew and Bouldin 1987), although rape roots are known to extrude protons even when supplied with NO_3^- (Hedley *et al.* 1982b). In young rape plants, the length of roots extruding acid and the total amount of protons released increased as P deficiency increased (Moorby *et al.* 1988). Protons released from the roots of rape solubilise acid-soluble P, however the impact of this on P supply to rape roots will depend on the properties of the soil on which the rape plant is growing (see Section 1.3.1.2).

1.3.2.3.3 *Enzyme release*

P deficiency may lead to increases in cellular levels of phosphatase (Dracup *et al.* 1984; Clarkson 1985), a hydrolytic enzyme that catalyses the hydrolysis of phosphate bonds in complex organic compounds (Caradus 1990). This has been demonstrated in many plant species, including subterranean clover (*Trifolium subterraneum*) (Dracup *et al.* 1984), white clover (Caradus and Snaydon 1987) and rape seedlings (Hedley *et al.* 1982b). Most phosphatase activity occurs on the surface of roots (Bieleski and Johnson 1972). The enzyme may leak into the cell wall at the root surface, and then leak into the rhizosphere (Dracup *et al.* 1984). The mechanism by which phosphatase activity increases the availability of P to plant roots is not clear. It is not known whether the enzyme releases organic P in the soil or traps P esters that may leak from roots, converting P into a reabsorbable form (Clarkson 1985).

The importance of phosphatase activity in the rhizosphere of one species on the acquisition of P by other adjacently-growing plant species is not known. It is possible that one species may benefit from phosphatase released into the rhizosphere by another species.

However, some researchers have argued that phosphatase activity may not be as important as has been previously thought. Hedley *et al.* (1982b) found that although phosphatase activity in the rhizosphere of rape seedlings increased 10-fold after 35 days, compared with in control soil, there was no marked increase in the concentration of soluble P in the soil solution of rhizosphere soil. Also, they found that the organic P fraction increased rather than decreased during the experiment, suggesting that hydrolysis of organic P by phosphatase was not significant. However, the conditions under which this research was carried out were unusual in that the plants were grown in thin layers of soil so that root densities were extremely high, creating a layer of rhizosphere soil. Root and microbial competition for soil P would have been severe under these conditions. It is possible that P released from organic matter by hydrolysis from phosphatase activity may have been quickly recycled.

1.3.2.3.4 *Microbes in the rhizosphere*

Microflora in the rhizosphere can affect plant growth by influencing the availability and uptake of nutrients (Curl and Truelove 1986). Microorganisms are more abundant in the rhizosphere than in the bulk soil due to nourishment from roots in the form of root exudates and root debris. This may be of benefit or harm to plants. Beneficial effects include the heterotrophic mineralisation of organic phosphate compounds resulting in the regeneration of orthophosphate, and the solubilisation of Al, Fe, and Ca phosphates (Curl and Truelove 1986). An example of harmful effects is the immobilisation of inorganic P by autotrophic and heterotrophic microorganisms which lowers the rhizosphere P supply (Curl and Truelove 1986), and may compete with plant roots for limited soil inorganic P (Barber *et al.* 1968) .

1.3.2.3.5 *Mycorrhizae*

Vesicular-arbuscular (V.A.) mycorrhizae are known to enhance the growth of many pasture species growing on soils of low P supply (Allen and Allen 1990; Bolan 1991), and have been known to tap phosphate several centimetres away from roots (Rovira 1978; Kucey *et al.* 1989), by increasing soil exploration (Kucey *et al.* 1989; Bolan 1991). It appears that mycorrhizal plants utilise the same sources of P as uninfected plants, but mycorrhizae utilise solution P sources more efficiently than uninfected roots by having an increased affinity for P ions which decreases the threshold concentration required for absorption (Bolan 1991).

Mycorrhizal infection of roots is known to affect the balance of plant communities, and plant responses to V.A.mycorrhizal infections in monocultures and mixtures are not always related (Allen and Allen 1990). Mycorrhizal infection may be deleterious to a species even at low soil P supply, if an associated species is able to take advantage of the symbiosis more effectively (Fitter 1977). For example, in a replacement series experiment, Hall (1978) found that the addition of P had a similar effect to inoculation with V.A.mycorrhiza, on competition between perennial ryegrass and white clover. Competition for soil P favoured the grass in mixtures with non-mycorrhizal white clover. This competitive effect of the grass on white clover was alleviated by the addition of P or by V.A.mycorrhizal infection of the white clover. This has also been demonstrated by other workers (Crush 1974; Buwalda 1980).

1.3.2.4 *Root cation exchange capacity and negative adsorption*

The cation exchange capacity (CEC) of roots is made up of fixed electro-negative sites in the root tissue (Epstein 1972), in particular, in the cell wall (Mouat 1983c). These negative charges influence the proportion of ions of different valencies present at the root surface. The importance of root CEC in the adsorption of cations onto root surfaces is well recognised (Epstein 1972), but its direct impact on the acquisition of anions, such as phosphate, is debatable.

In theory, the root CEC will affect the migration of anions, such as phosphate, to the root surface. The effect of root CEC on phosphate concentration at the root surface has been termed phosphate exclusion or negative adsorption of phosphate (Mouat 1983c). The level of repulsion of anions at the root surface will depend on the root CEC and this should affect the concentration of phosphate ions found at the root surface available to plants roots to take up (Butler *et al.* 1962; Mouat 1983c).

The CEC of roots of browntop has been found to be lower than white clover (Mouat and Walker 1959b). Mouat and Walker (1959b) argued that severe competition for soil P by browntop with white clover was due to the difference in root CEC between these two species, resulting from a greater repulsion of P anions from root surfaces of white clover than browntop (Mouat and Walker 1959b; Mouat 1983a,c). However, the uptake of P is an active process, moving phosphate ions against concentration and electrical gradients by an electrogenic pump (Bowling and Dunlop 1978; Dunlop 1980). Also the concentration of P at the root surface is far lower than in the bulk soil so that there is a net movement of P towards roots. Therefore the significance of repulsion of phosphate anions by root electro-negative charges is open to debate. This casts some doubt on the importance of root CEC in altering the balance of competition for soil P acquisition between roots of plants of two or more species. The question of how the concentration of P at the root surface, as influenced by the root CEC, will affect the active uptake of P into roots needs to be considered.

Root CEC has been shown to be negatively correlated with P concentration in hybrids of perennial ryegrass and Italian ryegrass (*Lolium multiflorum*) (Butler *et al.* 1962). However, P concentrations in these hybrids have also been found to be strongly correlated with the ability to respond to decreasing P supply by decreases in shoot:root ratios (Mouat 1983a). The smaller a plants root CEC, the greater the change in shoot:root ratio. Browntop has a lower CEC than white clover, and also a greater capacity to increase relative root growth (in relation to shoot growth) in response to limiting P supply (Mouat 1983a). The correlation between root CEC and the ability of plants to respond to decreases in P supply by decreasing shoot:root ratios casts further doubt on the importance of root CEC in explaining the competitive effects of browntop on white clover at low soil P supply because changes in shoot:root ratios may be more important than root CEC.

1.3.2.5 *Metabolic activity of plants*

The partitioning between inorganic and organic pools of P in roots, and transport to shoots of absorbed P may play an important role in the regulation of P uptake rates by roots (White 1973). Plant growth rate, and thus plant demand, is also known to have a substantial influence on the rate of uptake of P, particularly at suboptimum levels of soil P supply. P uptake capacity varies between species largely due to differences in plant demand (Lambers and Poorters 1992). A low demand, related to low growth rate as a consequence of plant investment in carbon storage and defenses against grazing (Campbell 1990), has long been recognised as an adaptive feature of plants able to grow on soils with deficient P supply (Rorison 1968).

1.3.3 Effects of defoliation on nutrient uptake by plants

Most work on the effects of defoliation on plant growth have focused on the above-ground plant parts, i.e., the sward canopy (King *et al.* 1978), probably due to the obvious difficulties associated with studying roots. However, the deleterious effects of shoot defoliation on roots has been known for many years (e.g., Jacques 1937; Butler *et al.* 1959; Davidson and Milthorpe 1966a,b). Defoliation is known to not only reduce the P uptake rate per plant, but also to decrease P uptake per unit root weight (Caradus and Snaydon 1986). The impact of defoliation on plant growth is more severe when soil nutrient supply is inadequate, because the removal of expanded leaves also removes an internal source of labile nutrients (Davidson and Milthorpe 1966a).

1.3.3.1 *Root death and rate of elongation*

The effects of shoot defoliation on root growth are rapid (Troughton 1973), with the root growth of some pasture grasses (cocksfoot and brome grass) known to be arrested within 24 hours of defoliation (Oswalt *et al.* 1959). Severe physiological shocks may stop root growth completely (Ennik 1966). Shoot defoliation has been reported to cause root death (Butler *et al.* 1959), reduce the growth rate of roots (Davidson and Milthorpe 1966b; Evans 1973a,b), produce thinner roots (Jacques 1937), and reduce the number of roots per tiller in pasture grasses (Jacques 1937).

The deleterious effects of defoliation on roots of browntop are more severe than for roots of white clover, red clover, perennial ryegrass, timothy, and cocksfoot (Evans 1973b). White clover roots are particularly tolerant of shoot defoliation, with the roots and nodules of white clover plants lost following recurrent defoliation being rapidly replaced by new root growth from stolons (Butler *et al.* 1959). The nitrogen fixing capacity of individual nodules on white clover roots is temporarily reduced by defoliation (Chu and Robertson 1974).

The ability of white clover roots to recover more rapidly than browntop roots to the deleterious effects of defoliation (Evans 1973b), may allow white clover roots growing in association with browntop to acquire a greater proportion of soil P when both species are defoliated than when undefoliated.

1.3.3.2 *Carbohydrate supply*

Plant roots require energy to actively absorb P (Davidson and Milthorpe 1966b; Ueno and Williams 1967; Harris 1978), and also for root growth. The severity of the effects of defoliation on inhibiting root growth depend on the amount of shoot material removed (Butler *et al.* 1959; Davidson and Milthorpe 1966b). Ueno and Williams (1967) found that white clover had a lowered uptake of P per unit of roots at nodes where leaves were removed. The main effect of defoliation on P uptake is due to reduced carbohydrate supply affecting active uptake processes (Davidson and Milthorpe 1966b). However, when soil P supply is low, nutrient availability may override the effects of carbohydrate depletion (due to leaf removal) in reducing regrowth, due to removal of labile sources of P in defoliated plant material (Davidson and Milthorpe 1966b).

1.3.4 Indirect mechanisms affecting P uptake by plant roots

The nature of interference occurring between plant roots is extremely complex. Factors other than the properties of the soil and of individual plants have a major influence on the acquisition of P by plant roots.

1.3.4.1 *Shading*

The ability of roots to acquire soil P is affected by shading of the shoots. The effects of shading and defoliation of the shoots on roots are similar. For example, heavy shading of white clover inhibits elongation of roots, and leads to a loss of roots and nodules (Butler *et al.* 1959) due to a reduction in the carbohydrate supply from shoots (as explained in Section 1.3.3.2).

Competition for light between shoots of two species, influences the interference occurring between their roots (Donald 1958; Snaydon and Baines 1981). Overshadowing of one plant species by another reduces the growth of the subordinate species by shoot competition, and also reduces the root competitive ability of the shaded species, worsening the immediate effects of shading (Newman 1973). For example, in a microsward and box trial, shading of white clover shoots by pasture grasses (perennial ryegrass, browntop and Yorkshire fog (*Holcus lanatus*)) prevented white clover from responding to P application (Snaydon and Baines 1981). Yet, white clover responded to applied P where roots-only (and not shoots) were associated with other species. Therefore grazing management strategies employed to reduce shading of white clover in pastures are important. Snaydon and Baines (1981) concluded that under their experimental conditions shoot competition was more important than root competition, although root competition is usually more severe than shoot competition (Snaydon and Howe 1986; Wilson 1988). Unfortunately Snaydon and Baines (1981) did not examine what the roots were competing for. Other types of root interferences may have been involved, as detailed below in Section 1.4.

Defoliation and thus grazing, has a significant effect on the level of shading occurring between plants. Generally, defoliation reduces the amount of shading by allowing more light to penetrate the canopy, reducing competition for light (Evans 1973b; Rhodes and Stern 1978).

1.3.4.2 *Grazing management and preference*

Most studies on the effects of grazing on pasture species have been interpreted in terms of competition for light. However, the effects of grazing on plant roots cannot be ignored. Defoliation is known to alter the balance of nutrient acquisition between roots of pasture species (O'Brien *et al.* 1967; Remison and Snaydon 1980a) and of desert species (Caldwell *et al.* 1987). Variation in the effects of defoliation on root death and on the rate of elongation of different pasture species (Evans 1973a,b; Remison and Snaydon 1980a), and therefore P uptake, has an important bearing on the nature of interference occurring between roots of different species. For example, the results of Remison and Snaydon (1980a) suggest that frequent defoliation reduced the root competitive ability (assessed by “aggressivity” (McGilchrist and Trenbath 1971)) of cocksfoot (*Dactylis glomerata*) more than perennial ryegrass despite the initially greater root competitive ability of cocksfoot.

Given the opportunity, grazing animals are selective in their choice of diet. Sheep tend to graze more discriminatingly than cattle (Frame and Newbould 1986). The chosen diet depends on palatability and accessibility of feed available (Watkin and Clements 1978). This has a marked effect on the botanical composition of pastures, partly by reducing the P uptake by roots of plants that are preferred feed. White clover is known to be grazed in preference to temperate grasses if available usually due to accessibility (Frame and Newbould 1986). White clover survives selection due to its plasticity in growth by developing a more prostrate, smaller leaved growth habit (Curll 1982), and by the ability of its roots to recover rapidly from the effects of defoliation (Butler*et al.* 1959; Evans 1973b).

1.3.4.3 *Supply of other nutrients*

P uptake by plant roots is influenced by the availability of other nutrients. For example, P uptake can be stimulated by the major cations and N, but is inhibited by sulphate (Andrew and Johansen 1978). In some species, in particular soybean and alfalfa (Aguilar and Van Diest 1981), the source of N supplied has an effect on soil pH. Nodulated legumes that fix atmospheric nitrogen (N_2), rather than relying on nitrate uptake, take up more cations than anions since uncharged N_2 enters roots, which in turn affects the soil P supply due to acidification of the rhizosphere (Marschner 1986), depending on the soils' mineralogy, as explained in Section 1.3.1.2.

Genetic differences are known to exist between plants in the interaction of P with other ions, in particular, trace elements (Andrew and Johansen 1978). For example, Al (aluminium) toxicity reduces P uptake in white clover (Dunlop and Hart 1987).

1.3.5 Summarising comments

The factors affecting the ability of browntop and white clover to acquire phosphate from soil and the effects of management practices on their persistence in hill country pastures have been considered. How this knowledge aids in understanding the nature of interference between plants, in particular between browntop and white clover, is demonstrated below.

1.4 Plant growth interference

Populations of plants of one or more species may interact in a number of ways. This is called “interference”, defined as “the response of an individual plant or plant species to its total environment as this is modified by the presence and/or growth of other individuals or species” (Harper 1964; Hall 1974). Interference between plants may be either detrimental or beneficial. Competition between plants is only one type of detrimental plant interference. Allelopathy is another. Positive interferences (beneficence) occur when either one, or both of two populations living together benefit from the association, with neither of the two species suffering from the association.

Many researchers often speak of competition when they are not actually aware of the nature of the interference occurring. Researchers should endeavour to use the term “interference” in preference to “competition” (Hall 1974; Harper 1977).

The term “interference” does not carry the ideas associated with the word competition (Harper 1961, 1977). Plants are sometimes unconsciously imagined behaving as human beings, in a competitive world, somewhat akin to the environment in which most research is carried out. In spite of much debate and criticism, competition tends to dominate as the type of plant interference used to explain plant and animal relations (Schoener 1982). Many studies in which competition has been alleged to have occurred have failed to truly elucidate the mechanisms of the interference occurring between plants perhaps due to ignorance of forms of plant interference other than competition.

In the study of plant interference, both beneficial and detrimental types of plant interferences should be considered. The simultaneous occurrence of negative interferences (competition and allelopathy) and positive interferences (beneficence) can have a significant effect on shaping the plant community structure (Hunter and Aarssen 1988). The term "beneficence" in this thesis refers to plant interference where neither plant or species is disadvantaged by the association, and one or both plants or species benefit from the association. This is an area of research that has not been studied in great detail.

1.4.1 Negative interference

There are three main types of negative interferences that may occur between plants: allelopathy, physical impedance, and competition. There is the direct interference of one plant on another by the release of toxic substances (allelotoxins) (Nye and Tinker 1977), or by physical means, such as the manner in which thick browntop mats prevent the spread of other pasture species (Harris 1974). Competition for nutrients can be more specifically referred to as exploitative competition. Exploitative competition is indirect, acting through shared limiting resources (Connell 1990). Certain situations, such as spatial segregation of two species in the field, are often attributed to exploitative competition or allelopathy, when it may be due to other mechanisms, such as the abundance of natural enemies of one species (Connell 1990). Connell (1990) referred to this as "apparent competition" where the mechanisms occurring are not due to "real competition", but mimic their effects.

1.4.1.1 *Competition*

Competition can be defined as "a negative interaction between individuals brought about by a shared requirement for resource in limited supply, and leading to reduced growth, reproduction, and/or survival of one or more individuals concerned" (adapted from Begon *et al.* 1986). The definition given above encompasses both the mechanism of the interference and the outcome of the interference. Although, this definition states that individuals suffer in a competitive situation, a more competitive species may perform better in mixtures with a less competitive species than in monoculture. This occurs when intraspecific competition for a resource of the more competitive species is more severe than interspecific competition.

Evidence of competition between browntop and white clover in hill country pastures is presented and discussed in detail below in Section 1.6.

1.4.1.2 *Allelopathy*

Allelopathy may be defined as “the detrimental effect of one plant on another by the production of harmful chemicals” (Nye and Tinker 1977). There have been few studies that have been truly conclusive in proving the mechanisms of allelopathy occurring. In order to show that allelopathic interference is occurring, several criteria must be met (adapted from Wardle 1987). Those criteria are:

[1] a toxin must be produced,
[2] the toxin must be identified,
[3] the plant must be capable of releasing the toxin,
[4] plants must be inhibited by the toxin,
and [5] other factors which could influence interference must be identified.

Research into the nature of allelopathic interferences between white clover and browntop has produced some conflicting results. Extracts of shoot and root tissue of browntop, and shoot tissue extract of white clover have been found to stimulate the germination of white clover, while inhibiting the germination of browntop (Scott 1975). Scott (1975) was not able to identify the allelotoxin involved. Allelopathic effects may depend on the amount of donor material (plant material or leachate from soil on which plant was growing) available (Macfarlane *et al.* 1982a). At high levels of donor material, white clover reduced the level of germination of browntop, perennial ryegrass, and white clover, while at low levels of donor material, the germination of perennial ryegrass, and white clover were not affected (Macfarlane *et al.* 1982a). However, shoot and radicle growth of both browntop and white clover were reduced. Phenolics were suggested as the probable toxin involved.

Although there is evidence to suggest that allelopathic effects of some species may cause a reduction in P uptake due to the presence of toxins (Newman and Miller 1977; Nye and Tinker 1977), this has not been demonstrated for white clover (Newman and Miller 1977) or browntop.

Auto-allelopathic effects of white clover on its own growth have been suggested by quite a few researchers (e.g., Newman and Rovira 1975; Macfarlane *et al.* 1982a,b). The allelopathic toxins involved have not been identified. However, there is evidence from the field to support the suggestion that auto-allelopathic effects of white clover are important, as it is known that local patches of white clover tend to migrate from year to year (Lieth 1960) and that white clover and perennial ryegrass (two auto-allelopathic species) are positively associated (Kershaw 1959; Harris 1974). Newman and Rovira (1975) suggested that if white clover was auto-allelopathic, it could grow better in mixtures than in monocultures, providing interspecific competition effects were not more severe than auto-allelopathic effects.

1.4.2 Positive interference

Positive interference usually arises when one plant ameliorates the physical, chemical, and/or biological environment of roots of another plant (Connell 1990). The direct stimulation of one species by another, e.g., nitrogen fixed by white clover becoming available to associated pasture grasses (Hall 1974; Vallis 1978), is a good example of positive interference. The better performance of grasses grown in association with legumes is due to niche differentiation in terms of nitrogen sources used when the nitrogen supply is limiting to the grass (Weiner 1980; Martin and Snaydon 1982).

Hunter and Aarssen (1988) suggested that the same neighbouring plants that may compete under conditions of resource demand exceeding supply may also simultaneously interact beneficially. However, positive interferences occurring below ground require further investigation because problems with studying roots have made progress in this area of research difficult (Hunter and Aarssen 1988).

1.4.2.1 *Phosphorus transfers between mycorrhizal plants*

Mycorrhizal connections between roots may provide a passage for phosphorus transfer from plant to plant. This has been demonstrated by injection of ^{32}P into shoots and roots of one plant, and detection of the labelled P in neighbouring plants of the same and different species (Chiarello *et al.* 1982; Whittington and Read 1982; Francis *et al.* 1986; Newman and Ritz 1986). Findings to date have not been conclusive in determining the transfer mechanism involved, i.e., whether the transfer is directly through mycorrhizal links or indirectly through leakage into the soil (Newman and Ritz 1986). However, there is good evidence to suggest that transfer in the short term is most likely through direct linkages rather than through leakage, reabsorption and translocation (Whittington and Read 1982).

Whether or not there is likely to be a net transfer of P in one direction through mycorrhizal connections from one species to another is still under debate. It has been suggested that mycorrhizal connections are most likely to be of significance in patchy environments, allowing transfer of P from a plant growing in a soil patch with higher P supply, to a neighbouring plant growing in a nutritionally poorer soil patch (Allen and Allen 1990). Similarly, one could envisage that a plant species with roots able to absorb large quantities of soil P under conditions of low supply, could transfer P through mycorrhizal connections to a not so well adapted neighbouring species. It is possible that for a net flow of P through mycorrhizal connections to occur, a nutrient concentration gradient must exist (Allen and Allen 1990). However, a net transfer of P would not be of benefit to the P donor plant.

Vesicular-arbuscular mycorrhizal fungal species common in New Zealand hill country soils include *Glomus fasciculatus* and *Acaulospora laevis* (Powell 1976). The significance of hyphal connections between roots of plants in a hill country pasture community is unknown. However, recent research suggests that the movement of phosphorus between plants may be too slow to have a significant effect on pasture plant community structures (Newman and Eason 1993).

1.4.2.2 *Rhizosphere effects*

A classic example of one species of plant benefiting for P acquisition by growing in association with another species, was demonstrated by Marschner *et al.* (1986). Wheat plants were shown to have a greater P uptake per unit length of root when grown in mixtures with white lupins (*Lupinus albus* L.) than when grown in monocultures. This was due to the mobilisation of sparingly-soluble iron and aluminium phosphates by exudation of chelating substances (probably citrate) and the net excretion of H^+ by proteoid roots of white lupin (Marschner *et al.* 1986). Lupin mobilised P in excess of its own requirement, allowing the wheat roots to acquire the surplus P.

The potential for growing mixtures of plants that would allow one species to benefit from the "mining" of less available pools of soil P by another species has also been suggested by other researchers (e.g., Clarkson 1985). This mechanism of beneficence has not been demonstrated in many studies, but it has often been suggested to explain the better performance of some species in mixtures compared with their performance in monocultures. However, although perennial ryegrass is able to remove more P from less readily-exchangeable pools of soil P than white clover, white clover does not appear to acquire any of the P mobilised by ryegrass (Sorn-Srivichai 1985). This may be simply due to the ability of ryegrass to obtain P from lower soil solution concentrations than white clover.

1.4.2.3 *Microorganisms in the rhizosphere*

Plant species differ in their rhizosphere microbial population due to the different substances that exude from their roots and leach from their shoots into the soil (Trenbath 1976). These microbes can affect nutrient uptake and plant growth (Rovira 1978), as explained in Section 1.3.2.3.4. However, Hedley *et al.* (1982b) argued that microbial effects in the rhizosphere could not wholly account for increased P availability in the rhizosphere of rape seedlings.

In terms of the effect of one species of plant on another, it has been demonstrated that fungi, and to some extent bacteria, are more abundant in the rhizosphere of mixtures of plant species than in monocultures (Christie *et al.* 1978). Through the beneficial and harmful effects of microorganisms on P supply to plants (Section 1.3.2.3.4), microorganisms may affect the balance of positive and negative interferences occurring between plant species.

The abundance of fungi and bacteria present in the rhizosphere of grassland plant species (sweet vernal (*Anthoxanthum odoratum* L.), perennial ryegrass, plantain (*Plantago lanceolata* L.) and white clover) is known to be influenced by the presence or absence of other species (Christie *et al.* 1978). When perennial ryegrass and plantain were grown in mixtures, fungi and bacteria in the rhizosphere were more abundant than when plants were grown in monocultures. Correlated with this was an increase in the growth rate and nutrient content of perennial ryegrass. It was not possible to determine whether this was due to the rhizosphere microbes influencing the growth of perennial ryegrass, or if the better physiological condition of perennial ryegrass grown in mixtures, compared with in monocultures, provided better conditions in the soil for the growth of rhizosphere microbes, although the latter mechanism seems most likely.

1.4.2.4 *Allelopathy*

The better growth of some plants in mixtures compared with in monoculture would suggest that there was a positive interference occurring between the two species, whereas the mechanism involved may actually be a negative interference occurring between plants of the same species, as explained in Section 1.4.1.2.

Positive allelopathic effects have also been suggested. Root exudates collected in leachates from white clover plants grown on sand have been shown to stimulate P uptake per unit root dry weight of sweet vernal (Newman and Miller 1977). The effects were not due to pH differences between the leachates (Newman and Miller 1977).

1.5 Methods of studying plant interferences

The methods developed to study plant interference are numerous. Only the most commonly used and most significant methods are described here.

1.5.1 Monocultures

The severity of intraspecific competition between plants in monocultures has been studied using increasing plant densities. As plant densities increase, and competition between plants becomes more severe, the mean size of surviving plants is reduced (Donald 1963; Firbank and Watkinson 1990). Some researchers have argued that the equilibrium reached in mixtures of species can be predicted from the performance of the individual species grown in monocultures (e.g., Tilman 1990). The basic assumption of this theory is that interspecific interference is consumer-resource based, and that the competitive ability of a plant species depends on various plant traits, such as relative growth rate, minimal tissue nutrient concentration required for plant growth, and proportion of biomass in the root (Tilman 1990).

This mechanistic approach may help to explain, in principle, the performance of individual plant species in mixtures. However, as demonstrated in previous Sections, interference between plants is often very complex; different species have different resource requirements, different growth patterns and most importantly, modify the environment of each other (Firbank and Watkinson 1990). Interference between plants may be non-competitive, for example, allelopathic interference (Newman and Rovira 1975), or one species may affect the colonization of roots of another species by bacteria and fungi (Christie *et al.* 1978). Competition by resource depletion is only one type of root interference known to occur between plants. It is not always possible to accurately predict the performance of a species in a mixture from its performance in monoculture (Harper 1977; Austin *et al.* 1985; Grace 1988).

Another approach to predicting the performance of individual species in mixtures from their growth in monocultures is Grime's model (Grime 1979; Grime *et al*. 1988) which considers plant growth strategies. The theory is based on classifying plant species into strategies they have adapted to survive in their habitats, as shown in Table 1.2. Because habitats of intermediary intensities of stress and disturbance exist, plants may have varying degrees of

Table 1.2 Primary strategies of plants found in habitats of high and low stress and/or disturbance (from Grime 1979)

Intensity of disturbance	**Intensity of stress**	
	Low	High
Low	Competitors	Stress-tolerators
High	Ruderals	no viable strategy

the three main strategies of competitors, ruderals, and stress tolerators. Grime's theory predicts landscape vegetation patterns well.

1.5.2 Additive series

The additive series design involves holding the density of one component (species) constant, while that of the other is varied. This method has been used widely (e.g., Clatworthy 1960), but it has also attracted much criticism. This is because both the plant density and proportion are varied at the same time, thus confounding the effects of total density and frequency (Harper 1964; Silvertown 1987; Snaydon 1991). By employing the additive series design, the effects of intraspecific interference cannot be separated from the effects of interspecific competition. However, Snaydon (1991) has argued that the additive design gives valid and interpretable values for indices of competition, in spite of changes in plant densities and proportions, stating that the design does not confound overall density with proportion of components "as the overall density has no biological or statistical meaning, in the same way that the total amount of toxin supplied has no meaning". The additive design may be useful in certain situations. For example, in a study of the effects of a weed infestation on a crop planted at fixed density (Silvertown 1987).

A study on the effects of perennial ryegrass and browntop on white clover provided an interesting example of the use of the additive design. Clatworthy (1960) found that only 40 plants of perennial ryegrass were required to prevent 10 plants of white clover from forming stolons, compared with 160 plants of browntop. These results highlight a problem with the use of various experimental designs, in that researchers employing a different design have found browntop to be more deleterious to the growth of white clover than ryegrass (Mouat and Walker 1959a). However, the conflicting findings may have been due to differences between the experiments in plant sizes or stages of growth (Firbank and Watkinson 1990).

1.5.3 Replacement series

A replacement series is created when a range of proportions of two plant components is obtained by replacing individuals of a monoculture of a first component by individuals of a second component, until a monoculture of the second component is achieved (Harper 1977). The total plant density is held constant.

The replacement series was extremely popular for many years and was accepted by some researchers as the only way to describe competition between two species (Hall 1978; Trenbath 1978). Part of the apparent elegance of this experimental design was that it allowed inter- and intraspecific plant interferences to be separated. The results can be described using easily-calculated indices, such as relative yields and relative crowding coefficients (Hall 1974; Harper 1977). However, it is now clear that the results obtained using a replacement series are density dependent (Weiner 1980; Firbank and Watkinson 1990; Snaydon 1991). For example, Taylor and Aarssen (1989) found that relative yield totals of pasture grasses grown in replacement series were sensitive to changes in the total density of the mixed stands. As a result this experimental design has recently received much criticism (e.g., Joliffe *et al.* 1984; Firbank and Watkinson 1985; Snaydon 1991). Despite this criticism, replacement series experiments have proven valuable in the study of effects of single factors on the outcome of interference between two species (Firbank and Watkinson 1990).

1.5.4 Addition series/bivariate factorial design

Plants respond differently to different combinations of frequency and density (Firbank and Watkinson 1990). Most competition experiments carried out to date have not fully described the nature of interference occurring between two species as this has not been taken into consideration in the experimental designs used. The addition series attempts to overcome this problem.

An addition series is created by setting up mixtures at a range of densities and proportions (Silvertown 1987). One approach taken is to replicate a replacement series (Firbank and Watkinson 1990) or additive series (Snaydon 1991) at a range of plant densities. A bivariate factorial design has been proposed by Snaydon (1991) as the best method to study plant interference. Using this method, results can be analysed as an additive mixture, giving measures of competitive ability, resource complementarity, and severity of competition, presented in two dimensional plots.The advantage of these designs is that the effects of both plant density and proportion are considered (Firbank and Watkinson 1990), but the number of treatments required to conduct such an experiment is many, and thus such trials are time consuming to conduct. Also in a field situation plants rarely occur as two- species mixtures, the addition series does not completely solve the problem of fully understanding the interferences that shape plant communities. Competition studies in the glasshouse should be conducted to elucidate possible mechanisms of interference occurring between plants in the field, rather than to completely mimic the field situation. Findings from the glasshouse can then be related to findings from field trials.

1.5.5 Dual labelling

Radio-isotopes have been used to study interference between plants for many years (e.g., Woods and Brock 1964; Whitcomb *et al.* 1969), but have generally not been recognised as an important tool in the study of root competition.

^{32}P has been used extensively to study the depths in soil from which plant roots acquire P (O'Brien *et al.* 1967; Newbould *et al.* 1971; Goodman and Collison 1981; Abbott and Fraley 1991). O'Brien *et al.* (1967) attempted to use results of this type of work to explain competition but were unable to elucidate the mechanisms involved. Others have used ^{32}P to study the effects of neighbouring plants on P acquisition (Ashokan *et al.* 1988), but using a single isotope does not allow intra- and interspecific interference to be compared concurrently.

Dual labelling of soil with ^{32}P and ^{33}P has proven far more useful than single labelling with ^{32}P in the study of plant competition. This involves the injection of ^{32}P and ^{33}P separately into soil interspaces on opposite sides of a central indicator plant, half way between the central plant and two plant species on either side (Caldwell *et al.* 1985). The two isotopes are then counted in the harvested material of the central plant. One advantage of this method is that it does not employ changes in competitive setting, one of the major drawbacks of other methods.

The dual labelling technique has advantages over other methods of studying plant competition, in that the mechanisms of plant root interference are examined rather than the manifestations of those interferences. Competitive settings need not be altered, intra- and interspecific interference can be observed simultaneously, effects of treatments such as defoliation can be easily examined, rapid plant responses to treatments can be observed, interference occurring between mature plants can be observed without the cumulative uptake of P in a plant's lifetime interfering, and the technique can easily be used in a field situation. Disadvantages include cost and risks involved in the use of isotopes.

Sorn-Srivichai (1985) also attempted to use dual labelling with radioactive P to study root competition between ryegrass and white clover. Unfortunately, the method employed involved the use of competitive settings in which changes in plant density confounded the plant association treatments. However, she did find that ryegrass and white clover acquired P from the same pools in both monocultures and mixtures, with ryegrass acquiring more P from less readily available pools than white clover.

1.5.6 Divided pots

The use of divided pots has been employed as a technique to separate the effects of root and shoot interferences. This involves the use of partitions to physically separate roots and/or shoots of individual plants or plant species. It is known that the effects of above- and below-ground associations of plants are not additive, but interactive (Donald 1958). As already mentioned, shading affects the ability of roots to acquire soil P. In the use of partitions, one needs to be aware of the complications that this technique introduces (Harper 1977), as explained below.

1.5.6.1 *Aerial partitions*

Aerial partitions have been used to prevent intermingling of the above ground parts of plants (e.g., Donald 1958; Rennie 1974), allowing root interference to be considered in the absence of shoot interference. A serious problem with the use of aerial partitions is that the radiation climate (Warren and Lill 1975; Harper 1977) and air flow are altered (Warren and Lill 1975), and may promote a more aggressive species and suppress a lesser species in a competitive situation (Yamada 1985), or vice versa, thus biasing the results of the experiment.

1.5.6.2 *Below ground partitions*

When employing below ground partitions, the soil weight per plant must be held constant to avoid confounding the effects of treatments with plant density. A partition below ground that prevents roots from intermingling may also divide soil resources and space for roots to spread, as was the case in the experiment conducted by Groves and Williams (1975) (Harper 1977). Experiments can be designed to avoid changes in the resources available per plant (e.g., Aspinall 1960). However, it has been suggested that intermingling of roots may result in a more efficient use of environmental factors in monocultures than when roots are separated (Rennie 1974). Grown at the same number of plants per soil weight, Rennie (1974) found that cotton plants (*Gossypium hirsutum* L.) grew better with other cotton plants than when grown separately. However, Rennie (1974) failed to consider root densities and the spread of roots throughout the growing containers. A more efficient exploratory pattern of root growth in the larger containers may have explained their findings.

1.6 Evidence of competition for P between roots of browntop and white clover

Research on the nature of interference occurring between roots of browntop and white clover has concentrated on competition for soil P. The likelihood of other forms of interference being involved has largely been ignored. Following is a critical review of the major work in this area of research.

1.6.1 Field surveys

In field surveys, associations between species are described. For example, positive associations occur when two or more species occur together more often than would be predicted by chance (Silvertown 1987). Perennial ryegrass and white clover have been found to be positively associated with each other, while both being negatively associated with browntop (Harris 1974; Simpson *et al.* 1987). Harris (1974) suggested that this was because browntop produced a dense mat of living and dead tillers, rhizomes, and root tissue which physically prevented the ingress and growth of other species.

Harris (1974) considered that competition for soil P, as suggested by Jackman and Mouat (1972a, 1974), was of less importance in explaining the absence of white clover from browntop-dominated hill country pastures than competition for light, and that grazing management was a major influence in manipulating the botanical composition of a hill country pasture. Grime's theory (Grime 1979) of plant growth strategies support Harris's claim. According to Grime (1979), white clover is a ruderal perennial herb/competitive ruderal (Table 1.2). The occurrence of white clover at a site is dependent on the intensity of disturbance (grazing/treading). That is whether the disturbance is sufficient to enervate more greatly the associated more strongly competitive perennial herbs. At adequate levels of nutrient supply, browntop is potentially fast growing and moderately to highly competitive (Grime 1979; Table 1.2). While under severe mineral deficiency, browntop is a stress tolerator (Table 1.2) due to its low growth rate when stress is high (Grime 1979).

Although Harris (1974) found that associations of white clover and ryegrass were found in soil patches of higher fertility and browntop in low fertility soil patches, this may not necessarily be due to root competition from browntop in the low fertility sites, but is possibly due to browntop plants being better adapted to low soil fertility conditions than white clover. It is possible that the occurrence of white clover in low fertility soil patches may have been low, even in the absence of browntop. In contrast to Harris (1974), Simpson *et al.* (1987) did not find any relationship between the distribution of white clover and soil P or K (potassium) supply in hill swards in mid-Wales.

1.6.2 Ability of roots to absorb P

The relative abilities of white clover and browntop to absorb P from low soil solution concentrations have already been described in Section 1.3.2.1. Using a mechanistic approach (Section 1.5.1), one would predict that browntop would be at a competitive advantage over white clover for P acquisition at low soil P supply. However, as already pointed out, it is not possible to predict whether browntop and white clover will compete in mixtures solely from their growth in monoculture. Whether or not competition for soil P will occur depends on the root densities and distribution of roots (Nye and Tinker 1977). Also, if they do compete, other types of plant interference may alter the balance of competition.

1.6.3 Competition experiments

Competition experiments carried out to study the importance of competition for soil P between roots of browntop and white clover have not been very extensive, and those that have been done have not been easily interpreted due to the experimental designs used. One needs to be aware that the results of an experiment based on changes in competitive settings may be a reflection of the design used. A competitive setting may be changed by altering one or more of a number of factors between treatments (e.g., absence or presence of partitions, absence or presence of associated species). A badly designed experiment can provide a setting that will guarantee the occurrence of competition between plants.

1.6.3.1 *Glasshouse trials*

The first detailed study on competition for soil P between browntop and white clover was undertaken by Mouat and Walker (1959a) using a pot trial carried out in a glasshouse. They claimed to have demonstrated severe competition for soil P between browntop and white clover. The results indicated that browntop did have a more deleterious effect on P acquisition by white clover than did perennial ryegrass. However, a close examination of the experimental design used, and the way in which the dry matter data were presented, reveals some flaws in the interpretation of this experiment.

The description given of the experimental design used by Mouat and Walker (1959a) was ambiguous. Either the experimental design used was a diallel series (replacement series), or the plant density in mixtures was twice that of the monocultures (variation of an additive series). No clear details were given about the plant densities in the monocultures and mixtures, although the description given did suggest that a replacement series design was used. The dry matter results were presented as "dry matter yield per pot". If the experiment was a diallel series, then the decreased yield per pot of white clover in mixtures with browntop, compared with in monoculture may have been simply due to there being less white clover plants in the pots with mixtures. If however, an additive type design had been employed, the lower yield of dry matter per pot of white clover in mixtures with browntop, compared with that in monoculture may have been due to a higher total plant density in the mixture.

Another shortfall was that a comparison of intra- and interspecific interference of browntop was not discussed by Mouat and Walker (1959a). An examination of the results for P concentration and P content in herbage reveals that browntop did not benefit in terms of P acquisition by growing in association with white clover, compared with when grown in monoculture. In a replacement series experiment, this would not have been the expected result if browntop was a more aggressive competitor for soil P than white clover. Had the experimental design used been a variation of the additive series, this result may have been due to a greater density of plants in mixtures than monocultures.

In spite of the problems with Mouat and Walkers' (1959a) experimental design, their results did suggest that competition for soil P may occur between browntop and white clover. They argued that as application of P increased white clover production increased when grown in association with browntop, this proved severe competition for soil P was occurring. The lower rate of response to P application of white clover grown in association with browntop compared with the white clover monoculture also suggests that interspecific competition was more severe than the intraspecific competition between white clover plants, but this may have been due shading from browntop, or, depending on the experimental design used, a greater plant density in mixtures than in monoculture. There is evidence that shoot competition between browntop and white clover may be more important than root competition (Snaydon and Baines 1981), particularly in mature swards where browntop has formed a thick mat that heavily shades white clover stolons (Harris 1974).

1.6.3.2 *Field trials*

Jackman and Mouat (1970) continued research into competition for P between browntop and white clover with a long term field trial. Convinced that browntop was a strong competitor for soil P (Mouat and Walker 1959a), they examined what they referred to as the "grass effect". The "grass effect" is the additional amount of superphosphate needed to produce the same amount of herbage and fixed N from clover growing with a grass as from clover growing in monoculture under the same conditions (Jackman and Mouat 1970, 1972a). The results of this particular field trial have been referred to in the literature (Jackman and Mouat 1972a) as evidence that browntop roots are strong competitors with white clover roots for soil P (e.g., Haynes 1980; Dunlop and Hart 1987; Caradus 1990). Competition for P from browntop roots affecting P acquisition by white clover was not the major finding of the trial. Evidence that competition for soil P does occur between roots of browntop and white clover was only suggestive because white clover had a greater soil P requirement than browntop (Jackman and Mouat 1972a), as noted by other researchers (Grant *et al.* 1981; Edmeades *et al.* 1984). Factors other than competition for P may also explain the effect of browntop on white clover. For example, shading of clover stolons by browntop (Jackman and Mouat 1972a), the effect of browntop on soil moisture (Jackman and Mouat 1972a), and a greater plant densitiy (and therefore possibly root density) in the mixtures of browntop and white clover than in white clover monocultures may have been important factors.

Jackman and Mouat were not able to show the mechanism of root competition for soil P if it was occurring. Below is a synopsis of the results of the trial, with a critique of the conclusions drawn. The field trial is described below in greater detail than would usually be appropriate for a literature review, in order to clarify the problems associated with it.

Two white clovers (*T. repens* L. var. "Grasslands Huia" and "Grasslands 4700") were each grown alone or with browntop on a P deficient soil at 15 rates of superphosphate. Browntop was used because it had been reported to be a strong competitor for soil P (Mouat and Walker 1959a), and partly because Jackman and Mouat (1970) claimed that, at low N supply and when mown, it would persist and would not shade white clover. However, they later realised that the clover growing with browntop produced longer and more erect petioles than the clover growing in monocultures because it was being shaded by browntop (Jackman and Mouat 1972a).

In the second paper detailing further harvests of the same trial, it became clear that shading was an important factor involved in the reduction in white clover herbage yield when grown with browntop (Jackman and Mouat 1972a). When large applications of superphosphate were applied, there was still a large reduction in the growth of white clover due to the presence of browntop (Jackman and Mouat 1972a). The P concentration in shoots of white clover was reduced by the presence of browntop in only two of the five harvests, both of which were during dry summers (N.Z. Meteorological Service). The reductions in P concentration in white clover herbage during those two summers were 0.027 and 0.021% in 1968-69 and 1969-70, respectively. As considered earlier, this may possibly have been due to browntop increasing the rate of soil drying on the availability of soil P. Note that the experiment was established in January, 1968. Shading, and drying of the soil by browntop in the mixtures (Jackman and Mouat 1972b), may explain the lower P concentration in the herbage of white clover in mixtures. Thus, the effect of browntop on P acquisition by white clover may not necessarily have been through direct competition between roots, but by an indirect mechanism that produced effects that mimic competition between roots for soil P ("apparent competition"). Of course, direct root competition may also have been involved, but apart from the obvious effects of shading of white clover stolons by the browntop mat (Jackman and Mouat 1972b), no definite conclusions can be made.

As the field trial progressed, the deleterious effects of browntop on white clover became less severe (Jackman and Mouat 1972a). Worthy of consideration is that negative associations between browntop and white clover are known to develop with time (Harris 1974). In order for one plant to interfere with another, they must be in close proximity. Initially, the swards would have had a fairly random distribution of browntop and white clover, so that interspecific plant interference would have been possible. However, as the two species became negatively associated with time, the level of interference between them would have lessened.

Thus the results of the field trial of Jackman and Mouat (1972a) suggest that "apparent competition" for P occurs between browntop and white clover but research to date has failed to prove that roots of browntop and white clover compete directly for soil P, although this may actually occur.

1.7 Concluding comments

The effects of management practices on the herbage production and botanical composition of hill country pastures are well known (Suckling 1964, 1975; Grant *et al.* 1981; Luscombe *et al.* 1981). However, to be able to make further advancements in hill pasture management, the mechanisms of the deleterious effects of browntop on the growth and persistence of white clover, must be known.

Research to date has failed to prove that exploitative competition occurs between roots of browntop and white clover. The exact nature of interferences, positive and/or negative, occurring between roots of browntop and white clover needs to be elucidated. The impact of preferential defoliation of white clover on these interferences is an area of research that has not been explored. The possibility that other factors (such as soil moisture, physical impedance of the growth of white clover by browntop mat, nutrient requirements of individual species (separate from root competition)) may be more crucial in determining the botanical composition and herbage yields in hill country pastures needs to be clarified.

The following four chapters present the results of four trials designed to examine the nature of root interferences between browntop and white clover by employing three different techniques. Firstly, the plant strategy theories of Grime (1988) were applied to the results of a monoculture P response trial of browntop and white clover, and related to their known performance in hill country pastures. The following two chapters (Chapters Three and Four) present findings of two dual labelling trials aimed at determining the mechanisms of root interferences between browntop and white clover as affected by P supply and defoliation. Finally, a competitive settings pot trial was carried out to compare with the findings of the dual labelling trial. The various experimental techniques used in the trials were assessed for their usefulness and ability to elucidate plant root interferences, and a summary of what has been learnt, and what is known, about root interferences between browntop and white clover has been presented.

CHAPTER TWO

RESPONSE OF THE GROWTH AND PHOSPHORUS UPTAKE OF BROWNTOP AND WHITE CLOVER TO INCREASING EXTERNAL PHOSPHORUS SUPPLY

2.1 Introduction

Browntop is known to be less sensitive to P shortages than white clover. The lowest solution concentration required to allow absorption of phosphate by roots has been measured using solution cultures and found to be only 0.04 μM P for browntop, and 0.54 μM P for white clover (Mouat 1983b), allowing browntop to grow at much lower soil solution phosphate concentrations than those required to sustain white clover growth. Also, browntop attains maximum production at a lower level of P application than white clover (Jackman and Mouat 1972b), and requires a lower P concentration in the plant than white clover at maximum production (Jackman and Mouat 1974). Browntop has also been noted for luxury consumption of phosphate, where P is absorbed beyond the needs for plant growth response (Jackman and Mouat 1974).

Response curves provide information about growth rates, phosphorus requirements (external and internal), and type of response for the species studied. Glasshouse response trials have been shown to be useful in highlighting gross differences between species (Caradus and Snaydon 1988), although they will not necessarily be related to responses measured in the field, due to the complexity of the field.

In the P response trial described in this chapter, observation of the effects of phosphorus supply on browntop and white clover will provide information on the relative abilities of the two species to obtain phosphorus from the high phosphate-fixing Ramiha soil, on the efficiencies of phosphorus use within the plants, and on their phosphorus response curves.

2.1.1 Objectives

The main objective of this trial was to examine the possible ways in which either browntop or white clover may have a competitive advantage when grown at low soil phosphorus supply. This was achieved by:
[1] determining the shape of the growth response curves to phosphorus for browntop and white clover in monocultures,
[2] determining if there was an internal and/or external threshold requirement for phosphorus for either species when grown on P deficient Ramiha subsoil,
[3] calculating arbitrarily chosen critical values for P concentration in (level of nutrient supply at which 50% and 90% of maximum yield is obtained) browntop and white clover,
[4] comparing the total P uptake and concentrations of P in shoots and roots between browntop and white clover to gain an insight into the abilities of the two species to desorb P from the soil and absorb P into the roots, and
[5] comparing the two species for efficiency of P utilization and allocation of phosphorus within the plant.

2.2 Materials and methods

The P response trial was carried out in pots in a glasshouse. The design of the experiment was a 5 x 2 factorial combination of treatments with four replicates in randomised blocks. There were 40 pots altogether. Two factors were examined; level of phosphorus supply: 0, 20, 50, 100 and 200 mg P/kg subsoil applied as monocalcium phosphate; and plant species: Browntop (*Agrostis capillaris*) and white clover (*Trifolium repens*).

2.2.1 The soil

Ramiha soil, a yellow brown earth-yellow brown loam intergrade (Cowie 1976) was collected from Tuapaka Farm, Aokautere, New Zealand. Ramiha soil was chosen for this study because phosphate deficiency is prevalent on this soil, and it supports hill country pasture where browntop is common. Soil was collected from a depth of 5-20 cm, and is referred to as Ramiha subsoil. This subsoil has a medium to high phosphate fixing capacity (a P retention of 68%, according to the method of Saunders 1965) and was extremely deficient in P (Olsen P = 5 $\mu g\ g^{-1}$ soil). Air dry soil (500 g), sieved to < 5 mm, was weighed into free-draining pots after being thoroughly mixed with the appropriate amount of monocalcium phosphate, as detailed above. Basal nutrients were applied in solution to the subsoil at the start of the experiment before planting. The basal nutrient solution contained 60 g/l $MgSO_4.7H_2O$, 80 g/l KCl, 0.192 g/l $Na_2MoO_4.2H_2O$, 1.3 g/l $MnSO_4.4H_2O$, 1 g/l $CuSO_4.5H_2O$, 1 g/l $ZnSO_4.7H_2O$ and 0.68 g/l $Na_2B_4O_7.10H_2O$. This solution was applied at a rate of 5 ml per 500 g of subsoil. Following application of the basal nutrients, the subsoil was allowed to dry and was thoroughly mixed. Before planting the subsoil was watered to a gravimetric water content of 40% (i.e., 75% of water content at 50 cm suction) and was maintained at this level by watering to weight. At 59 days from planting a solution of 5.4 g/l NH_4NO_3 was applied at a rate of 50 ml/500 g subsoil to provide supplementary N.

2.2.2 Planting

Ramets were used to propagate plants from four single parent plants of each species. The parent plants were collected from the established hill country pasture at the site from which the subsoil was collected. Small leaved white clovers were selected as they are more common on hill country pastures than larger leaved ecotypes (Kemp, P.D. pers. comm.). The ramets were planted as monocultures into the pots at a rate of 7 plants/500 g subsoil, and were thinned to 6 plants/500 g subsoil two weeks later. All white clover plants were inoculated with *Rhizobium trifolii* at planting.

2.2.3 Harvesting

All plants were harvested 93 days from planting. Shoots were cut at soil level, and the roots were washed from the subsoil using a hydro-pneumatic elutriation system of root washing (Smucker *et al.* 1982), an automatic root washer that uses jets of water to remove subsoil from the roots.

Strictly speaking, plant material considered to be shoot material was "above ground material"; rhizomes and tillers of browntop, and stolons, petioles and leaves of white clover. Browntop shoots were further separated into rhizomes and tillers.

All plant material was dried to constant moisture percentage in an oven at 65°C, weighed and then ground. The Kjeldahl-type digest mixture of 100 g of potassium sulphate and 1 g selenium powder in 1 litre of concentrated sulphuric acid (Twine and Williams 1971) was used to digest samples. Phosphorus and nitrogen contents were measured on a Technicon auto-analyser (Technicon 1973).

2.2.4 Mitscherlich response curves

Mitscherlich curves were fitted to the data for shoot dry weight responses to P application. The Mitscherlich equation is a widely used model that is often applicable to plant responses to nutrients (Campbell and Keay 1970; Bennett and Ozanne 1972; Spencer *et al.* 1980). This form of the response curve implies that each successive increment in nutrient supply produces a diminishing increment in yield. The Mitscherlich equation is described by:

$$Y=A[1-Be^{-Cx}] \qquad [2.1]$$

where Y is yield at a given level of P application, x is level of P application, A is maximum yield of the plant when the nutrient (e.g., P) is not limiting.

Rearrangement of Equation 2.1 gives

$$B = (A - Y_0)/A \quad [2.2]$$

where $0 < B < 1$, and

$$C = -1/x[\ln(A - Y_x/A.B)] \quad [2.3]$$

Factors which affect *A* include plant species, climate, soil type, pests, management and adequacy of supply of other nutrients (Bennett and Ozanne 1972). However, due to the asymptotic shape of the Mitscherlich curve, an arbitrary level of yield below the maximum is generally chosen to make comparisons between plant species and genotypes, for example, 90% of maximum yield.

Factors which affect *B* include the P status of the virgin soil, P history and plant species. *B* is related to the difference between yield on the unamended soil and the maximum possible yield, and (1 - *B*) is the proportion of maximum yield when no P is applied. *B* can be thought of as the responsiveness of the plant on that soil or at that site (Bennett and Ozanne 1972).

C reflects the curvature of response, and reflects the rate of approach of the curve to the asymptote. It depends on the amount of P that becomes available to plants in a soil (e.g., sand has a high *C* value because little P is adsorbed) and the ability of the plant to take up P, and can be thought of as indicating the effectiveness of the applied nutrient (Bennett and Ozanne 1972). *C* depends largely on root structure (Bennett and Ozanne 1972).

On occasions, in soils with low initial soil P status and/or for some plant species, a response curve with a sigmoidal shape is observed. In such cases a Mitscherlich-type response curve is only appropriate at P additions above a certain threshold level of P applied.

2.2.5 Statistical analyses and curve fitting procedures

Analysis of variance using a General Linear Model procedure was used for analysis of the experiment. The computer programme used for the statistical analyses was SAS (Statistical Analysis System) on the computer network system available at Massey University. The results were analysed as a randomised complete block design (Zar 1984). Curves were fitted to the individual replicate data using an iterative procedure with a computer programme, Curvefit (Kolb 1979).

The LSD presented in the graphs can be used to make comparisons between all means, unless otherwise stated.

2.3 Results

The results of the glasshouse P response pot-trial have been presented below as P-response curves. Yield responses have been presented first (Figures 2.1, 2.2, and 2.3, and Tables 2.1, 2.2, and 2.3), followed by P concentrations (Figures 2.3, 2.4, and 2.5). Lastly, the means for N concentration have been presented (Figures 2.6, 2.7, and 2.8)

2.3.1 Yield response curves

2.3.1.1 *Shoot yields*

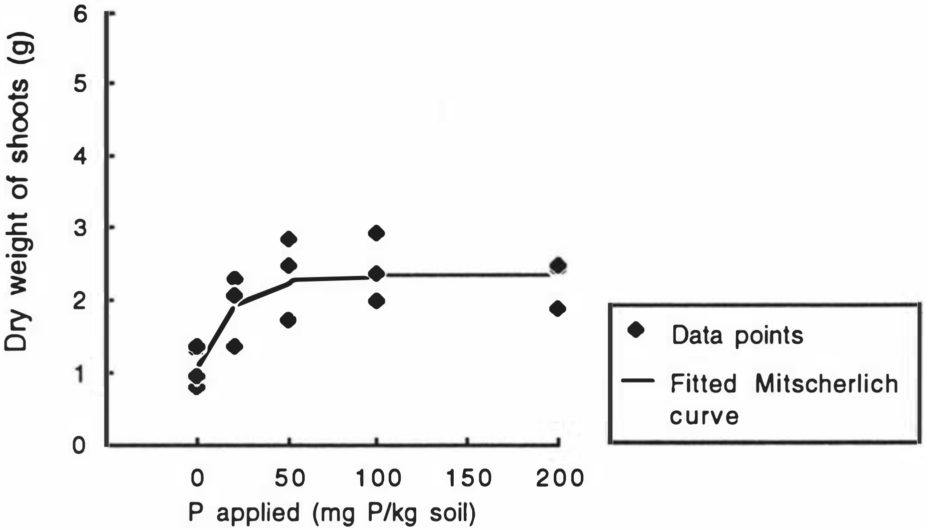
The growth responses of browntop and white clover shoots followed a Mitscherlich-type response curve (Figure 2.1). The predicted maximum yield (*A* value) for white clover was greater than for browntop (Table 2.1, Figure 2.1), and was attained at a higher rate of P applied (Figure 2.1). The maximum yield of white clover was not reached at 200 mg P applied/kg subsoil. The rate of increase in shoot yield towards the maximum shoot yield with increasing P supply was greater for browntop than white clover, as indicated by the more pronounced curvature of the response curve for browntop (Figure 2.2), and by the lower *C* value for white clover than for browntop (Table 2.1).

The increase in shoot dry weight with P application for white clover was more substantial than for browntop (Figure 2.1), as indicated by the greater *B* value for white clover (Table 2.1). The addition of monocalcium phosphate to unamended Ramiha subsoil produced a 3.4 fold increase in shoot dry weight to the maximum possible predicted yield of white clover, but only a 2.2 fold increase for browntop. This yield increase was estimated using the fitted Mitscherlich curves, and, as mentioned earlier, the maximum dry weight of shoots for white clover was not reached at 200 mg P applied/kg subsoil.

2.3.1.2 *Tiller and rhizome yields of browntop*

The response curves of tiller and rhizome weights of browntop were the same, and, like the shoots, followed the Mitscherlich response curve with around 70% of the shoot weight allocated to the tillers at all levels of P applied (Table 2.2).

a.



b.

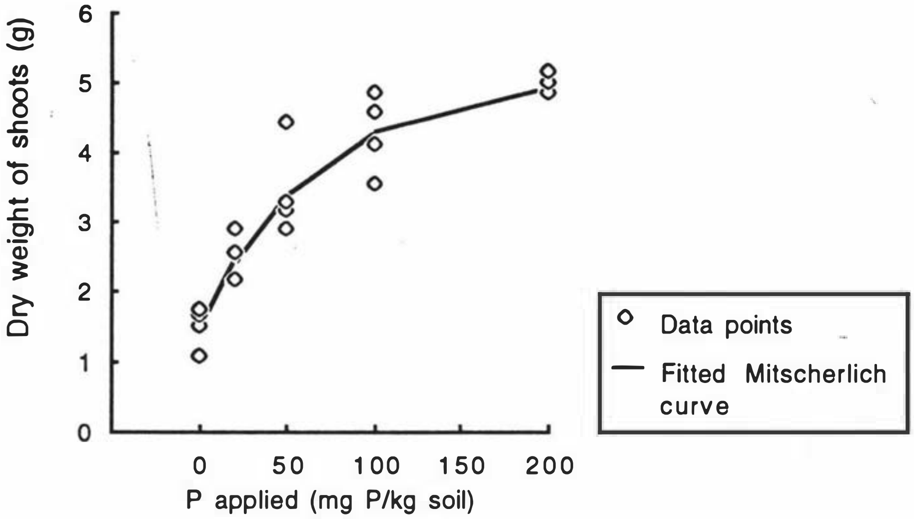


Figure 2.1 Actual and fitted response of dry weights of shoots of (a) browntop, and (b) white clover grown in monocultures on Ramiha subsoil to P application as monocalcium phosphate. Values for Mitscherlich coefficients of fitted curves are given in Table 2.1.

Table 2.1 Coefficients of the fitted Mitscherlich curves for shoot dry weights of browntop and white clover, including t test comparisons between the coefficients for the two species.

Mitscherlich coefficient	**Browntop**	**White clover**	**t**
A	2.33	5.14	36.97 $p \geq 0.001$
B	0.54	0.71	8.50 $p \geq 0.001$
C	0.056	0.015	7.28 $p \geq 0.001$

$t_{0.001(2),38} = 3.566$

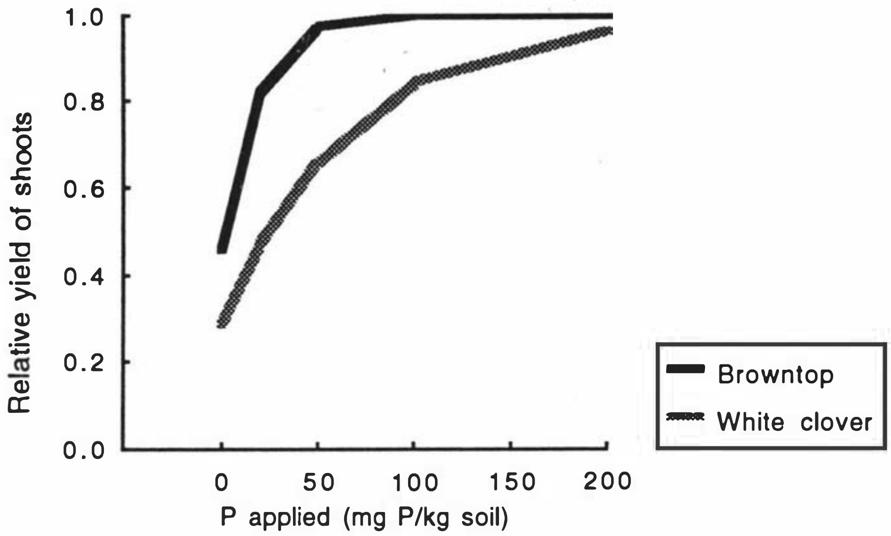


Figure 2.2 Relative yield responses of dry weights of shoots of browntop and white clover grown in monocultures on Ramiha subsoil to P application as monocalcium phosphate, calculated from the fitted Mitscherlich response curves.

Table 2.2 Effect of P application as monocalcium phosphate to Ramiha subsoil on the dry weights of tillers and rhizomes of browntop grown in monoculture.

Dry weight(g/pot) of tillers and rhizomes of Browntop

P applied (mgP/kg subsoil)	**Tillers** mean(st.error) n=4	**Rhizomes** mean(st.error) n=4
0	0.79 (0.08) a	0.29 (0.10) a
20	1.37 (0.20) b	0.54 (0.19) ab
50	1.60 (0.13) b	0.68 (0.16) b
100	1.67 (0.08) b	0.71 (0.14) b
200	1.60 (0.06) b	0.66 (0.13) b
P comparison	LSD: 5% 0.47	LSD: 5% 0.26

Means within each plant division (i.e., tillers or rhizomes) with different letters differ significantly ($p \leq 0.05$).

Most of the dry weight produced in browntop shoots was allocated to the tillers rather than to the rhizomes (Table 2.2), with the proportion allocated to the rhizomes being, on average, around 28% of shoot dry matter.

2.3.1.3 *Root:shoot ratios*

The root:shoot ratio of browntop plants was greater than for white clover (Table 2.3). There was no effect of P application on the root:shoot ratio of white clover, while the root:shoot ratio of browntop was increased by P addition (Table 2.3). However, it is generally accepted that root:shoot ratios decrease with increasing P supply (e.g., Bradshaw *et al.* 1960; Hart 1981). The data suggest that a substantial amount of root material may have been lost during the root washing process (Appendix 5) because the root:shoot ratios were lower than expected (Mouat 1983a). Also there appeared to be greater root loss at lower levels of P supply where roots may have been finer.

Due to the atypical nature of the response in the browntop root:shoot ratios to increasing P supply, the results presented here concentrate on the data for weights and P uptake by shoots. However, means for P and N concentration in roots were presented on the assumption that the samples of roots collected were representative of the whole root system.

2.3.2 External P requirements for shoot growth

Browntop required a lower application of P as monocalcium phosphate to reach the same relative yield as white clover (Figure 2.2). From the fitted curves (Figure 2.1), the level of P application required to produce an arbitrary level of growth relative to the predicted maximum possible yield was calculated. To produce half of the predicted maximum yield of shoots, 1.4 and 23 mg P applied/kg subsoil were needed for browntop and white clover, respectively. Similarly, the level of P application required to attain 90% of predicted maximum shoot growth was determined. These predicted values were 31 and 131 mg P applied/kg subsoil for shoots of browntop and white clover, respectively. Also, 90% of predicted maximum growth of browntop tillers was attained at 28 mg P applied/kg subsoil. The higher *C* value (greater curvature of response) and lower *B* value (greater relative yield) of browntop were a reflection of its lower external P requirement.

2.3.3 P content of shoots and internal P requirements

2.3.3.1 *P concentration in shoots*

The P concentration in shoots of browntop and white clover increased linearly with P applications (Figure 2.3). The rate of increase in P concentration in shoots with increasing P supply was greater for browntop than white clover (Figure 2.3). Browntop had an internal P concentration requirement of only 0.12% P in shoots (tillers and rhizomes), to produce maximum yield, while white clover required greater than 0.15% P. The exact internal P requirement for white clover shoots could not be determined from this trial as the top level of P applied was not enough to produce maximum yield.

Up to 50 mg P applied/kg subsoil, there were no significant differences between the two species for P concentration in shoots at the same level of P applied. However, at 100 and 200 mg P applied/kg subsoil the P concentration in shoots of browntop was greater than in white clover (Figure 2.3).

2.3.3.2 *P content of shoots*

For white clover the P content of shoots responded to P application in a similar manner to the shoot dry weights. All additions of P applied produced an increase in the P content of shoots of white clover. Up to 50 mg P applied/kg subsoil, the P content of shoots of browntop also responded to P application in a manner similar to the shoot dry weights. Nevertheless, with P applications greater than 50 mg P/kg subsoil there was a marked increase in the P content of shoots of browntop that was not observed for the shoot dry weight (Figure 2.1a), due to the increase in P concentration in the shoots with all applications of monocalcium phosphate (Figure 2.3).

There was no difference between the two species for P content at 0 and 20 mg P applied/kg subsoil, with the P contents being 1.1 and 2.1 mg P/pot, respectively. At higher levels of P application the P content of shoots of white clover was greater than in shoots of browntop due to the greater shoot dry weights of white clover (Figure 2.1). At 200 mg P applied/kg subsoil the P contents of browntop and white clover were 5.1 and 7.6 mg P/pot, respectively.

Table 2.3 Effect of application of P as monocalcium phosphate to Ramiha subsoil on the root:shoot ratio of browntop and white clover plants grown in monocultures (LSD = 0.11 for P by species interaction).

P applied (mgP/kg subsoil)	Browntop	White clover
0	0.23	0.16
20	0.29	0.12
50	0.32	0.15
100	0.39	0.14
200	0.38	0.14

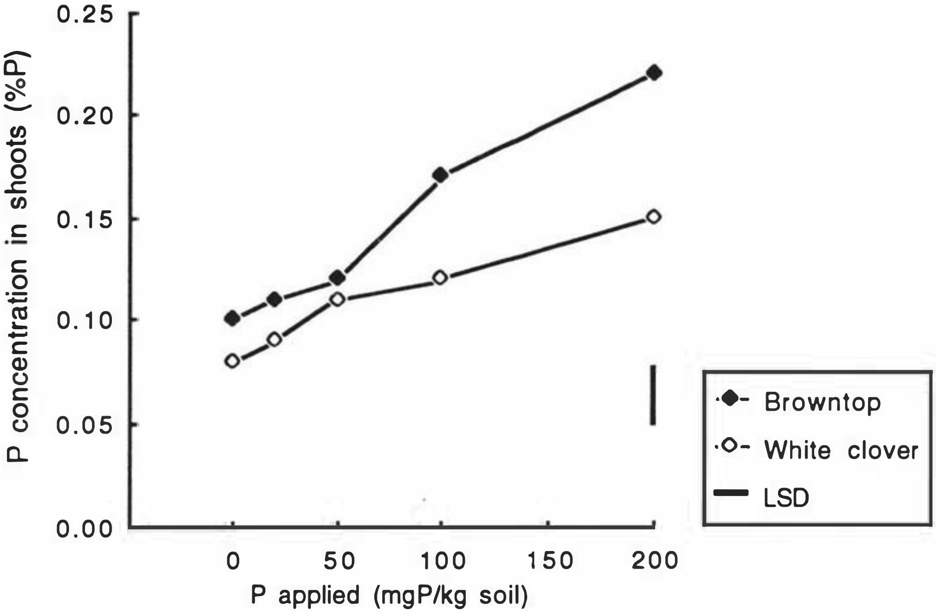


Figure 2.3 Effect of application of P as monocalcium phosphate to Ramiha subsoil on the P concentration in shoots of browntop and white clover grown in monocultures.

2.3.3.3 P content of rhizomes and tillers of browntop

The P concentration in rhizomes and tillers increased with P applications of 100 and 200 mg P/kg subsoil ($p \leq 0.05$) (Figure 2.4). The P concentration in rhizomes was significantly lower than in tillers ($p \leq 0.0001$) at all levels of P application. Interestingly, the P concentration in rhizomes was the same as the P concentrations found in the roots at the lower levels of P application, but was greater than in roots at 200 mg P applied/kg subsoil (Figure 2.4).

2.3.4 P concentration in roots

The P concentration in roots of browntop was lower than in white clover ($p \leq 0.0001$) (Figure 2.5). At 90% of predicted maximum shoot dry weight, the P concentration in roots of browntop was 0.08% P, about half that found in roots of white clover (0.18% P). The response of P concentration in roots of both browntop and white clover to P application appeared slightly sigmoidal. Applications of 100 and 50 mg P/kg subsoil were required to produce significant increases in P concentration in roots of browntop and white clover, respectively (Figure 2.5).

2.3.5 N concentration in shoots and roots

The responses to P application of N concentration in shoots of browntop and white clover contrasted (Figure 2.6). The N concentration in shoots of browntop was reduced from 2.4% in plants grown on unamended subsoil to 2.0% with the first application of P (20 mg P/kg subsoil), most likely due to dilution with increased shoot dry weight. Further applications of P did not affect the N concentration in browntop shoots significantly. The N concentration in white clover shoots did not increase significantly until 50 mg P/kg subsoil had been applied, and did not increase above that level although the roots were nodulated.

At all levels of P applied, except in plants grown on unamended subsoil, the N concentration in white clover shoots were higher than in browntop plants (Figure 2.6). The N concentration in browntop shoots (tillers and rhizomes) was low, below 2% at 50, 100 and 200 mg P applied/kg subsoil. Despite this, browntop plants showed no symptoms of nitrogen deficiency (Plate 2.1). However, the extremely low N concentrations found in the rhizomes of browntop (Figure 2.7) may account for the lack of N deficiency symptoms in shoots with the N concentration in tiller material-only being higher (2.2 to 2.8% N) than for the whole shoots. The allocation of N between rhizomes and tillers was similar to the allocation of P, with a greater concentration of N and P being found in the tillers.

There was no effect of P application on the N concentration found in roots of either browntop or white clover (Figure 2.8). Over all levels of P applied the N concentration in roots of white clover was greater than in roots of browntop.

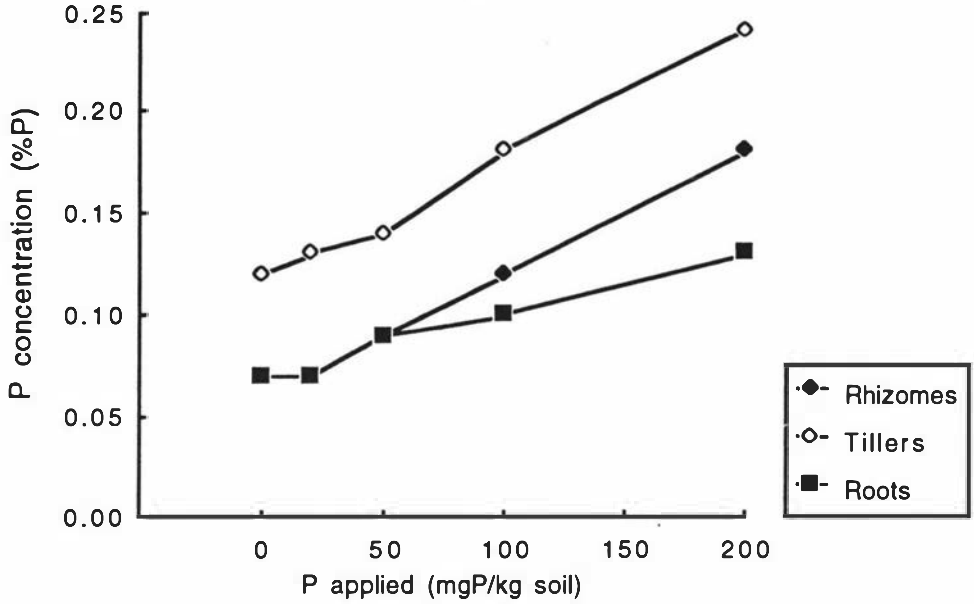


Figure 2.4 Response of P concentration in tillers, rhizomes, and roots of browntop grown in monoculture to P application as monocalcium phosphate to Ramiha subsoil. (LSD = 0.02, $p \leq 0.05$).

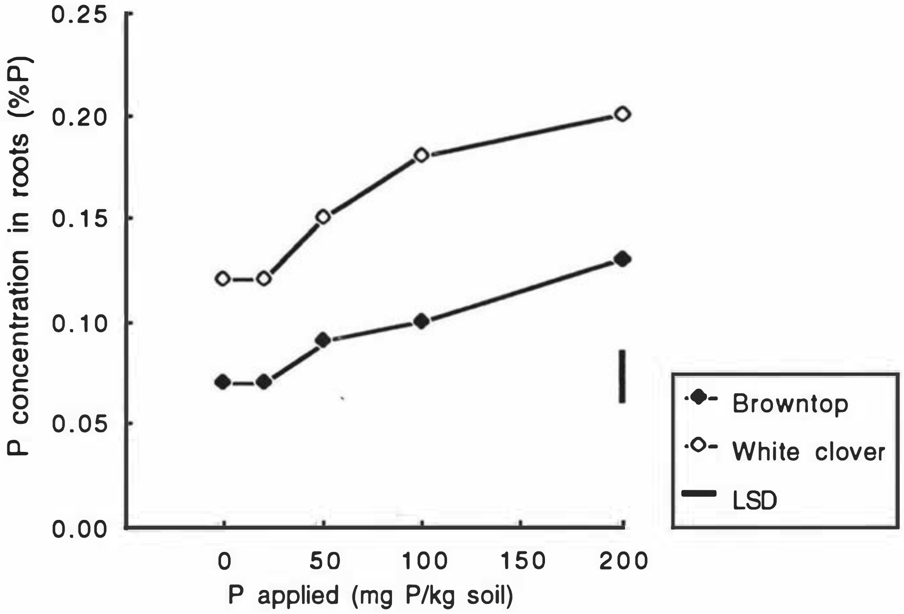


Figure 2.5 Effect of application of P as monocalcium phosphate to Ramiha subsoil on the P concentration in roots of browntop and white clover grown in monocultures.

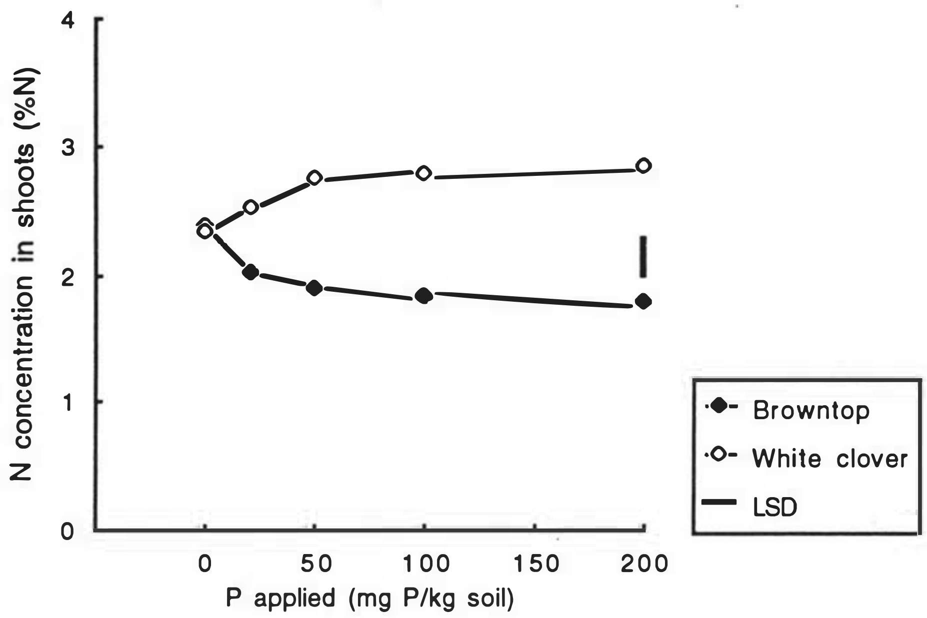


Figure 2.6 Effect of application of P as monocalcium phosphate to Ramiha subsoil on the N concentration in shoots of browntop and white clover plants grown in monocultures.

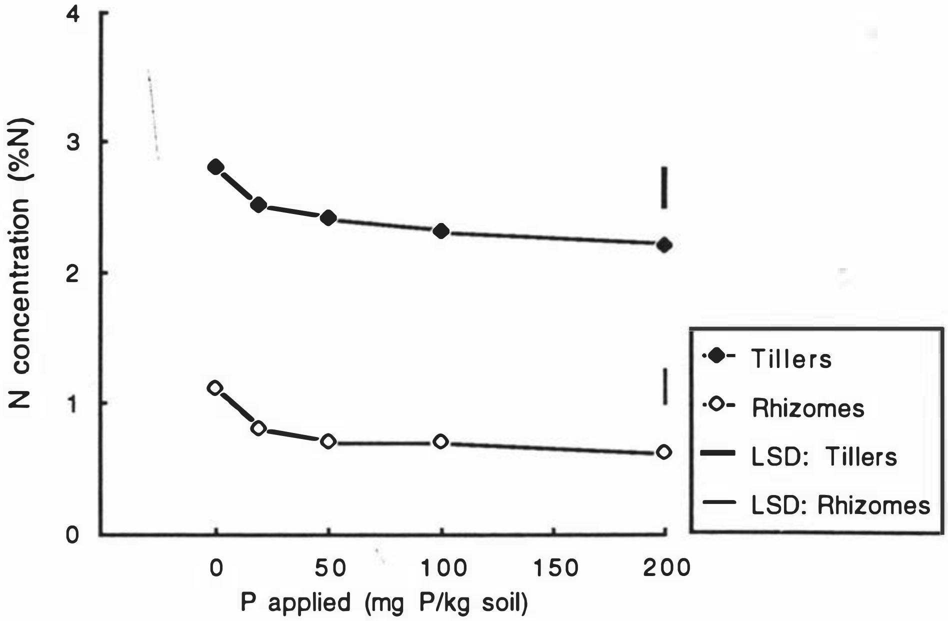


Figure 2.7 Effect of application of P as monocalcium phosphate to Ramiha subsoil on the N concentration in tillers and rhizomes of browntop plants grown in monocultures.

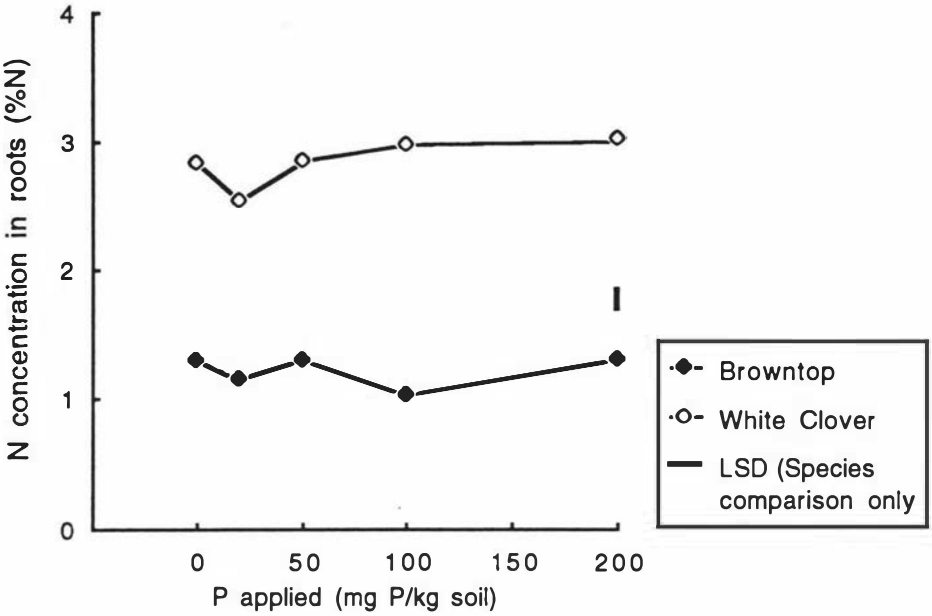
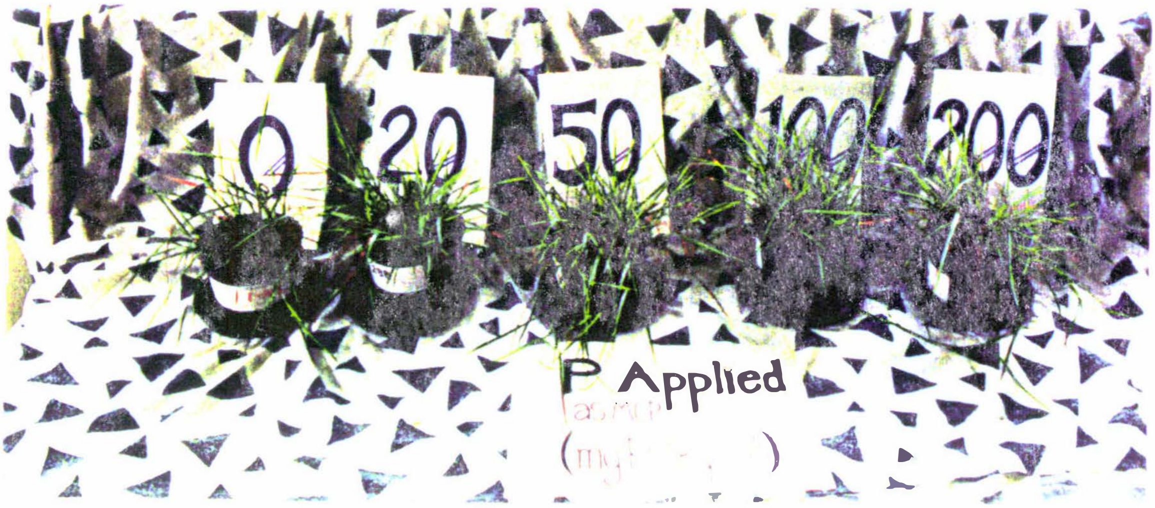


Figure 2.8 Effect of application of P as monocalcium phosphate to Ramiha subsoil on the N concentration in roots of browntop and white clover plants grown in monocultures.

a.



b.

Plate 2.1 Photographs of (a) browntop, and (b) white clover plants in the P

2.4 Discussion

In order to understand the possible ways in which either browntop or white clover may be at an advantage to dominate pastures at low soil P supply, we need to consider plant traits that are known to enable survival under conditions of high P stress. The findings from the P response trial have been discussed below from an ecological viewpoint.

The P response trial was conducted under conditions of extremely deficient soil P supply. The P concentrations measured in shoots of browntop (tillers plus rhizomes) and white clover grown on unamended soil were only 0.10 and 0.08 %P, respectively. Although these P levels may be considered to be too low for the survival of plants, especially white clover, the plants in this trial did not appear to be near death (Plate 2.1). The plants were cloned from naturalised ecotypes that may have adapted to survive and grow on low fertility soils at lower tissue P concentrations than bred lines.

Browntop ecotypes collected from low P soils have been found to be more efficient at utilising P when P supply is low than ecotypes collected from soils of higher P supply (McCain and Davies 1983). However, it is generally accepted that less productive genotypes of plants tend to have higher shoot P concentrations when grown under favourable conditions, as found in a studies of white clover ecotypes (Snaydon and Bradshaw 1962; Spencer *et al.* 1980; Caradus 1986). Yet, the results of the P response trial suggest that at very deficient levels of soil P supply, white clover ecotypes collected from low fertility hill country sites may be able to survive under these conditions by being able to grow at very low shoot P concentrations, and by partitioning a large proportion of P to the roots (Caradus 1986; Figure 2.5).

2.4.1 P stress tolerance

Under conditions of nutrient stress, the ability of plant species to withstand stress is considered to be more important than competitive ability. According to Grime (1979), plants indigenous to infertile habitats conserve nutrients, as opposed to maximising the rate of capture of nutrients.

Browntop is considered to be a competitor, a stress-tolerator, and a ruderal, while white clover is a competitor and a ruderal (Grime *et al.* 1988). Because the P response trial was conducted under conditions of high P stress, stress tolerance is the most important plant trait to compare between browntop and white clover under these conditions. Below is an examination of how the findings of the present trial compare with the theory of Grime (1979; Grime *et al.* 1988).

The lower shoot growth measured for browntop compared with white clover (Figure 2.1) agreed with the findings of other researchers that some *Agrostis* species have low growth rates (Clarkson 1967). Slow growth is considered to be an adaptive feature of low fertility-tolerant grasses (Clarkson 1967) and other nutrient stress tolerant species (Hobbie 1992) when grown under conditions of low soil nutrient supply. The growth of browntop plants in this trial was not limited by N deficiency as indicated by the N concentrations measured in the tillers (Figure 2.7; Andrew and Johansen 1978).

Low growth rate creates a low demand for soil nutrients (Hobbie 1992). The importance of a low growth rate to survival on low P soils has been emphasised by some plant ecologists, who have concluded that interspecific differences in P metabolism, uptake and utilisation are not as important as growth rate (Chapin and Bieleski 1982; Chapin *et al.* 1982).

The high shoot dry matter production of white clover (Figure 2.1) posed a problem in that the roots of white clover were unable to acquire phosphorus in large enough quantities to avoid suffering from P deficiency (Figure 2.3), which also inhibited the fixation of N in roots, reflected by the lower N concentration in shoots of white clover grown on unamended soil (Figure 2.6). Notice that the P concentration in roots of white clover was higher than in roots of browntop (Figure 2.8) due to the high requirement for P in root nodules to allow nitrogen fixation to occur (Dunlop and Hart 1987). Furthermore, the lower N concentration in white clover shoots at low P supply demonstrated that a high P concentration is required to allow efficient N fixation.

The lower shoot growth rate of browntop compared with that of white clover may partly explain the higher P concentration found in browntop shoots (Figure 2.3) in terms of dilution. The low efficiency of P use by browntop compared with white clover (shoot dry weight produced per unit of P absorbed), may be partly a reflection of the slow growth rate of browntop. The higher P concentration in browntop compared with white clover would give browntop an advantage for survival, particularly during future periods of P shortage. Low efficiency of P utilisation has also been found with other P stress-tolerant species (Chapin and Bieleski 1982).

Browntop appears to have a mechanism which allows its roots to continue phosphate absorption even when the concentration of phosphate required to achieve maximum growth has been reached; a process termed "luxury consumption" (Hommels *et al.* 1989). Luxury consumption allows browntop to conserve P by maintaining an internal supply. Consistent with the findings of previous research (Sprague 1934), surplus absorbed P was stored in the shoots of browntop (Figures 2.3 and 2.4). Being mobile within the plant, phosphorus can be efficiently retranslocated to growing tissues (Epstein 1972). Even with the low levels of soil P supply in the present trial, browntop, but not white clover, was able to store P because with its slow growth it was able to acquire P in quantities greater than its requirement for growth, resulting in luxury consumption at the levels of P applied of 100 and 200 mg P/kg subsoil (compare Figure 2.1a and Figure 2.3).

Luxury consumption is considered to be an adaptive feature of plants able to grow under conditions of low nutrient supply (Chapin 1980). Luxury consumption of phosphorus was observed for browntop as early as 1934 (Sprague 1934). The delayed onset of P deficiency in times of future shortage of soil P supply by retranslocation of stored P would have a great impact on browntop because of its low growth rate. By conserving P internally browntop plants would be able to sustain growth for a longer time than white clover (Clarkson 1967; Rorison 1968; Chapin 1980).

2.4.2 External P requirement

Another trait that contributed to browntops' tolerance of low P soils (Mouat 1983b) was the lower P application required to produce maximum shoot dry matter of browntop compared with white clover (Figure 2.2), as found by previous research (Jackman and Mouat 1972b). The lower soil P supply required for browntop to attain maximum possible shoot yield was due to the more pronounced curvature of response to P application compared with white clover (Figure 2.2), as indicated by the greater Mitscherlich *C* value for browntop (Table 2.1). According to the predictions made from the fitted Mitscherlich curves, P deficiency in browntop was alleviated with an application of only 31 mg P/kg subsoil on the extremely deficient Ramiha subsoil. This would enable browntop to survive well in the field when growing on poor and/or unfertilized soils. The maximum shoot yield for white clover was not even attained in this trial, probably because the P supply was low in the Ramiha subsoil and P would have been strongly adsorbed by the subsoil.

Differences in curvature of response to P application by plant species may be due either to differences in the amount of P in the soil that becomes available to the plant or to the ability of the plant to take up P (Bennett and Ozanne 1972). In comparing browntop and white clover, the most obvious of these explanations, one that relates to the ability of the plant to take up P, is that browntop has a more extensive root system than white clover. Browntop roots are more extensive than white clover in terms of root and root hair length, fineness of roots, surface area of roots, and length of roots with root hairs (Evans 1977). For a nutrient of low mobility in the soil, such as P, these parameters are extremely important in terms of depletion of P from soil. The more fibrous nature of browntop roots (Caradus 1980) provides access to a larger pool of available P than would be available to white clover (Fitter and Hay 1987).

A problem with the comparison made above for P uptake efficiency is that the functionally active part of the root system (which is far less than the total root length) is not known. However, as apical regions are known to be more active than other regions of roots, the greater number of root growing tips of browntop than white clover (Evans 1977) support the above argument.

Browntop is better adapted to soils of low P supply than white clover, due to the lower productivity of browntop (Figure 2.1) and slower relative root growth rate of white clover (Caradus 1980).

Edmeades *et al.* (1984) found that browntop dominated pasture did not respond as well as white clover/ryegrass pasture to P application on P deficient soil. Not only was the production of shoot dry matter lowest in the reverted browntop-dominated pasture, but a higher application of P was required to reach maximum possible yield. The reason for this seemingly conflicting finding to the present glasshouse trial, may be that with a low proportion of legume in the browntop dominated pasture, higher rates of P application were needed to stimulate increased white clover production.

2.4.3 Nutrient cycling

Another important, and often overlooked, plant trait that affects the distribution of plant species is the rate of nutrient recycling. The rate of recycling of nutrients from litter decomposition is affected by the amount of lignin in, and the C:N ratio of, the litter (Hobbie 1992). The rate of decomposition of plant litter is inversely proportional to the C:N ratio of the litter (Hobbie 1992). Low fertility-tolerant plants tend to produce litter that decomposes slowly. Considering that browntop has a lower N concentration than white clover in both shoots (Figure 2.6) (except at 0 mg P applied/kg subsoil) and roots (Figure 2.8), it would be expected that the rate of return of P from browntop to the soil would be slower than from white clover. The advantage of a slow rate of decomposition of browntop litter would be a reduction in loss of nutrients to leaching and other organisms (Grime 1979).

The consequence of a slow rate of cycling of P within a browntop dominated pasture would be that because browntop has the capacity to absorb P in luxury quantities, even when grown on an extremely deficient soil (as shown in this P response trial), large quantities of P may become tied up in organic forms, living and dead.

In terms of loss of P due to grazing, allocation of P to roots may be of some advantage to white clover plants. With severe grazing, browntop plants would lose large amounts of P stored in the shoots, particularly as the P content and P concentration in tillers is substantially greater than in the rhizomes (Figure 2.4 and Table 2.2), which are less likely to be removed when browntop plants are grazed. However, the greater root/shoot ratio of browntop (Table 2.3) may offset this difference.

2.4.4 Summarising comments

Despite the extensive amount of work on the deleterious effects of browntop on P uptake by white clover (Mouat and Walker 1959a; Jackman and Mouat 1970, 1972a, 1972b, 1974), the comparative responses of browntop and white clover to P applied have only been briefly discussed. A general, and somewhat ambiguous, conclusion was drawn by Jackman and Mouat (1972b), based on the lower external P requirements of browntop, that "the clovers were less efficient, ..., in their response to P than were the grasses". This was the basis of their explanation for browntop decreasing the ability of white clover to acquire soil P. However, it should be realised that the finding that white clover requires a much higher application of P than browntop to produce similar relative shoot yield, also found in the present trial, may simply be a reflection of browntop being better adapted to grow under conditions of low soil P supply than white clover. It does not suggest that browntop is capable of reducing the availability of soil P to white clover roots. For this to occur an examination of the ability of browntop to reduce the supply of P in the soil is required. However, it seems that in the long term, browntop may "lock up" large quantities of P. The impact of this on the botanical composition of hill country pastures would depend on the rate of cycling of P from browntop back into the soil (Hobbie 1992). Further investigation into the likelihood of P depletion zones overlapping between roots is also required in order to conclude that direct competition for P could occur. This would require consideration of the root densities within the soil, determined from root lengths.

The findings of this trial highlight the problems involved in attempting to predict the effect of soil P supply on root interactions between two species from their responses to P application when grown in monocultures (Harper 1977). That browntop is better adapted than white clover to grow on soils of low P fertility does not prove that browntop roots will compete directly with white clover roots. The results do suggest that browntop would be the "winner", and white clover the "loser" if grown together at low P supply. Slow growing plants of low phosphorus requirement may actually be poor competitors when grown in fertile soils with high producing nutrient demanding plant species. Etherington (1983) claimed that in order to avoid such competition, low fertility-tolerant species may take refuge in harsh environments where the higher producing plant species cannot perform well, however it is inelegant to bestow the power of reason upon a plant.

Although there is no evidence from the P response trial that browntop reduced the P supply in the soil to a greater extent than white clover, this did not discount the possibility that browntop roots may compete directly with white clover roots in the field through an overlapping of depletion zones (Grinsted *et al.* 1982). The greater root length and root hair length of browntop compared with white clover (Evans 1977) makes this a strong possibility. In the following Chapters Three and Four the nature of intra- and interspecific root interactions of browntop and white clover are considered.

CHAPTER THREE

THE EFFECTS OF SOIL PHOSPHORUS SUPPLY AND DEFOLIATION ON THE INTER- AND INTRASPECIFIC ROOT INTERFERENCES OCCURRING IN MINISWARDS OF BROWNTOP AND WHITE CLOVER

3.1 Introduction

Plant interference can be defined as the response of an individual plant or plant species to its total environment as modified by the presence and/or growth of other individuals or species (Hall 1974). Competition does not embrace all the mutual influences of plants growing together (Hunter and Aarssen 1988). Root competition refers to the specific situation where soil resources are in limited supply and the amount of a soil resource obtained by one plant is directly reduced by the presence of roots of another plant, which may be of the same or different species. If one species is more competitive than another species, that species will obtain more of a limited resource when grown with the less competitive species than when grown with other plants of the same species (Hall 1974). The direct stimulation of one species by another is an example of non-competitive interference (Allen and Allen 1990).

It can be tempting to explain the distribution of plant species in terms of plant competition, especially if the exclusion of one species is associated with the presence of another. It has long been accepted that white clover is a weak competitor for phosphorus (Haynes 1980) because of its poor ability to acquire P at low levels of soil phosphorus supply (Jackman and Mouat 1972a). This conclusion has been used to explain the absence of white clover in browntop-dominated hill country pastures in New Zealand (Mouat and Walker 1959a; Jackman and Mouat 1970). Nevertheless, the evidence supporting the dominance of browntop over white clover due to superior competitiveness for P is not as good as is often assumed (refer to Section 1.6). The effect of browntop on phosphorus uptake by white clover has been associated with indirect effects of shading (shoot competition for light) and soil moisture stress (interference by browntop on the environment of white clover roots) (Jackman and Mouat 1974). Also there is no evidence to date that browntop gains a competitive advantage for phosphorus uptake by growing in the presence of white clover. There is no doubt, however, that browntop can absorb phosphorus from extremely low soil solution concentrations (Mouat 1983a), and that a reduction in soil P availability would have a more detrimental effect on white clover than on browntop (Jackman and Mouat 1974; Chapter Two). However, it is not possible to predict the interactions that will occur between species from their growth in monoculture (Harper 1977; Firbank and Watkinson 1990).

There is good evidence that grazing may interact with competition for phosphorus (Caldwell *et al.* 1987; Banyikwa 1988). The preferential defoliation of one plant may lead to the greater uptake of phosphorus from a limited supply by a neighbouring undefoliated plant of the same or different species. Using a dual labelling technique, Caldwell *et al.* (1985) found that Sagebrush (*Artemisia tridentata*) absorbed 86% of ^{32}P or ^{33}P from an interspace shared with a prairie bunch grass (*Agropyron spicatum*), while absorbing only 14% from an interspace shared with another species of prairie grass (*Agropyron desertorum*), and concluded that *A. desertorum* was more competitive with Sagebrush than *A. spicatum.* This work was extended to show that competition for P was involved, by demonstrating that defoliation of the neighbouring *Agropyron* prairie grasses lead to an increase in isotope acquisition by the central Sagebrush plant within 14 days (Caldwell *et al.* 1987).

Under the same defoliation treatment white clover is known to maintain root elongation better and suffer less root death than perennial ryegrass (Evans 1973a), and browntop (Evans 1973b). However, it is not known how defoliation affects the interactions that occur between roots of browntop and white clover in relation to phosphorus uptake.

Most studies on competition between plants measure the performance of plants in various competitive settings, for example, additive and replacement series experimental designs, or by the use of various aerial and/or soil partitions (Harper 1977) In these types of experiments, competition may be inferred from how the performance of the plants is affected by the various experimental settings, but the mechanisms of direct interactions between roots remains unclear.

A dual isotope labelling technique has been used to demonstrate direct competition between roots of plants in the field, and the rapid effects of defoliation on root competitive ability (Caldwell *et al*. 1987). The objective of the present study was to determine whether a technique of dual isotope labelling with ^{32}P and ^{33}P could be used to determine the nature of the interactions between roots of browntop and white clover.

The questions to be answered in this trial were:
[1] Does browntop gain a competitive advantage for soil phosphorus uptake by growing with its roots associated with roots of white clover plants compared with when grown with its roots associated with the roots of other browntop plants?
[2] Is white clover at a competitive disadvantage for soil phosphorus uptake by growing with its roots associated with browntop roots compared with when grown with its roots associated with the roots of other white clover plants?
[3] Can defoliation of either browntop or white clover or both alleviate the severity of competition for soil phosphorus with outer simulated sward plants of the same or different species?
[4] Is there any type of inter- or intraspecific root interference occurring in browntop-white clover swards other than competition?

3.2 Materials and methods

3.2.1 Experimental design and treatments

The basis of the design was to determine the relative amounts of phosphate that a row of plants (either browntop or white clover) can absorb from two adjacent soil spaces, one dominated by white clover roots and the other by browntop roots. ^{32}P was injected into the soil on one side of a central row of indicator plants and ^{33}P into the other side (Figure 3.1). The relative uptake of ^{32}P and ^{33}P was assessed by harvesting the shoots and roots a short time after labelling, in this case seven and fourteen days, and then counting the two isotopes.

The design of the experiment was a randomised complete block, with four replicates. The treatments imposed were; level of phosphorus: 0 and 100 mg P/kg soil, applied as monocalcium phosphate; central indicator species: browntop or white clover; and defoliation: outer simulated sward browntop or outer simulated sward white clover defoliated, or not defoliated. The treatments were imposed in twelve factorial combinations.

The defoliation treatments were imposed on the same day as the injection of the carrier-free radioactive ^{32}P and ^{33}P. The defoliation treatments and P isotope injections were carried out over four consecutive days, with one replicate being treated each day. The defoliation treatment involved cutting the outer simulated swards on one side of the row of central indicator plants to a stubble height of 20 mm. This defoliation was considered to simulate sheep grazing (Clark *et al.* 1984).

3.2.2 The soil

Ramiha subsoil (see Section 2.2.2), collected from Tuapaka Farm, Aokautere, New Zealand, was passed through a 5 mm sieve to remove debris, such as plant material, and to make it easier to create a homogeneous soil mixture. Soil was weighed into rectangular plastic trays of the dimensions 300 mm by 400 mm, and 100 mm depth. The trays were separated into two sections with a partition made of perspex and sealed with silicon (Figure 3.1). 4.5 kg of Ramiha soil was weighed into each sealed half after being thoroughly mixed with the appropriate amount of monocalcium phosphate. The soil in each sealed half of the tray was further partitioned by pushing two plastic partitions into the soil (Figure 3.1) separating the roots of plants in each sealed half of the tray into two harvests.

A closed system (i.e., trays were not free draining) was used to allow control of the soil water content and to reduce possible problems associated with the use of radioisotopes, e.g., to prevent leakage. The bulk density of the sieved soil was 1.036 g/ml. At 50 cm suction the gravimetric water content was 50%.

3.2.3 Watering

The water content across the tray was carefully monitored and kept constant because soil water content is known to have an effect on the availability of phosphate to plant roots (Low and Piper 1960).

The soil water content was monitored daily throughout the experiment by weighing, and was occasionally checked across the tray by using an IRAMS time domain reflectometer (Topp *et al.* 1982). At planting the soil was watered up to 35% gravimetric water content. Later, as the demand for water by the plants increased, the soil was watered up to a gravimetric water content of 40%.

3.2.4 Planting

Ramets of browntop and white clover were used to propagate plants from four single parent plants of each species. The chosen parent plants were collected from the same site as the soil, on Tuapaka Farm. For white clover, the more stoloniferous small leaved ecotypes were chosen as they are more common in hill country pastures than the larger leaved ecotypes. The ramets were planted into the trays as shown in Figure 3.1. The soil was inoculated with *Rhizobium trifolii* to encourage nodulation of white clover roots.

3.2.5 Aerial partitions

Aerial partitions were used to separate the above ground parts of the central row of either browntop or white clover plants from the outer simulated swards of browntop on one side and white clover on the other (Figure 3.1 and Plate 3.1). The aerial partitions were made of sturdy cardboard covered in reflective

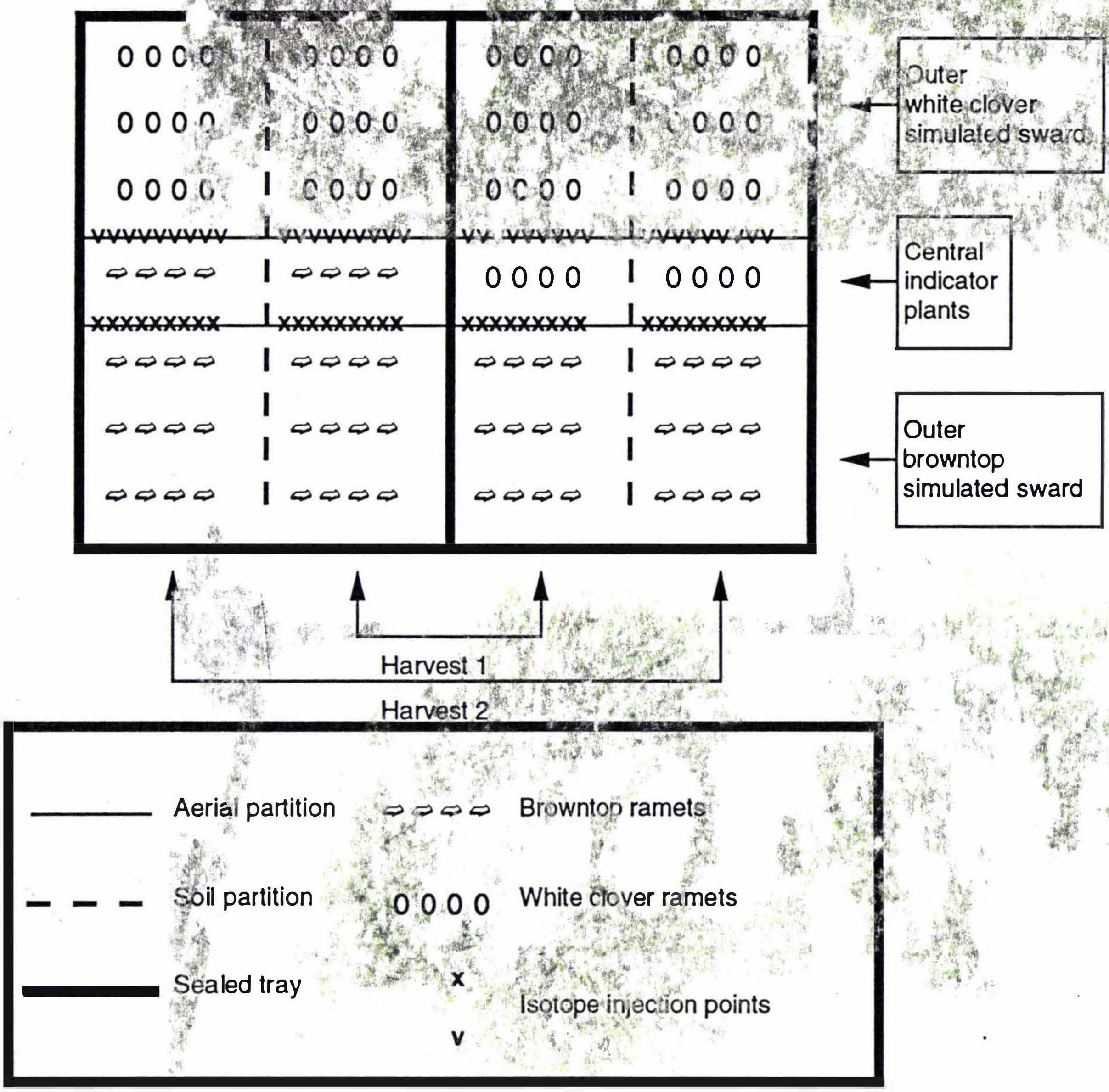


Figure 3.1 The planting arrangement of ramets of browntop and white clover, detailing positioning of points of P isotope injection, aerial partitions, and the soil partition used to separate the tray into two harvests.

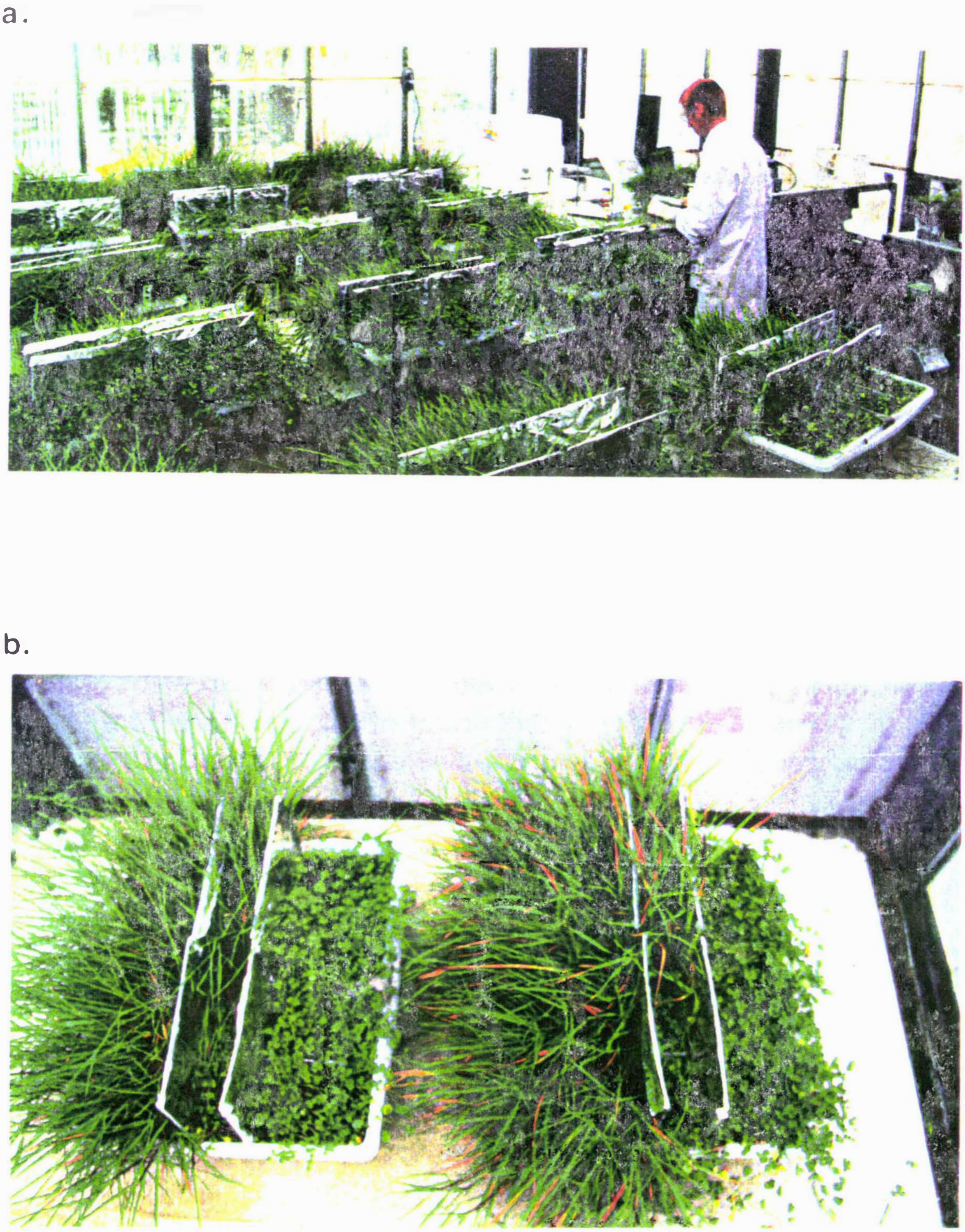


Plate 3.1 Photographs showing (a) glasshouse view, and (b) close up view of simulated sward dual labelling trial. Plants were grown at 0 and 100 mg P applied/kg soil in the left and right trays, respectively.

aluminium foil, 100 mm high, and ran across the whole width of the tray. One aerial partition was placed on each side of the central row of indicator plants, arranged between the three sections of the tray (Figure 3.1 and Plate 3.1). The partitions were held in place with the aid of thin bamboo skewers.

No shoot interactions occurred between the three sections of each tray so that only any root interactions between the central row of indicator plants and the outer simulated swards could be examined. There were still shoot interactions occurring between plants within each of the three sections in each tray.

3.2.6 Basal nutrients

A basal nutrient solution, similar to that recommended by Middleton and Toxopeus (1973) was applied to the subsoil. The basal solution contained required plant nutrients, minus N, P, and Ca. The major nutrients stock solution contained 7.35 g/l of K_2SO_4, 2.22 g/l of $MgSO_4.7H_2O$, 36 ml/l of 1N HCl. The minor nutrients stock solution contained 3.0 mg/l of H_3BO_3, 1.0 mg/l of $CuCl_2.2H_2O$, 20.0 mg/l of $MnCl_2.2H_2O$, 0.4 mg/l of $(NH_4)Mo_7O_{24}.4H_2O$, 1.5 mg/l of $ZnCl_2$ and 0.14 mg/l of Fe citrate. Nutrient solution was made up by diluting 200 ml of each stock solution to a volume of 4.5 l before application to the trays. The nutrient solution was applied at a rate of 300 ml per half-tray at 16, 22, 30, 61, and 95 days from planting.

3.2.7 Injection of carrier free radioactive phosphates

The method for P isotope injection into the soil was similar to that recommended by other researchers (Caldwell *et al.* 1985, 1987; Abbott and Fraley 1991). P isotopes were injected on the same day the defoliation treatments were imposed, 109 days after the ramets were planted. The original carrier free solutions of ^{32}P and ^{33}P were diluted so that the two solutions would be at a similar level of activity (Table 3.1). Carrier free ^{32}P or ^{33}P was injected into the soil, midway between the central row of indicator plants and the first row of plants in the outer simulated swards, based on the ramet planting points (Figure 3.1).

Holes were made prior to injection in order to avoid the problem of soil blockages in the micro-pipette tip. From each of two stock solutions of ^{32}P and ^{33}P, 0.1 ml was injected directly into the injection points in the soil to a depth of

40 mm with a micropipette. There were nine injection points on either side of the four central indicator plants in each sub-plot (Figure 3.1). The injection depth of 40 mm was chosen as both browntop and white clover are known to have most of their P uptake activities above a depth of 70 mm in a New Zealand hill country pasture (Jackman and Mouat 1974). In replicates 1 and 3, ^{32}P was injected into the side with the browntop simulated sward adjacent, and ^{33}P into the side with the white clover simulated sward adjacent. In replicates 2 and 4 the sides were reversed; ^{32}P was injected into the side with the white clover simulated sward adjacent, and ^{33}P into the side with the browntop simulated sward adjacent. This was done in case there was a preferential uptake of one of the P isotopes over the other. Following the injection of P isotope solution, the holes were carefully filled to prevent root channeling (Abbott and Fraley 1991).

The radioisotopes were essentially carrier free and the concentrations of the added radioactive phosphorus were too low to influence plant growth (Schenk and Barber 1979). Also, the amount of phosphate in the 0.1 ml aliquot was too low to be detected by the Murphy and Riley (1962) test for phosphorus. Because phosphate ions are rapidly adsorbed by Ramiha soil, the P isotopes would not have moved appreciably from the point of injection.

3.2.8 Harvesting

Plants were harvested either seven or fourteen days after imposing the defoliation treatments and the P isotope injections. The edge sub-plots were harvested after seven days and the middle sub-plots were harvested after fourteen days (Figure 3.1). The shoots of the central row of indicator plants of browntop and white clover, and the inner row of browntop plants in the outer simulated sward (i.e., directly adjacent to the aerial partitions), were harvested by cutting at ground level. Shoots of white clover in the outer simulated swards were not harvested due to the difficulties involved in determining which plant material belonged to the inner row of white clover plants directly adjacent to the aerial partition, because of the stoloniferous prostrate growth habit of white clover. Shoot samples were then dried in an oven at 65°C to constant weight. All shoot samples were ground using a hand-held Braun Aromatic KSM 2 coffee grinder.

Root sub-samples were collected by taking soil core samples with a specially designed sampling core of the dimensions 2700 mm^2 area (90 mm long and 30 mm wide), and 100 mm depth, i.e., a volume of 270 000 mm^3. The soil was washed from the roots, using the hydropneumatic elutriation system of root harvesting (Smucker *et al.* 1982). Root samples were taken from the central indicator row of plants and from the middle row of the outer simulated swards, parallel to the central indicator row of plants.

The roots from the outer simulated swards were sampled to allow an assessment of any differences in root density between the central indicator plants and the outer simulated sward plants that may have existed due to shading of central indicator plants by the aerial partitions. Fresh weights and root volumes (measured by displacement of water) were recorded. These roots were then preserved in 80% ethanol.

Root lengths were measured using a Comair root length scanner (Richards *et al.* 1979) and the root density, L_V, (in cm cm^{-3} soil) and specific root length, *SRL* (in m g^{-1} root weight) were calculated. The effective potential zone of soil exploitation around each root, calculated as the half mean distance between any two roots, was given by the mean radius r of a cylinder, where $r = 1/(\pi L_V)$ (Grinsted *et al.* 1982). The calculation of soil exploitation zone assumed completely random distribution of roots throughout the soil. As plant roots are said to compete for P when P depletion zones around roots are equal to, or greater than, the effective zone of soil exploitation around roots (Grinsted *et al.* 1982), the time taken for depletion zones between roots to overlap was calculated using the mean square distance, x, where $x = \sqrt{(2D.t)}$ (Bieleski 1976; Appendix 1), and D (diffusion coefficient in soil in $cm^2\ s^{-1}$).

$D = D_l.Ø.f_l.\Delta C_l/\Delta C.\ D$, where:

t = time in seconds,

D_l = diffusion coefficient in free solution (0.9×10^{-5} $cm^2\ s^{-1}$),

$Ø$ = volumetric water content of soil,

f_l = impedance factor,

ΔC_l = change in concentration of solution (mol ml^{-1} solution), calculated from adsorption/desorption isotherm (Appendix 1),

ΔC = amount of P removed from the soil (mol ml^{-1} soil) for concentration change ΔC_l, calculated from adsorption/desorption isotherm (Appendix 1).

Data were presented as time (in days) predicted for P depletion zones of roots to overlap in the outer simulated swards of browntop and white clover. The harvested roots of the central indicator row of plants were dried in an oven at 65°C to constant weight. Phosphorus (^{31}P) and nitrogen contents were measured on an auto-analyser, after Kjeldahl type digestion (Twine and Williams 1971). The digestion mixture used was 100 g of potassium sulphate and 1 g selenium powder in 1 l of concentrated sulphuric acid.

Table 3.1 Activities of ^{32}P and ^{33}P in the total 0.9 ml of diluted stock solutions injected into each sub-plot, at the time of P isotope injection.

Date of defoliation and P isotope injection	**Block number**	**Total activities of P isotopes injected per sub-plot(kBq)** ^{32}P	^{33}P
22/5/1990	1	343	149
23/5/1990	2	327	145
24/5/1990	3	312	141
25/5/1990	4	297	137

3.2.9 Counting radioactive phosphorus-32 and -33

The technique used was to ash the plant material at 450°C and dissolve the ash in hydrochloric acid. Samples were weighed into round-bottom lipped crucibles. Lids were placed on the crucibles, leaving a gap for air movement. The crucibles were placed in a cold muffle furnace and slowly heated to 300°C. The temperature was held at 300°C for four hours, then it was increased to 450°C and left at this temperature overnight (12 hours). No problem of incomplete ashing was encountered by this technique.

Approximately 2 ml of 2.5M HCl was added dropwise to the ash in each crucible, taking care to avoid excessive effervescence. The crucibles were then placed on a heating block and gently warmed for 30 minutes. The resulting solution was filtered through a Whatman No. 541 filter paper into a 10 ml volumetric flask. The volume was made up to 10 ml with the 2.5M HCl.

6 ml of the 2.5M HCl digest was pipetted into a 20 ml glass scintillation vial and 14 ml of scintillation cocktail was added. Patterson and Greene's (1965) scintillation cocktail 4 g of PPO (2,5 Diphenyl oxazole) and 0.1 g Di-methyl POPOP (2,3,3,6-0,0 1,4-Di-(2-(5-phenyl-oxazoyl))benzene) dissolved in 670 ml of toluene and 330 ml of Triton-X 100] was used. The lid was screwed on and the mixture was shaken vigorously by hand forming a white opaque gel.

It was necessary to make standard solutions equivalent to the 0.1 ml aliquots that were injected into the soil. To make these, 0.1 ml of carrier free ^{32}P and ^{33}P solution was pipetted separately into two glass scintillation vials. To this 5.9 ml of 2.5M HCl was added and gently shaken by hand to mix. Then 14 ml of scintillation cocktail was added to make the final volume 20 ml. These solutions were used to set the channel readings required to separate the counts for ^{32}P and ^{33}P in the shoot and root samples, as well as to determine the total counts of ^{32}P and ^{33}P that were injected into the soil. Quenching effects of the ash were measured by counting ^{32}P and ^{33}P standards containing varying known weights of non-radioactive plant material and 0.1 ml of either ^{32}P or ^{33}P. ^{32}P and ^{33}P were counted simultaneously on a three channel liquid scintillation counter. Details of channel settings and calculations are given in Appendix 4.

Shoots of central indicator plants and outer simulated sward browntop plants, shoots of central indicator white clover plants and roots of central indicator browntop and white clover plants were counted. As all samples were not counted on the same day, the unquenched data for both ^{32}P and ^{33}P were adjusted for decay to a single point in time, calculated using the half lives of ^{32}P and ^{33}P. Activities of ^{32}P and ^{33}P were normalised in relation to the original carrier free solutions that were injected into the soil, by dividing the activity of each sample by the total activity of each P isotope injected into each tray containing 4.5 kg of soil. The data were then expressed as normalised activity per gram of shoot or root, and as total normalised activity of shoots and roots for both ^{32}P and ^{33}P. It was not possible to calculate absolute units of P uptake. However, the relative differences in uptake of ^{32}P and ^{33}P between samples was assumed to be proportional to phosphate uptake, from each side, from the soil labile pool for the duration of the experiment from the time of P isotope injection to harvest (Newbould *et al.* 1971; Caldwell and Virginia 1989). The assumption that ^{32}P and ^{33}P were translocated similarly within the plant and that roots have the same affinity for absorbing the two tracers was made (Caldwell and Virginia 1989).

3.2.10 Frequency distributions of normalised activities of phosphorus-32 and -33

The frequency distributions for the sample populations of radioactivity measurements were positively skewed. The non-normality of the distribution of the activities was associated with a non-constant variance. In order to perform statistical tests that assume a constant variance, the data were transformed to stabilise the variance, s^2. Plots of ln s^2 against ln(sample value) showed points clustered along a line passing through the origin and of slope 2, suggesting that a log transformation was the most appropriate transformation (White *et al.* 1987). Log transformation of data for count rates (cpm) has also been used by other researchers studying uptake of radioactive phosphate by roots in an inter-cropping system (Ashokan *et al.* 1988). Because the sample variances were large and the number in each sample relatively small, Sichel's (1952) estimator (X_S) (equation 3.1), a maximum likelihood estimator, was used to give a more efficient measure of the true mean, than the arithmetic mean, and variance(X_S) (equation 3.2) was used to give a more efficient measure of the true variance of the population from which the sample was drawn.

$$X_s = \exp(x).\left[1 + V/2 + (n\text{-}1)V^2\big/2^2 2!(n+1)\right.$$
$$\left. + (n-1)^2 V^3\big/2^3 3!(n+1)(n+3) + \ldots\right] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.1$$

$$V_{Xs} = \exp(2x+V).\left[\exp(V/n)\left(1 + (n-1)V^2\big/2n^2\right.\right.$$
$$\left.\left. + (n-1)^3 V^4\big/2^2 2! n^4 (n+1) + \ldots\right) - 1\right] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

Where	X_s is Sichel's maximum likelihood estimator, V_{Xs} is the variance of X_s, x is the sample mean of logarithmically transformed data, i.e., mean of ln$X$, V is the sample variance of logarithmically transformed data, i.e., variance of ln X,
and	n is the number of observations

3.3 Results

The ^{32}P-^{33}P dual isotope labelling technique removed the need to determine pools of P isotope in the plant, assuming that the two isotopes behaved in the same manner within the plant. One problem encountered was the large variability in uptake of P isotopes, which has been experienced by other researchers (Caldwell *et al.* 1985; Abbott and Fraley 1991). A few samples with exceptionally high activities, possibly due to individual roots being directly contaminated with P isotope during the injection process, were ignored and treated as missing values in the statistical analyses.

3.3.1 Effect of species association on phosphorus acquisition

The data for the effect of species association on P isotope acquisition by the central indicator species were averaged over the defoliation treatments. The species association by defoliation treatment interaction was not significant. When grown on unamended soil, central indicator browntop plants acquired more radioactive phosphorus (either ^{32}P or ^{33}P) from soil space shared with white clover roots in the outer simulated sward than from space shared with roots of other browntop plants in the outer simulated sward, at both 7 and 14 days from P isotope injection (Figure 3.2). At the lowest level of phosphorus supply, i.e., on unamended soil, central indicator white clover plants acquired less radioactive phosphorus from the soil space shared with roots of browntop in the outer simulated sward than with roots of other white clover plants in the outer simulated sward, when harvested 7 days following P isotope injection (Figure 3.3a), however the difference was not significant after 14 days (Figure 3.3b).

Unexpectedly, at 100 mg P applied/kg soil, central indicator white clover had a greater uptake of phosphorus from soil space shared with browntop roots in the outer simulated sward than with roots of other white clover plants in the outer simulated sward, at both 7 and 14 days from P isotope injection (Figure 3.3).

The data for P isotope uptake into roots were not presented because there were no significant effects of species in the outer simulated sward on the P isotope concentration in roots.

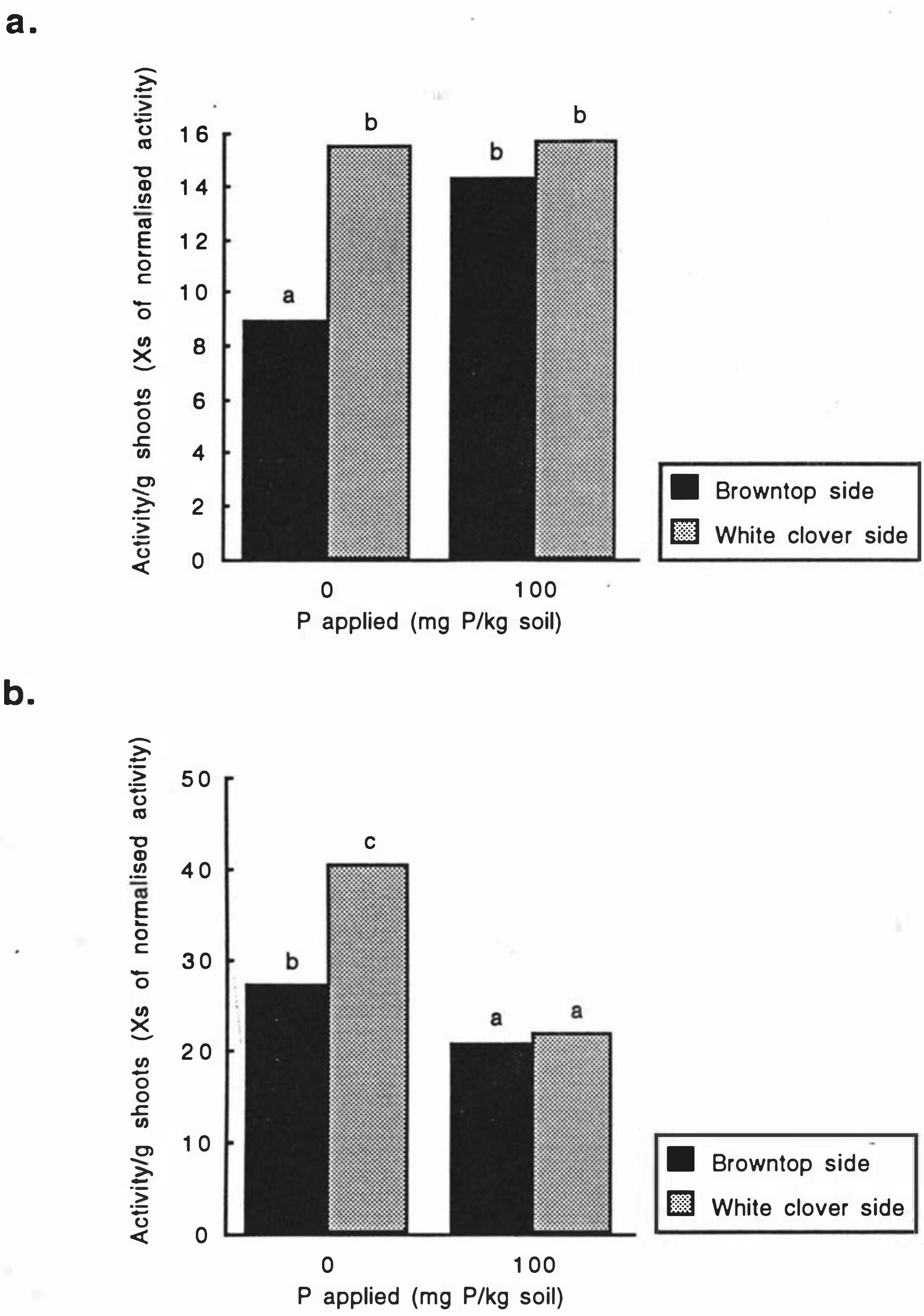


Figure 3.2 Normalised activities ($x10^{-5}$) per g of shoot dry weight of P isotope absorption from soil space shared with browntop and white clover by central indicator browntop plants, (a) 7 days (harvest one), and (b) 14 days (harvest two) following the imposition of defoliation treatment and P isotope injection. Means with different letters were significantly different for comparisons within each harvest, as indicated by the standard errors (calculated from V_{Xs}) of Sichel's estimator (X_s).

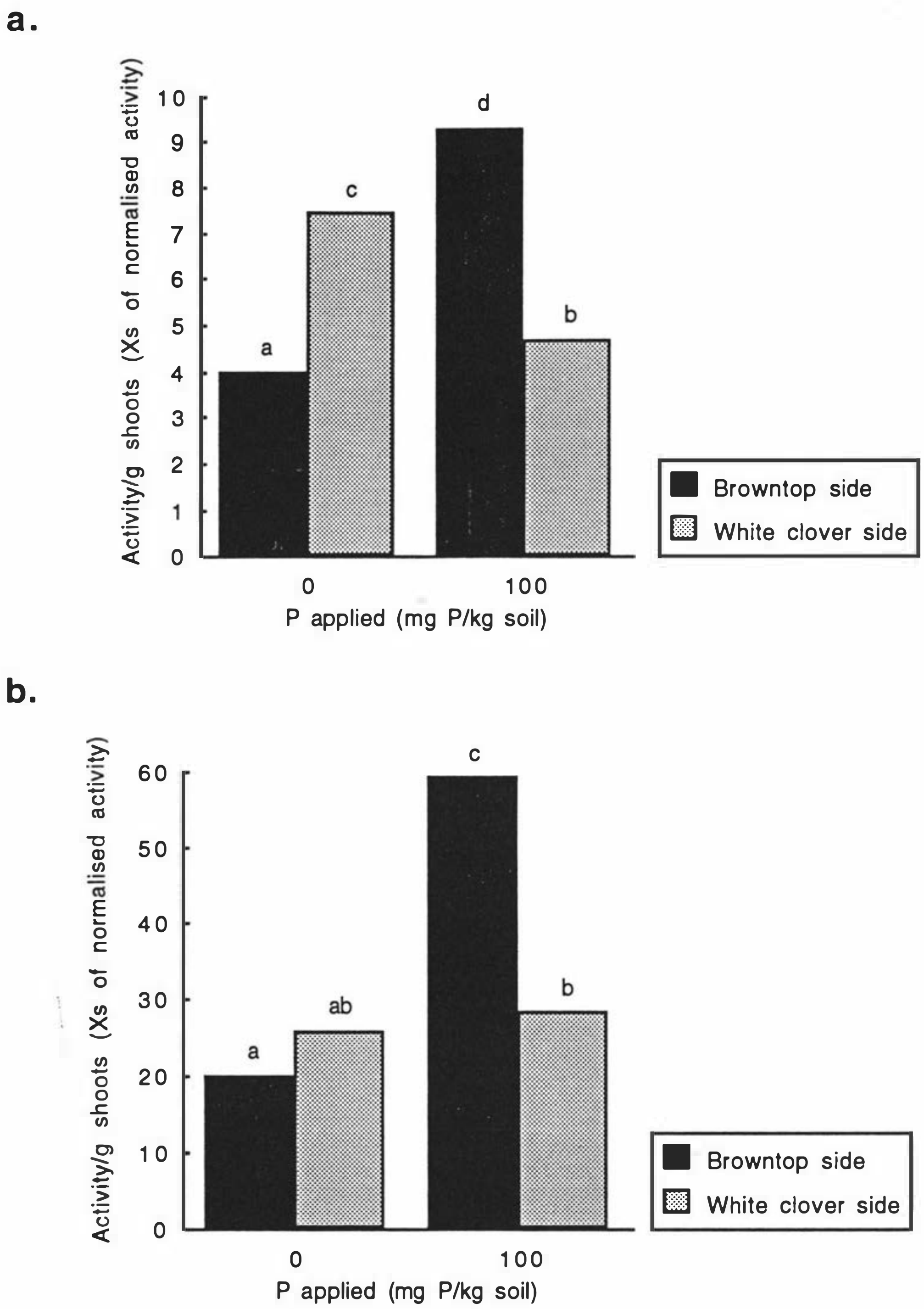


Figure 3.3 Normalised activities (x10^{-5}) per g of shoot dry weight of P isotope absorption from soil space shared with browntop and white clover by central indicator white clover plants, (a) 7 days (harvest one), and (b) 14 days (harvest two) following the imposition of defoliation treatment and P isotope injection. Means with different letters were significantly different for comparisons within each harvest, as indicated by the standard errors (calculated from V_{X_S}) of Sichel's estimator (X_S).

3.3.2 Effects of defoliation on acquisition of radioactive phosphorus-32 and -33

3.3.2.1 *Effects of defoliation of outer simulated sward species on P isotope acquisition by central indicator browntop and white clover*

There were no significant effects of defoliation of outer simulated sward plants on either the total P isotope activity in shoots of central indicator plants, or on the activity per g of shoots of central indicator plants, of either browntop or white clover. Acquisition of P isotope was considered for each side separately, and for the total uptake from both sides. The activities were highly variable and there was no clear pattern apparent.

3.3.2.2 *Outer simulated browntop swards*

The results for P isotope acquisition by the browntop plants in the outer simulated swards were the same irrespective of the central indicator species present. Therefore, the data for outer simulated browntop swards grown with browntop as central indicator species were pooled with those of outer simulated browntop swards grown with white clover as central indicator species. The results for the acquisition of P isotopes, from the browntop side only, by the first row of plants in the outer simulated browntop sward (directly adjacent to the aerial partitions) have been presented (Figures 3.4, and 3.5). Similar data for white clover was not available due to the difficulties described in Section 3.2.8.

When browntop plants in the outer simulated swards were defoliated, the P isotope concentration in shoots of these outer sward browntop plants (i.e., in the stubble and regrowth) was greater than when compared with undefoliated outer sward browntop plants, grown at both 0 and 100 mg P applied/kg soil at both harvests (Figure 3.4).

Also, at the first harvest with 100 mg P applied/kg soil, the activity per g in shoots of browntop plants in the outer simulated swards was greater when outer simulated white clover swards (i.e., on the opposite side of the tray) were defoliated than when there was no defoliation treatment, but not as great as when the outer simulated browntop sward, itself, was defoliated (Figure 3.4a).

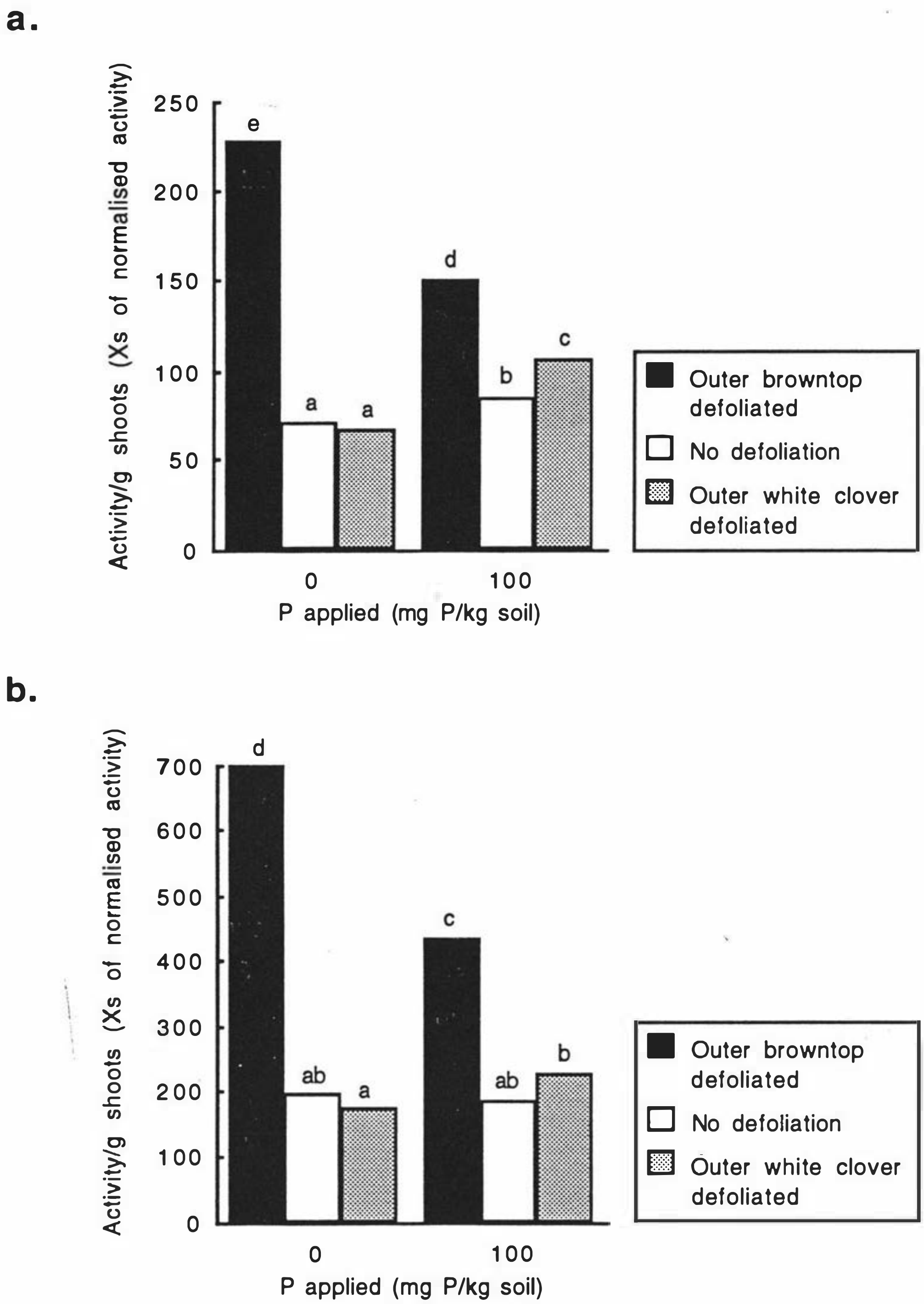


Figure 3.4 Effect of defoliation treatments on normalised activity (x 10^{-5})/g in shoots of browntop plants in the outer simulated sward, (a) 7 days, and (b) 14 days following imposition of the defoliation treatments and P isotope injections. Activity data given for P isotope injected into browntop side only. Means with different letters were significantly different for comparisons within each harvest, as indicated by the standard errors (calculated from V_{X_S}) of Sichel's estimator (X_S).

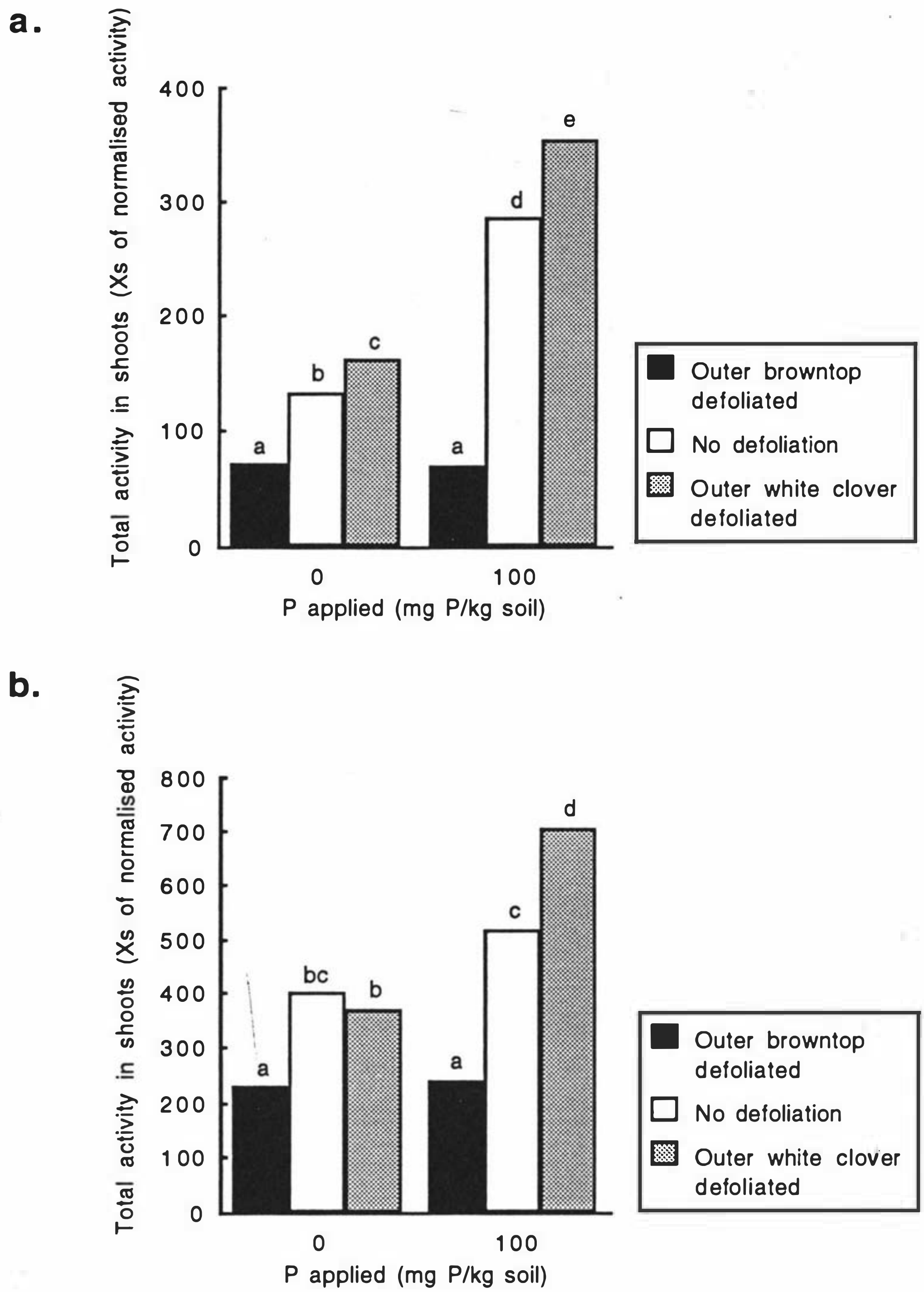


Figure 3.5 Effect of defoliation treatments on the total normalised activity ($x10^{-5}$) in shoots of browntop plants in the outer simulated swards, (a) 7 days, and (b) 14 days following imposition of the defoliation treatments and P isotope injections. Activity data given for P isotope injected into browntop side only. Means with different letters were significantly different for comparisons within each harvest, as indicated by the standard errors (calculated from V_{Xs}) of Sichel's estimator (X_s).

The total amount of P isotope found in the shoots of defoliated browntop plants in the outer simulated sward (i.e., in the stubble and regrowth) was lower than in shoots of undefoliated browntop plants in the outer swards at both harvests (Figure 3.5). Interestingly, the defoliation of white clover in the outer sward led to an increase in the total P isotope acquisition by browntop plants in outer swards (i.e., on the other side of the central indicator plants) compared with when there was no defoliation treatment, at both P levels at the first harvest (Figure 3.5a), and at 100 mg P applied/kg soil at the second harvest (Figure 3.5b).

3.3.3 Root properties

The root density, L_V, in outer simulated swards of browntop was approximately four times greater than for white clover. The overall average L_V for browntop was 37 cm/cm^3, while for white clover it was only 9.2 cm/cm^3. Root density increased with P application for both species (Figure 3.6). There was also a significant interaction ($p < 0.05$) between outer sward species and P application. The root density of white clover was more responsive to the P application of 100 mg P/kg soil to unamended soil than browntop (Figure 3.6).

In contrast to the root density, the specific root length (*SRL*) of browntop roots in the outer swards decreased by 18% with an application of 100 mg P/kg soil to unamended subsoil. There was no significant effect of P application on the *SRL* of white clover plants in the outer simulated swards (Figure 3.8b). The *SRL* of browntop was 2.4 times that of white clover when grown on unamended subsoil, and double at 100 mg P/kg soil.

3.3.3.1 *Effect of central indicator species on root properties of plant species in the outer simulated swards*

There was no effect of central indicator species on any of the root properties examined for browntop plants in the outer swards; root density, root length, and specific root length (Figures 3.6a, 3.7a, and 3.8a). Similarly, there was no effect of central indicator species present on root properties of white clover plants in the outer swards when grown on unamended subsoil (Figures 3.6b, 3.7b, and 3.8b).

However, when browntop was present as central indicator species, at 100 mg P/kg soil, there was a greater root density of white clover plants in the outer swards than when the central indicator species was white clover (Figure 3.6b). This increased root density was due to an increase in total root length (Figure 3.7b) and root weight because there was no effect of central indicator species on the specific root length of white clover plants in the outer swards (Figure 3.8b).

3.3.3.2 *P depletion zone around roots*

The estimated effective zone of potential soil exploitation around each root of browntop plants in the outer swards was less than around roots of white clover plants in the outer swards, at both levels of P supply (Figure 3.9).

The large distance between roots of white clover plants in the outer swards grown on unamended soil (2 x 0.23 mm = 0.46 mm), meant that the time predicted for P depletion zones around roots to overlap was extremely long (>4000 days, Figure 3.10).

When grown on unamended subsoil, the estimated time taken for depletion zones around roots to overlap was far less for browntop than for white clover, whereas there was no significant difference in time between the two species at 100 mg P applied/kg soil (Figure 3.10). Although the time for P depletion zones to overlap was much lower at 100 mg P applied/kg soil than at no P applied, it was still longer than the total time that the trial was conducted. Clearly, based on this criteria, the roots were not apparently competing for P. However, there is the possibility that roots may have competed for P because the root length measurements made may have failed to include the total root length of fine root hairs which may have been lost during the washing process, and, if present, may not have been detected and recorded by the root length scanner.

3.3.3.3 *Root volume of central indicator plants and plants in the outer simulated swards*

Root volumes were measured for root sub-samples from central indicator plants and from the outer swards. Root volumes were measured at the first harvest to determine whether there were any differences in root density

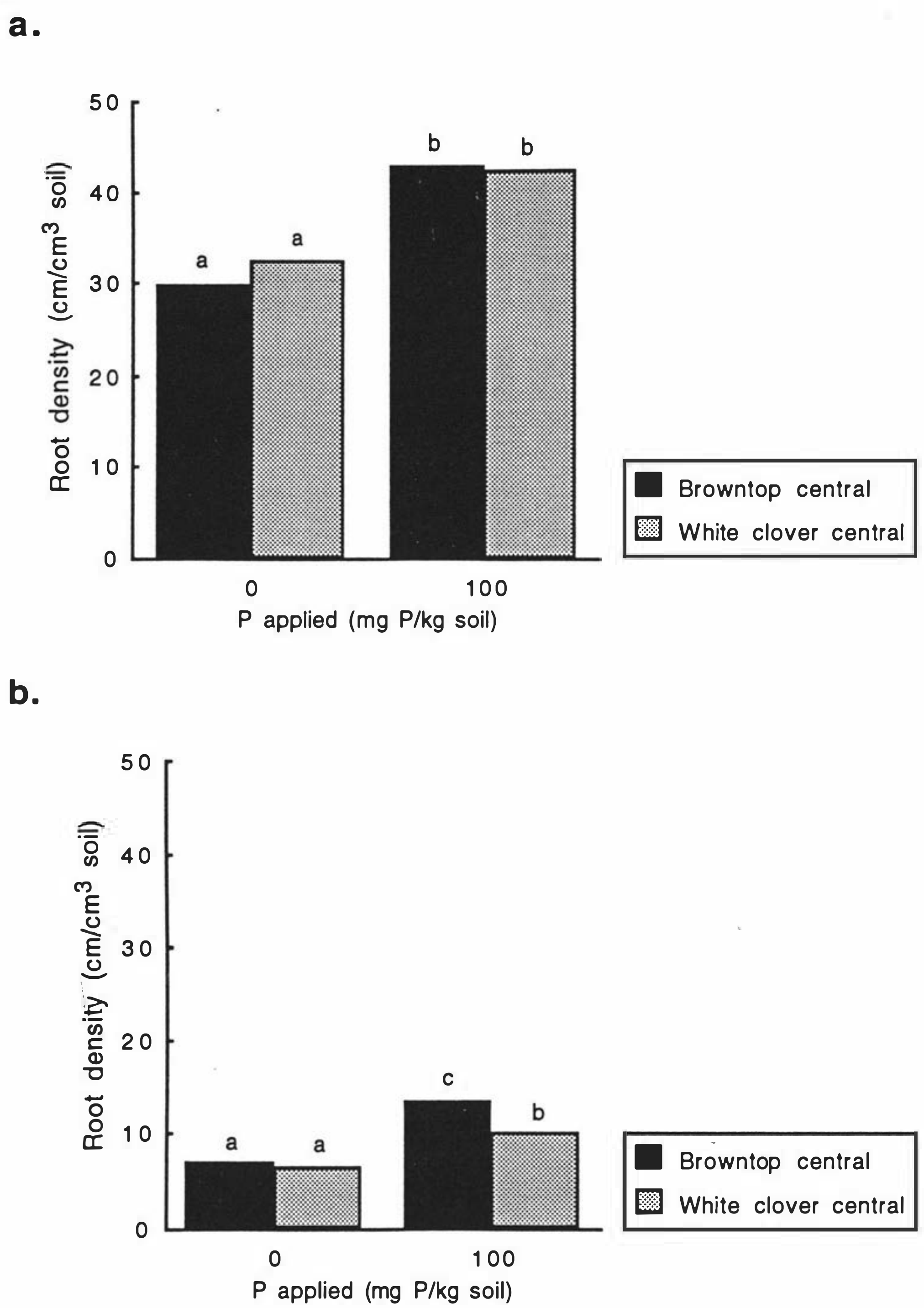


Figure 3.6 Effect of central indicator species and P application, as monocalcium phosphate, to Ramiha subsoil on the root density of (a) outer sward browntop plants (LSD = 8.2, $p < 0.05$), and (b) outer sward white clover plants (LSD = 2.5, $p < 0.05$) at the first harvest. Means with different letters were significantly different for comparisons within each outer sward species.

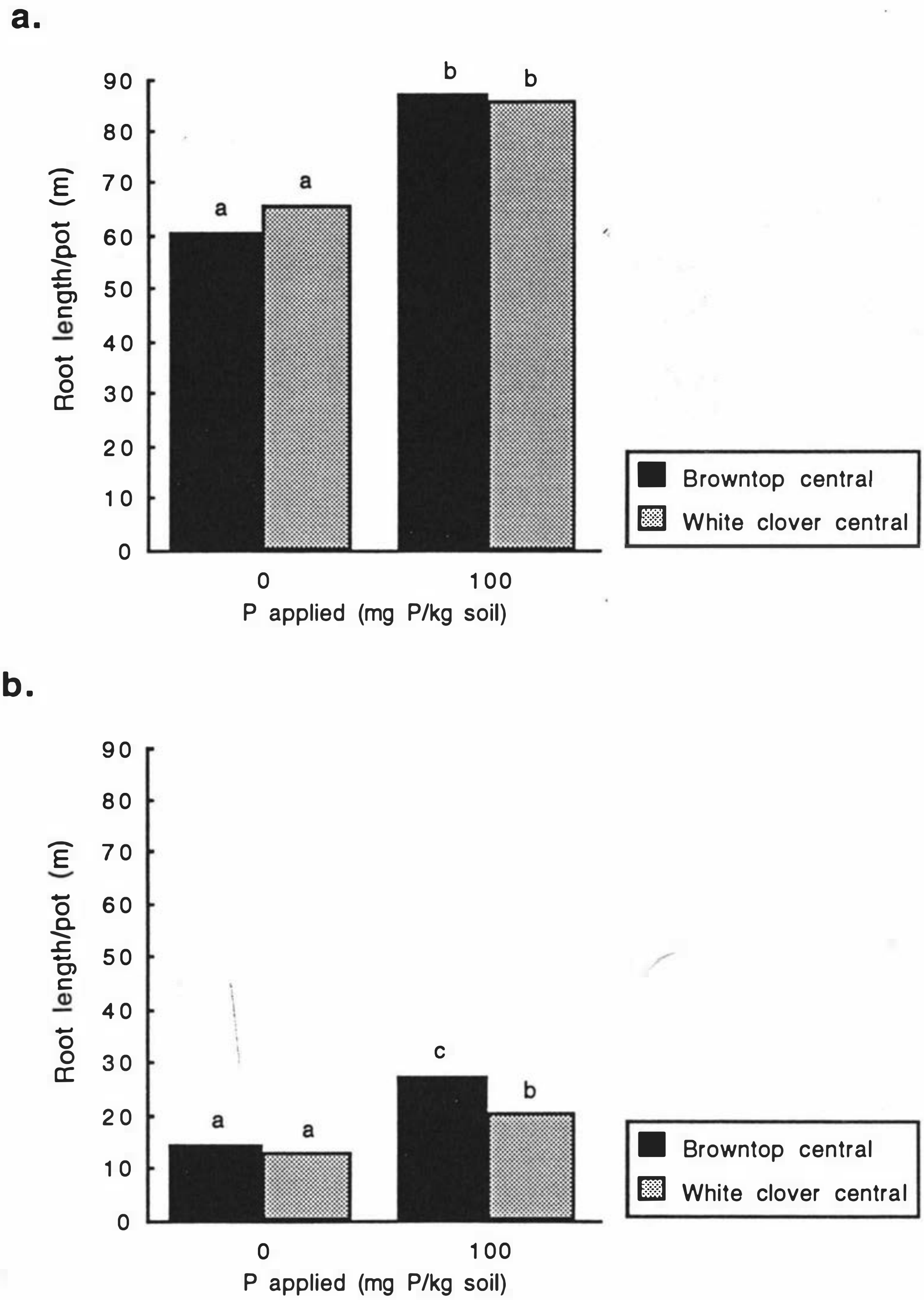


Figure 3.7 Effect of central indicator species and P application, as monocalcium phosphate, to Ramiha subsoil on the root length per pot of (a) outer sward browntop plants (LSD = 16.6, $p < 0.05$), and (b) outer sward white clover plants (LSD = 5.0, $p < 0.05$) at the first harvest. Means with different letters were significantly different for comparisons within each outer sward species.

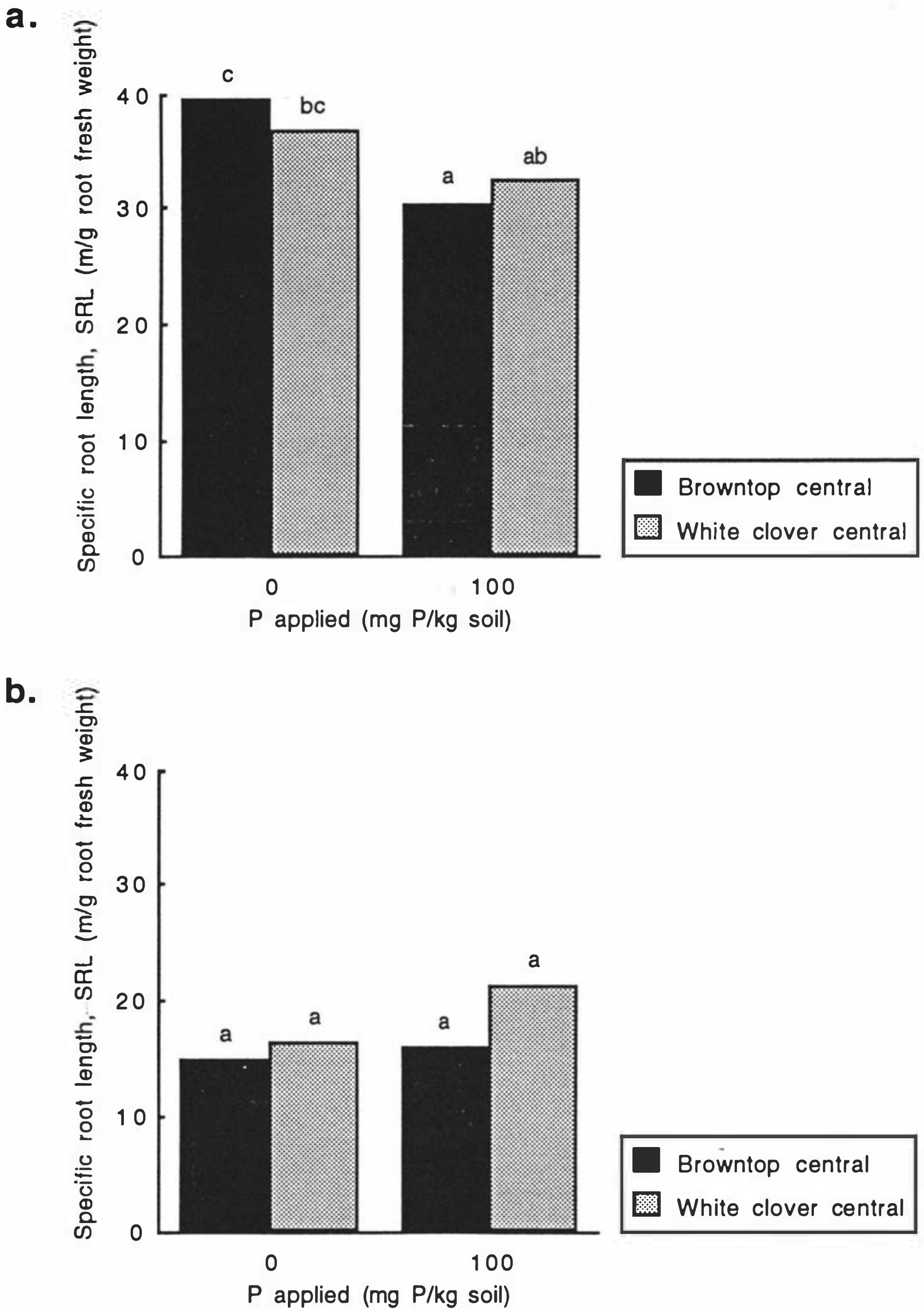


Figure 3.8 Effect of central indicator species and P application, as monocalcium phosphate, to Ramiha subsoil on the specific root length (m/g root fresh weight) of (a) outer sward browntop plants (LSD = 5.4, $p < 0.05$), and (b) outer sward white clover plants (LSD = 12.1, $p < 0.05$) at the first harvest. Root fresh weights were used to calculate specific root length because root dry weights were not available. Means with different letters were significantly different for comparisons within each outer sward species.

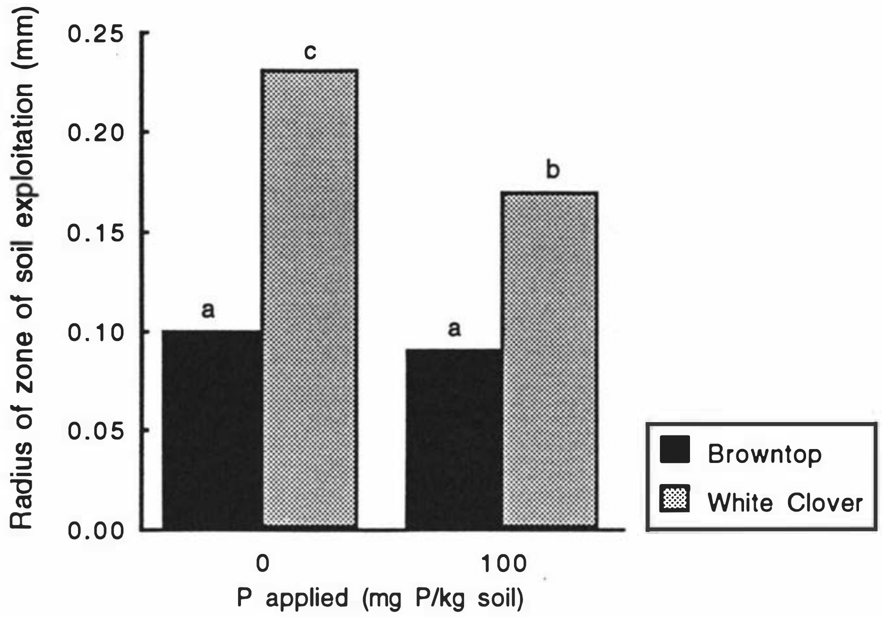


Figure 3.9 The effect of P application, as monocalcium phosphate, on the zone of soil exploitation around roots of browntop and white clover plants in the outer simulated swards at the first harvest (LSD = 0.016, means with different letters were significantly different, $p < 0.05$).

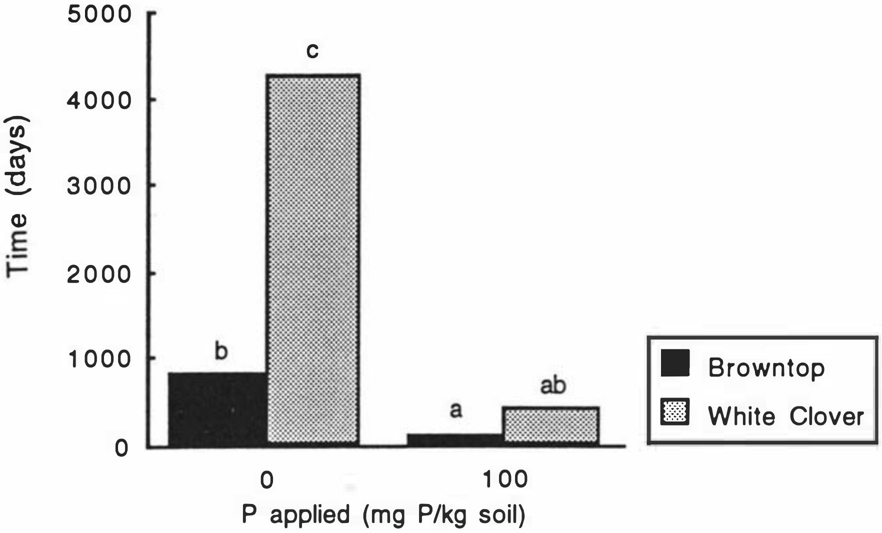


Figure 3.10 The effect of P application, as monocalcium phosphate, on the predicted time taken for P depletion zones around roots of browntop and white clover in the outer simulated swards to overlap, calculated from root densities and soil P buffer capacity, (LSD = 503, means with different letters were significantly different, $p < 0.05$).

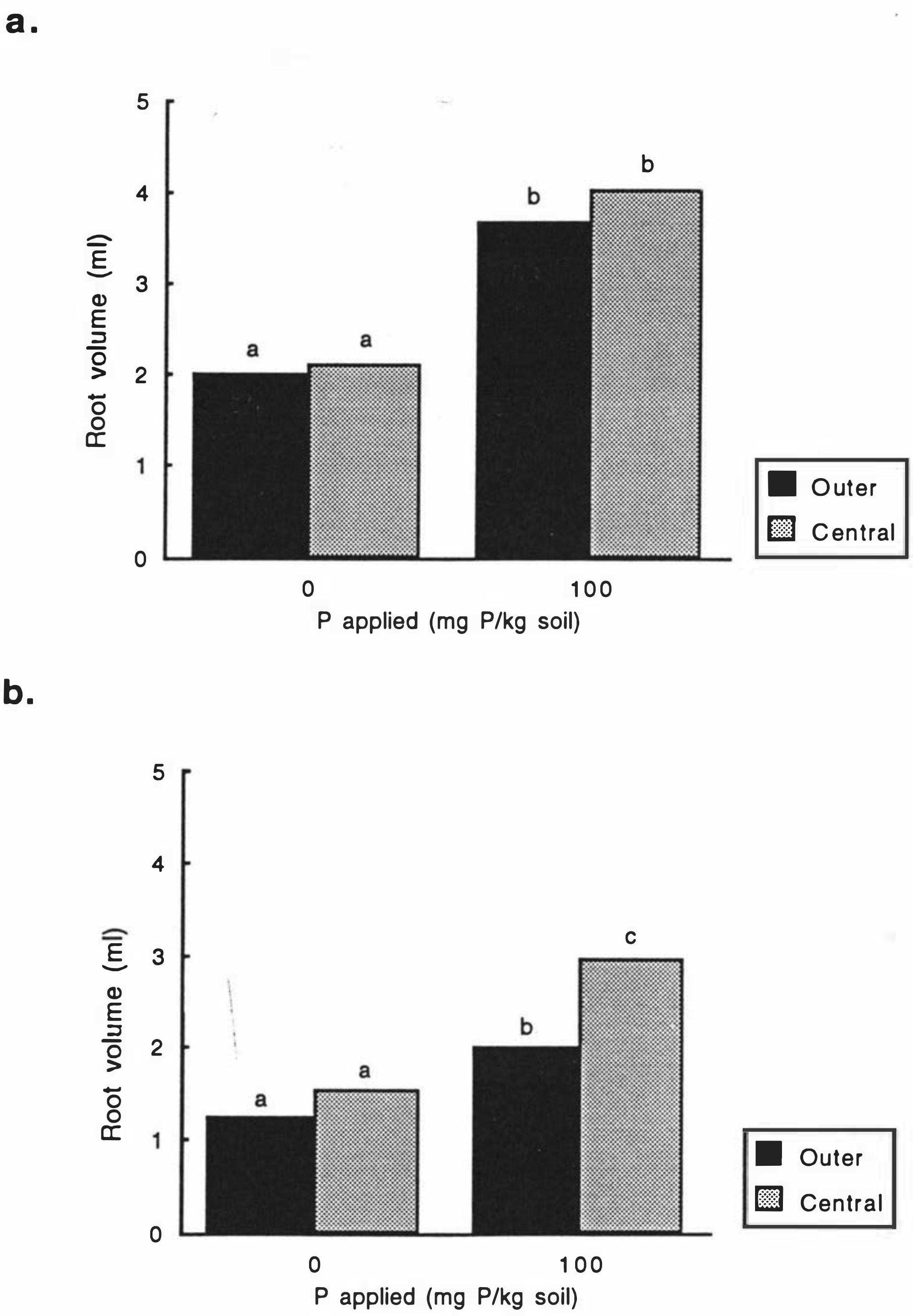


Figure 3.11 Effect of P application on the root volume of (a) browntop (LSD = 0.6), and (b) white clover (LSD = 0.4) plants in the central indicator and outer simulated sward positions for the first harvest, (means with different letters were significantly different for comparisons within each species, $p < 0.05$).

between different sections of the tray. At 100 mg P applied/kg soil, the volume of roots of white clover was greater in the soil below the central row of indicator plants than in the middle row of the outer simulated sward (Figure 3.11b). However, when grown on unamended subsoil there was no significant effect of plant position in the simulated sward on the root volume of white clover (Figure 3.11a). There were no effects of plant position on the root volume of browntop at either level of P application (Figure 3.11a).

3.3.4 Shoot and root weights of central indicator plants

The shoot dry weights of central indicator browntop plants were greater than for central indicator white clover plants (Figure 3.12). The root dry weights of central indicator browntop were also greater than for white clover (Figure 3.13).

Both shoot and root dry weights increased with P application (Figures 3.12 and 3.13) with white clover shoots being more responsive to P than browntop shoots. White clover roots were as responsive as browntop roots.

The root/shoot ratio of central indicator white clover plants was greater than that of central indicator browntop plants when grown on unamended subsoil (Figure 3.14). The root/shoot ratios were considered atypical, and may have resulted due to the root harvesting method used, as explained in Appendix 5. There was no significant difference in root/shoot ratio between the two species at 100 mg P applied/kg soil. For white clover the ratio decreased with increasing P supply. However, for browntop there was no significant effect of P application on the root/shoot ratio of browntop (Figure 3.14).

3.3.5 P and N content of shoots

The P concentrations in browntop shoots were 0.10 and 0.17% at 0 and 100 mg P applied/kg soil, respectively, while in white clover shoots the concentrations of P were 0.09 and 0.12% at 0 and 100 mg P applied/kg soil, respectively (Figure 3.15a) at the first harvest. Although these P concentrations were low, they were consistent with the P concentrations measured in the P response trial (Section 2.3.3.1). On unamended soil there was no difference in the P concentration in the shoots of the two species, however, at 100 mg P applied/kg soil the P concentration in browntop shoots was greater than in white clover shoots (Figure 3.15a). There was no effect of defoliation of outer sward plants on the P concentrations in shoots for either central species.

The total uptake of phosphorus into shoots by browntop was always greater than white clover (Figure 3.15b), largely because of browntop's greater shoot weight (Figure 3.12a), and also because of the greater P concentration in shoots of browntop at 100 mg P applied/kg soil (Figure 3.15b). As expected, the total P uptake into shoots of both browntop and white clover increased with P application (Figure 3.15b) due to corresponding increases in shoot weight (Figure 3.12a) and P concentration (Figure 3.15a).

The N concentration in white clover shoots was substantially higher than in browntop shoots (Figure 3.16). Browntop plants grown at both levels of P application and white clover plants grown on unamended subsoil would have been suffering from N deficiency. The N concentration in white clover shoots increased from 1.7% to 2.3% with an application of 100 mg P/kg soil (Figure 3.16). The average overall concentration in shoots of central indicator browntop plants was only 0.9%.

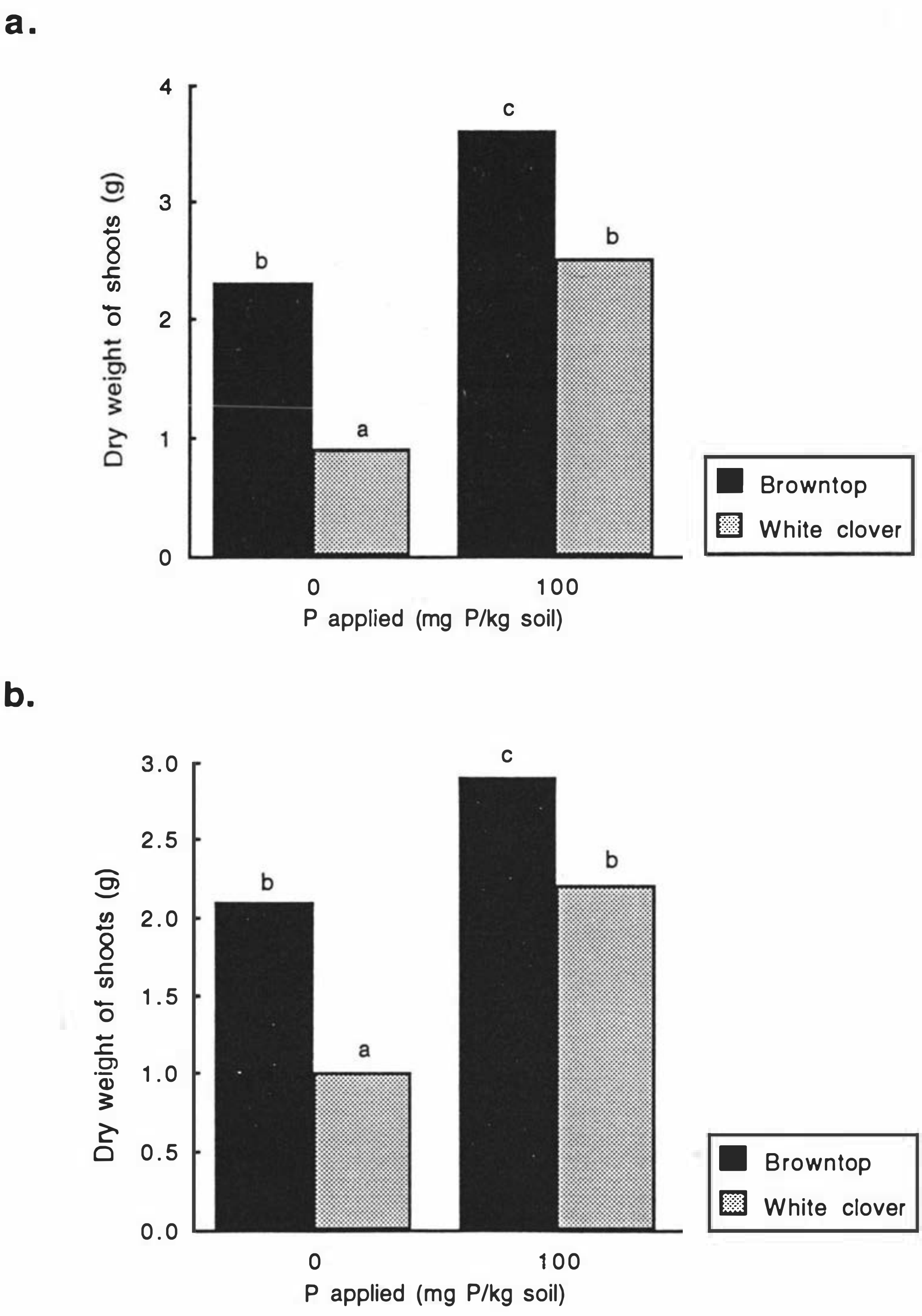


Figure 3.12 Effect of P application on shoot dry weights of central indicator plants of browntop and white clover for (a) the first harvest (LSD = 0.6), and (b) the second harvest, (LSD = 0.6). Means with different letters were significantly different for comparisons within each harvest, $p < 0.05$).

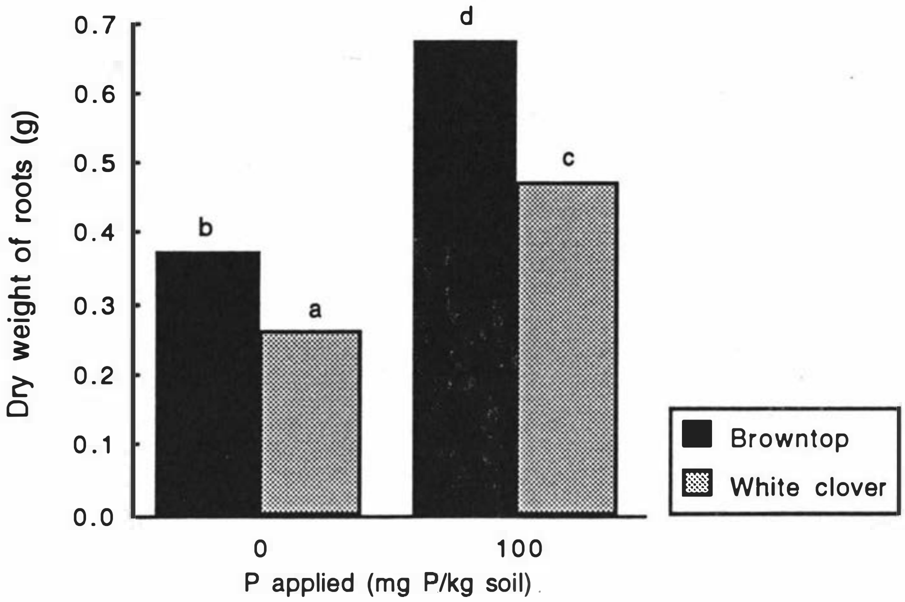


Figure 3.13 Effect of P application on root dry weights of central indicator plants of browntop and white clover for the first harvest, (LSD = 0.07, means with different letters were significantly different, $p < 0.05$).

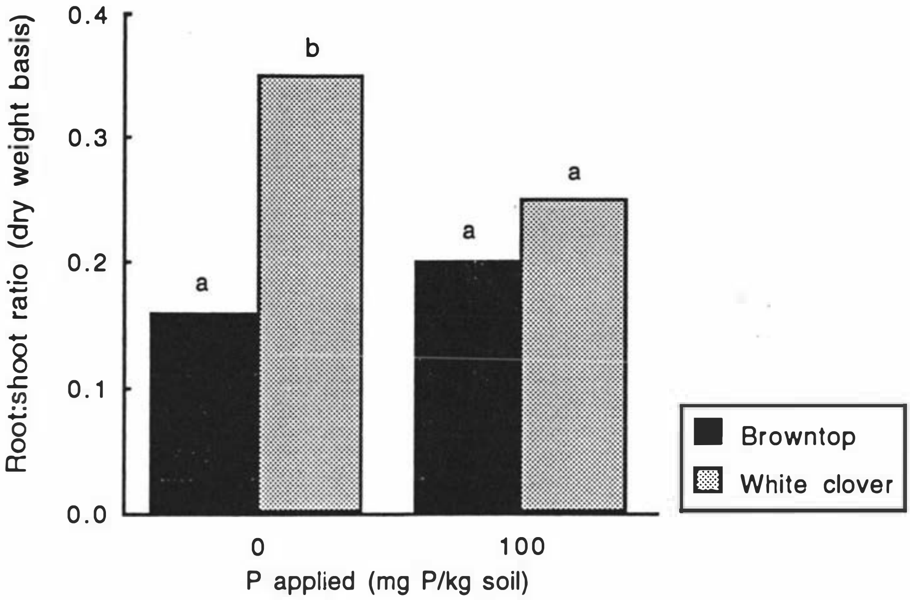


Figure 3.14 Effect of P application on the root:shoot ratio of central indicator plants of browntop and white clover at the first harvest, (LSD = 0.10, means with different letters were significantly different, $p < 0.05$).

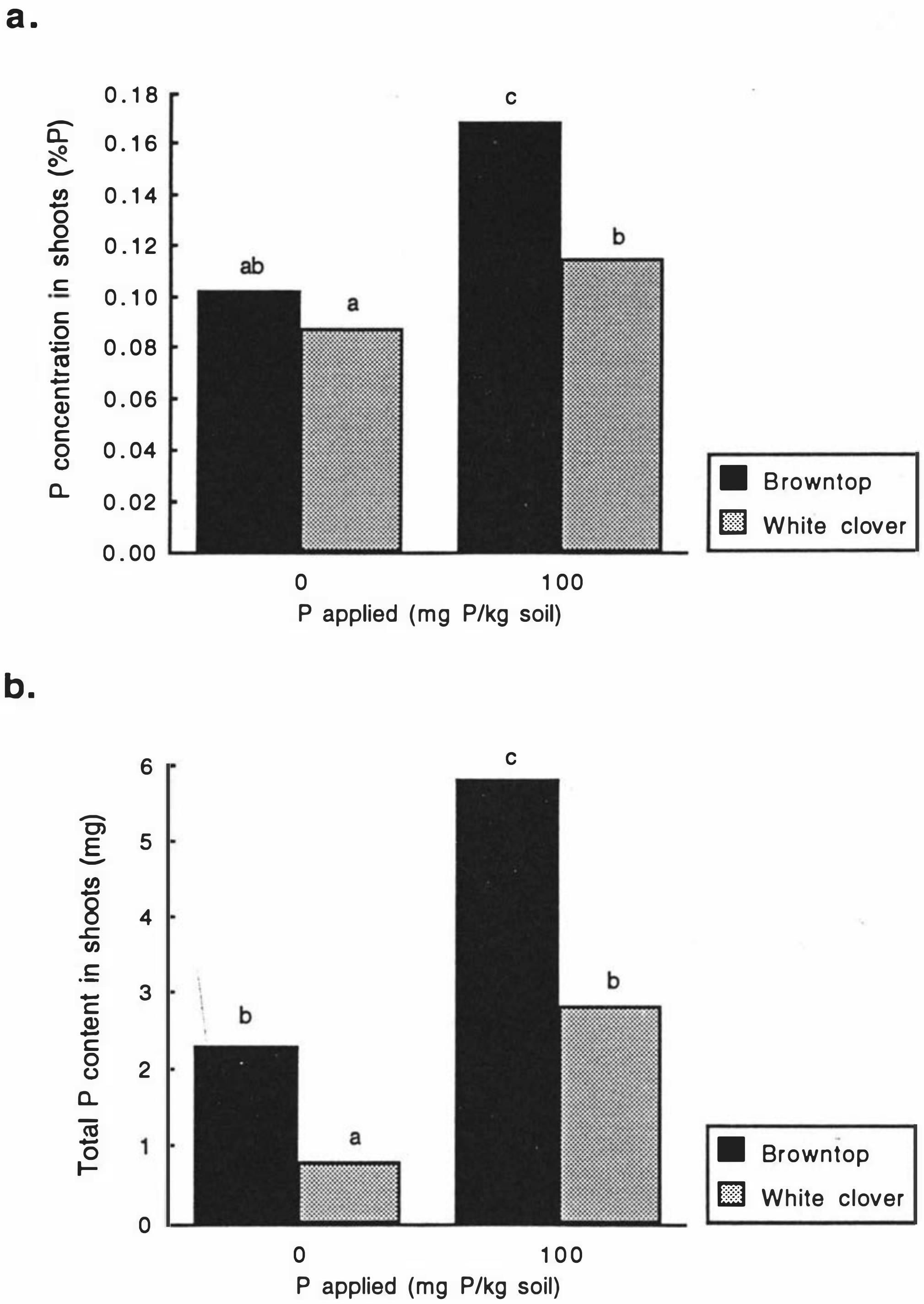


Figure 3.15 Effect of P application on (a) the P concentration (LSD = 0.02) and (b) P content in shoots (LSD = 1.1) of central indicator plants of browntop and white clover at the first harvest (means with different letters were significantly different, $p < 0.05$).

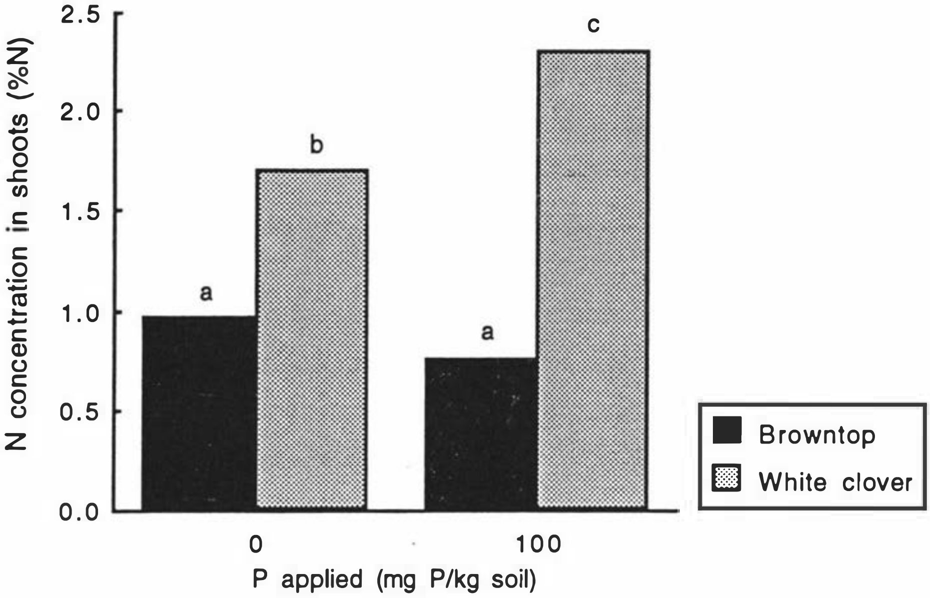


Figure 3.16 Effect of P application on the N concentration in shoots of central indicator plants of browntop and white clover at the first harvest, (LSD = 0.24, means with different letters were significantly different, $p < 0.05$).

3.4 Discussion

3.4.1 Evidence of root competition for soil P

Browntop roots had a deleterious effect on P acquisition by white clover roots when grown on unamended subsoil (Figure 3.3a). Browntop roots appeared to compete with white clover roots on unamended subsoil at the first harvest because central indicator browntop plants acquired more P isotope from the white clover side than the browntop side (Figure 3.2a), and central indicator white clover plants acquired less P isotope from the browntop side than from the white clover side (Figure 3.3a). At the second harvest the deleterious effect of browntop plants in the outer swards on P isotope acquisition by central indicator white clover plants grown on unamended subsoil was not significant (Figure 3.3b), although browntop did obtain more P isotope from the white clover side (Figure 3.2b). This suggested that browntop was more competitive for P than white clover.

However, the root densities of plants grown on unamended subsoil were low, and the predicted time taken for P depletion zones around roots (even in the browntop monocultures where root densities were highest) to overlap was much longer than the duration of the trial, (i.e., >> 116 days) (Figure 3.10). Therefore, if the assumption that roots were randomly distributed at the depth of the P isotope injections is made, suggesting that it was unlikely that roots of browntop and white clover would have competed directly with each other for P. The relevance of predicting the onset of root competition for P between individual roots by determining when depletion zones overlap may need to be investigated further using plants grown at realistic densities. Grinsted *et al.* (1982) used the theory of overlap of P depletion zones of roots to predict the onset of competition for P between roots of plants grown at unrealistically high root densities.

However, because both browntop and white clover acquired less P isotope when grown on unamended subsoil (Figures 3.2 and 3.3) with roots associated with browntop than with white clover roots, the results do suggest that browntop may compete with white clover by reducing the availability of P isotope in the soil to a greater extent than white clover. Supportive of the explanation that browntop reduced the soil P availability to a greater extent than white clover was that the application of P removed the deleterious effect of browntop on white clover. The greater P content of browntop shoots than white clover shoots (Figure 3.15) also suggested that this was a possibility. The significance of browntop reducing the P availability in soil to a greater extent than white clover would be of even more importance in the field where the rate of cycling of P back to the soil (see Section 2.4) would be slower with a greater proportion of browntop than white clover making up the sward botanical composition in hill country pastures in New Zealand (Harris 1974), because *Agrostis* species are known to be associated with slowly decomposing litter in grasslands (Williams and Gray 1974).

The finding that defoliation of outer browntop swards did not alleviate the deleterious effects of outer sward browntop plants on P isotope acquisition by central indicator white clover or browntop plants, is supportive of the conclusion that browntop roots were not competing directly with white clover or browntop roots, especially when the competitive ability of browntop roots in the outer swards was reduced by defoliation (Figure 3.5). However, browntop had the ability to maintain a reasonable rate of P uptake following the severe defoliation, and had a greater concentration of P isotope in shoots of defoliated browntop plants (i.e., in the stubble) than in the shoots of the undefoliated plants (Figure 3.4).

The greater uptake of P by browntop from the white clover side than from the browntop side may have been partly related to N supply, with the N supply in the soil being greater on the N-fixing white clover side than on the browntop side. Obviously, browntop plants were suffering from N deficiency (Figure 3.16) which would influence P uptake. However, this explanation is highly speculative.

It is feasible that browntop could reduce the P concentration in the unamended subsoil to a lower level than white clover because browntop is able to absorb P from much lower soil solution concentrations than white clover (Mouat 1983b). Therefore, P isotopes would have been adsorbed more strongly onto subsoil with browntop growing on it than with white clover growing on it. It is not clear whether browntop could permanently reduce the P concentration in the soil within 116 days or if browntop acquires more P by regulating the remaining P supply in the soil solution to a low P concentration as it becomes available, although the latter explanation seems more feasible. Had this been the case, the P isotope availability would have been greater on the white clover side than the browntop side. Unfortunately, soil samples were not taken from under the growing swards to assess P availability.

3.4.2 Evidence of other non-competitive root interference

The application of 100 mg P/kg soil caused a marked change in the nature of interference occurring between roots of browntop and white clover. Browntop acquired similar amounts of P isotope from each side (Figure 3.2), so that with the P application of 100 mg P/kg soil browntop P acquisition no longer benefitted from growing with white clover.

The major finding of this trial was that, at 100 mg P applied/kg soil, white clover acquired more P when with browntop roots than when associated with white clover roots (Figure 3.3). The greater acquisition of P isotope by central indicator white clover plants from the browntop side than from the white clover side can be explained quite simply. Increasing local root density is effective in increasing the uptake of immobile ions, such as phosphate (Fitter 1976; Bolan 1991), due to a decrease in the distance of diffusion of phosphate ions and an increase in the surface area of roots for absorption (Nye and Tinker 1977). However, the reason for the greater root growth of white clover when grown closer to browntop plants is not so easily explained. Either, white clover was a greater competitor against white clover than against browntop, or some non-competitive interference was involved. The application of 100 mg P/kg soil removed the competitive effect of browntop on white clover. Considering that browntop has long been recognised to be a highly aggressive competitor for soil P, especially with white clover, it was not expected that the competitive effect of browntop would be alleviated at such low levels of P supply that produced plants with P concentrations in their shoots of 0.12% and 0.18% for white clover and browntop, respectively.

Surprisingly, the root yield of white clover plants was greater the closer they were to roots of browntop when grown at 100 mg P/kg soil, but not on unamended subsoil. This effect was evident even when the white clover plants were not growing directly adjacent to browntop, suggesting that it might not have been in response to browntop reducing the P availability in the soil. When browntop was present as the central indicator species, white clover roots collected from the middle row of the outer sward were more dense than when white clover was the central indicator species (Figure 3.6). The only difference between these sub-plots was a small extra width of one species or the other. That is, a few extra plants in the central strip apparently had an effect on plants in the middle row of the outer sward.

The greater root density of white clover was due to an increase in total root length (Figure 3.7) and root weight. Similarly, the volume of roots of white clover was greater in the central indicator position than in the outer sward position (Figure 3.11). However, there was no effect of central indicator species on the root morphology (assessed by specific root length) of white clover plants in the outer sward (Figure 3.8).

Intraspecific competition of white clover may be more severe than interspecific competition with browntop at 100 mg P applied/kg subsoil. It is generally accepted that intraspecific competition is more severe than interspecific competition, because the more similar the needs of two plants, the greater the contest (Haynes 1980). However, this seems unlikely if the P concentration in the soil was lower under browntop than under white clover. Also, there is diminutive evidence to support the assumption that adding a resource will reduce competitive effects (Wilson 1988). Applying P may have altered the competitive balance.

Either white clover may have had a deleterious effect upon its own root growth (autotoxicity), or browntop had a beneficial effect on the root growth of white clover (Figure 3.17). Because the effect occurred a relatively large distance from the browntop outer sward (> or = 3 cm), autotoxicity of white clover would seem to be the most feasible explanation, especially considering that there were not substantial quantities of browntop roots found in the soil cores taken from the middle row of the outer white clover swards. It has been suggested that a water soluble factor in raw humus of some plants may inhibit root growth through interfering with respiration of root tissue (Robinson 1971).

Some species are able to proliferate roots in nutrient rich patches (Lambers and Poorter 1992). As mentioned above, however, white clover had less roots in the soil below outer white clover sward than near browntop. This soil was most likely more rich in nutrients than in the outer browntop sward, providing more support to the argument that autotoxicity of white clover may have been occurring.

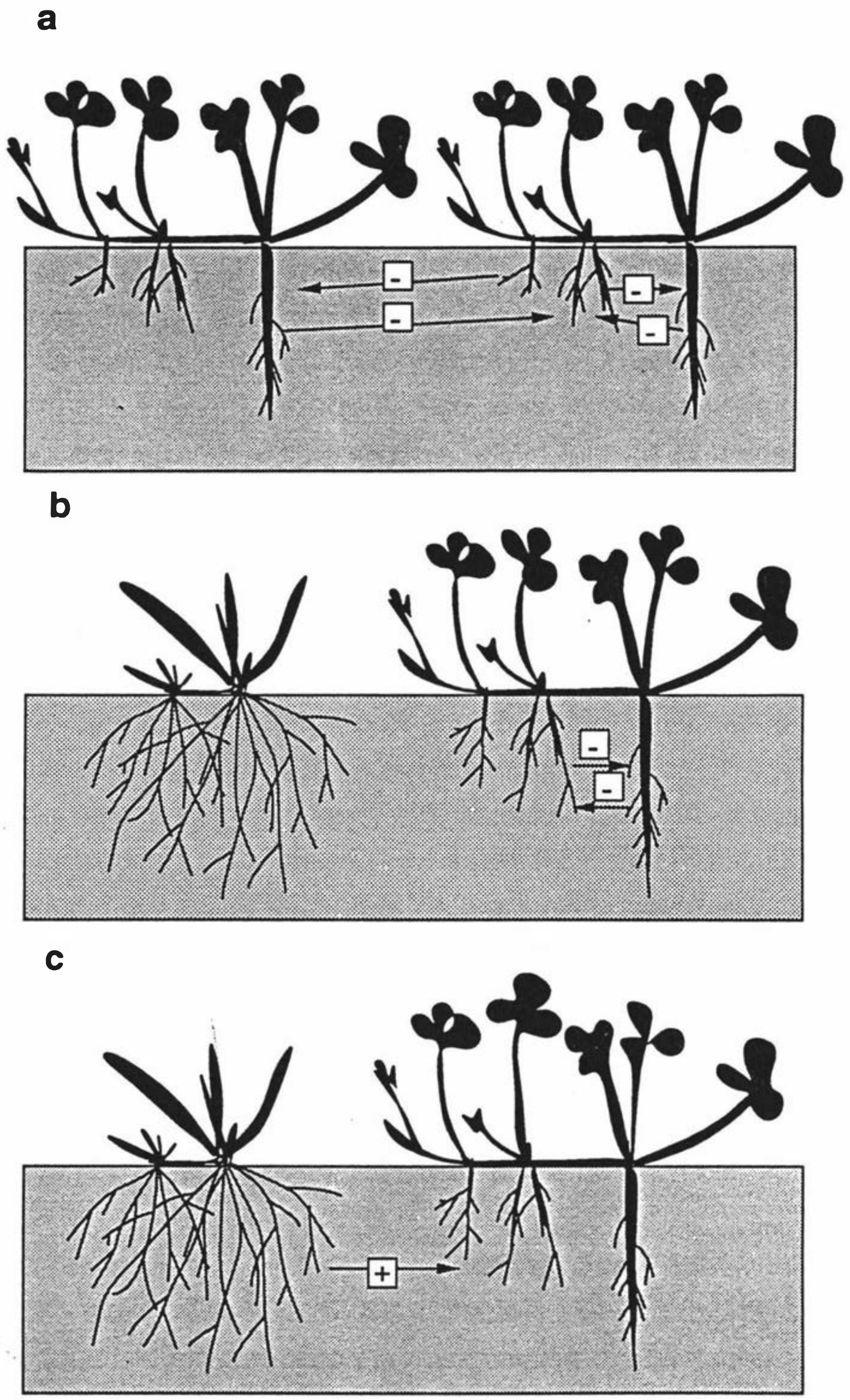


Figure 3.17 Possible inter- and intraspecific allelopathic root interactions occurring within a browntop-white clover sward; (a) white clover autotoxic with inter- and intra-plant toxicity, (b) white clover autotoxic with intra-plant toxicity, and (c) browntop produces chemical that is stimulative to white clover, i.e., inter-species stimulation.

The effects of closeness of white clover plants to browntop on white clover root growth are analogous to the pattern of growth of autotoxic plants in monocultures. When autotoxic plants are grown in monocultures, edge plants grow larger than central ones (Fitter and Hay 1987, pp. 329). There is evidence from the glasshouse (Newman and Rovira 1975; Macfarlane *et al.* 1982b) and the field (Lieth 1960; Kendall and Stringer 1985) to suggest that white clover is autotoxic. White clover plants have been found to dominate local patches, however these patches have been found to migrate from one year to the next (Lieth 1960). Also, white clover is known to be positively associated with ryegrass (Harris 1974; Simpson *et al.* 1987), another species that appears to be autotoxic (Newman and Rovira 1975).

Phenolics were suggested by Macfarlane *et al.* (1982a) to be the chemical involved in the toxic property of white clover. Phenolics and other short chain fatty acids (water soluble) may accumulate in soil and are known to be detrimental to root respiration, growth and root hair formation (Marschner 1986). These toxins are products of decomposition of organic substances, and their phytotoxicity increases with decreasing pH (Marschner 1986). The pH of rhizosphere soil of white clover was likely to be more acidic than the rhizosphere soil of browntop, because soil pH decreases when legumes are N_2 fixing due to H^+ extrusion to offset a cation/anion uptake imbalance (Marschner 1986). Therefore, it is possible that phytotoxins would have been more inhibiting in the soil adjacent to white clover than with browntop growing on it. Another possibility is that white clover may be more sensitive to phytotoxins than browntop. Further research is required to investigate these possible explanations.

Interestingly, there is also some evidence that browntop may be stimulative to white clover. Scott (1975) found that extracts of browntop shoots and roots had a stimulative effect on the germination of white clover plants. However, in the field white clover has not been found to be positively associated with browntop (Harris 1974; Simpson *et.al.* 1987), although this may be due to other factors such as shading of white clover by browntop or soil moisture stress.

Autotoxicity of white clover is largely speculative. These results do not show that phytotoxicity was involved, as no toxin has been isolated or identified. Yet there does not appear to be any other feasible explanation.

Competition for P did not appear to be an important plant interference at 100 mg P applied/kg soil. The time taken for P depletion zones to overlap (Figure 3.10) suggested that direct competition for P could not have occurred, however if fine root hairs were able to be measured by the root length scanner, the root length measurements would have been longer and the P depletion zones around roots could possibly have been overlapping before the conclusion of the trial. Also the assumption that roots were evenly spaced is not very likely. In any case, had competition for P been occurring between roots of browntop and white clover, the greater root length of white clover in association with browntop compared with in association with white clover had an overriding influence, and this also removed the benefit that browntop had for P acquisition from growing with white clover which was evident when grown on unamended subsoil (Figure 3.2).

3.4.3 Ecologically significant plant traits

The specific root length of browntop was greater than white clover, which was expected. Slow growing species tend to have higher specific root lengths (Lambers and Poorter 1992), contributing to a more efficient use of biomass allocation for soil exploration. The high specific root length of browntop would be important in terms of P acquisition because of the low mobility of P in the soil. Although the yield of browntop shoots in this trial was greater than that of white clover shoots, browntop is considered to be a slow growing species relative to white clover (Grime *et al.* 1988).

The specific root length of browntop decreased with P application, thus the specific root length of browntop was more plastic than for white clover. This was unexpected because generally, white clover would be expected to be more plastic than browntop. Fast growing plant species tend to display a greater plasticity in response to environmental factors (Lambers and Poorter 1992). However, specific root lengths tend to be unpredictable (Lambers and Poorter 1992).

3.4.4 Summarising comments

The dual labelling trial was useful to address all of the questions to be answered in this chapter. It was also useful in elucidating some possible plant interactions that were not detected by the simple P response trial, therefore highlighting the problems of attempting to predict the nature of interference between plants in mixtures from their growth in monoculture, as discussed in Chapter Two. The nature of interferences occurring between browntop and white clover is far more complex than has been shown by previous work which has concentrated on field trials and competitive settings trials that have not attempted to separate the effects of root interactions from shoot interactions.

The results of this dual labelling trial suggest that the deleterious effects of browntop on white clover observed in the field are possibly not the result of direct exploitative competition between roots of browntop and white clover, providing that soil P supply is not of an extremely severe deficient level. However, root densities measured for browntop-dominated hill country pasture in New Zealand have been found to be substantially higher (80 to 160 cm/cm^3) (Barker *et al.* 1988) than those measured in the dual labelling trial (10 to 40 cm/cm^3) (Figure 3.6). Therefore, it is possible that competition for P could play an important role at the higher root densities found in the field, especially considering that, in the present trial, browntop was shown to acquire more total P isotope than white clover. The deleterious effects of competition for P from browntop on white clover would be in balance with autotoxic effects of white clover on its own root growth if the suggestion that white clover is autotoxic is correct.

The non-competitive interference elucidated in this dual labelling trial needs to be considered further. In the following chapter, root interference between browntop and white clover grown at adequate to luxury levels of soil P supply were investigated using the dual labelling technique to determine whether non-competitive root interferences between white clover and browntop occur at higher levels of soil P supply.

CHAPTER FOUR

THE EFFECTS OF SOIL PHOSPHORUS SUPPLY AND DEFOLIATION ON THE INTER- AND INTRASPECIFIC ROOT INTERFERENCES OCCURRING IN MINI-SWARDS OF BROWNTOP AND WHITE CLOVER GROWN AT ADEQUATE TO LUXURY LEVELS OF SOIL P SUPPLY

4.1 Introduction

At deficient levels of soil P supply, direct competition for P between roots of browntop and white clover was found to be not as important as has been previously suggested (Chapter Three). Some type of root interference, other than interspecific root competition was occurring in the browntop-white clover simulated sward. The unidentified interference was revealed by white clover roots acquiring more P isotope when they were associated with browntop roots than with roots of other white clover plants, when grown on Ramiha subsoil at 100 mg P applied/kg soil. The possibilities of intraspecific root competition being greater than interspecific root competition and autotoxic effects of white clover on its own root growth were considered (Section 3.4).

A second dual labelling trial was conducted at higher soil P supply to determine whether the unknown root interference occurred at luxury levels of soil P supply, where direct competition for soil P is unlikely to occur because soil P supply should be greater than the total demand for P by all plants.

The effect of defoliation of outer swards on P isotope acquisition by central indicator plants was examined again, despite the lack of effects of defoliation found in the previous dual labelling trial. The effects of defoliation are known to be rapid (Caldwell *et al.* 1987) and the root densities in the soil in the first dual labelling trial were not considered high enough to prove that direct competition between individual roots was occurring. To overcome these problems encountered in the first dual labelling trial, central indicator plants were harvested only four days after the defoliation treatments were imposed, and it was assumed that root densities would be higher at the higher level of P supply. Using this approach the effects of direct root competition and toxicity (allelopathy) could be examined simultaneously if they both occur.

4.1.1 Objectives

The objectives of this trial were:

[1] To determine whether white clover acquired more P isotope from a soil space dominated by browntop roots compared with when the soil was dominated by roots of other white clover plants, at adequate to luxury levels of soil P supply.

[2] To determine whether direct competition for soil P was occurring between roots of browntop and white clover at higher levels of soil P supply than used in the previous dual labelling trial.

[3] To elucidate whether there was evidence of plant root interference(s) other than root competition for P occurring in browntop-white clover mini-swards.

4.2 Materials and methods

4.2.1 Experimental design and treatments

The design of the second dual labelling trial was similar to that outlined in Chapter Three (Section 3.2). The major difference between the two trials was that the level of soil P supply in the present trial was greater than in the first trial. This was achieved by using Ramiha topsoil, instead of subsoil, and by applying higher rates of P to the topsoil.

The experiment was a randomised complete block with four replicates and a factorial combination of twelve treatments. The treatments imposed were; level of phosphorus supply (68 and 344 mg P/kg soil, applied as monocalcium phosphate), central indicator species (browntop or white clover), and defoliation (outer browntop or white clover simulated sward defoliated, or no defoliation). There was one harvest.

Once again, the defoliation treatments were imposed on the same day that the isotopes were injected into the soil. This was done over four consecutive days, with one replicate being treated each day. The outer simulated swards were cut to a height of 20 mm when the defoliation treatments were carried out.

4.2.2 The soil

Ramiha topsoil was collected from Tuapaka Farm, Aokautere, New Zealand. The soil had a much higher Olsen P value (15 μg P/g soil) than the subsoil (Olsen P 5 μg P/g soil). Topsoil was passed through a 5 mm sieve.

The same plastic trays of the dimensions 300 mm by 400 mm and 100 mm depth were used. The trays were separated into two sections with perspex partitions (Plate 4.1). 4.5 kg of Ramiha topsoil was weighed into each half of the tray after mixing with the appropriate amount of monocalcium phosphate.

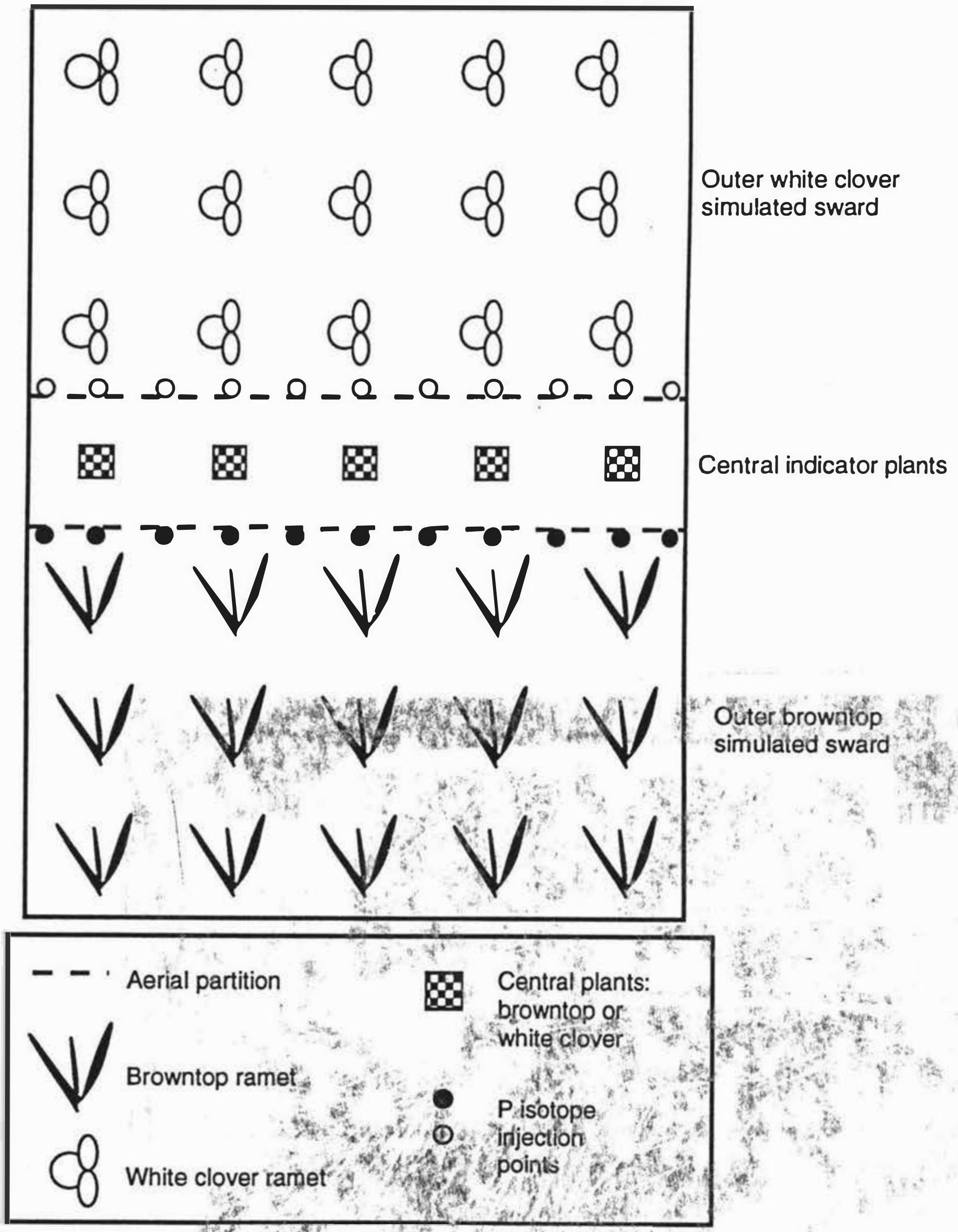


Figure 4.1 Planting arrangement of ramets of browntop and white clover in the half-tray, showing positioning of aerial partitions and points of injection of radio-isotopes.

Plate 4.1 Photograph of trays used to grow simulated swards, showing separation of tray into two halves, the left side with browntop as central indicator species, and the right side with white clover as central indicator species. The tray pictured above had outer white clover simulated sward

4.2.3 Watering

The soil was originally watered to a gravimetric moisture content of 35% at planting, and was increased up to 40% when the demand for water by the plants increased. The water content across the tray was monitored using an IRAMS time domain reflectometer (Topp *et al.* 1982), and by weighing daily.

4.2.4 Planting

Ramets were used to propagate browntop and white clover plants from four single parent plants of each species. The planting arrangement is shown in Figure 4.1. The overall planting density was 35 ramets per 4.5 kg of soil, equivalent to a sub-plot. White clover plants were inoculated with *Rhizobium trifolii* to ensure good nodulation of roots.

4.2.5 Aerial partitions

Aerial partitions were used in the same manner as the first dual labelling trial (Figure 4.1 and Plate 4.1), as described in Section 3.2.5.

4.2.6 Basal nutrients

The basal nutrient solution applied was the same as that detailed in Section 3.2.6. Nutrient solution was applied at planting, 43, and 65 days after planting.

4.2.7 Injection of carrier-free radioactive phosphorus

The method of isotope injection used is outlined in Section 3.2.7. There were eleven injection points on either side of the row of five central indicator plants (Figure 4.1). 0.1 ml of stock carrier-free solution was injected into each injection point at a depth of 40 mm. Isotopes were injected on the same day that defoliation treatments were imposed, 94 days after the ramets were planted. In replicates one and three, ^{32}P was injected into the browntop side and ^{33}P was injected into the white clover side (see Figure 4.1). This process was reversed in replicates two and four.

4.2.8 Harvesting

The central indicator plants were harvested four days after imposing the defoliation treatments and the isotopes were injected. The tops were harvested by cutting all of the shoots (above ground plant material) from the roots. Root sub-samples were collected by taking a core sample directly over the crown of each plant with a soil sampling core of diameter 27 mm. The soil was washed from the root samples by hand using a fine sieve and a hose. The harvested plant material was then dried to constant weight in an oven at 65°C and weighed. The shoot samples with more than 1 g available were ground using a grinding mill. Smaller shoot samples and root samples were cut with scissors.

Samples were measured for counts due to ^{32}P and ^{33}P, and nitrogen and phosphorus concentrations were measured on an auto-analyzer, after sample digestion by the Kjeldahl method (Twine and Williams 1971). The digestion mixture used was 100 g of potassium sulphate and 1 g selenium powder in 1 l of concentrated sulphuric acid.

4.2.9 Counting radioactive phosphorus-32 and -33

The method used for counting ^{32}P and ^{33}P is outlined in detail in Section 3.2.9. Counts were measured for shoot and root samples.

4.2.10 Frequency distributions of normalised activities of radioactive phosphorus-32 and -33

The frequency distribution for the activities of the radioactive phosphates was positively skewed, as found in the previous dual labelling trial (Chapter Three). A log transformation was performed on the P isotope activity data to stabilise the variance, as described in Section 3.3.1. Log transformations were not required to be performed on other data (e.g., shoot dry weights and P concentrations). Analysis of variance, using a general linear model was performed on the data and log transformed activity data. Results for P isotope activity were presented using X_S, Sichel's (1952) estimator. The computer programme employed to carry out the statistical analyses was SAS, available on the network computer system at Massey University.

4.3 Results

4.3.1 Effect of species association on phosphorus isotope acquisition

Browntop acquired more radioactive P than white clover, with both species obtaining more isotope from the side with simulated swards of the other species, at both levels of P application, irrespective of the defoliation treatment (Figure 4.2). That is, there was a significant central species by side (outer species associated) interaction. The P isotope concentration in central indicator browntop shoots was greater at 68 mg P applied/kg topsoil than at 344 mg P applied/kg topsoil (Figure 4.2a). The central species by P level interaction was significant.

There were significant differences between the replicates (clones). Therefore the variance of the P isotope count means was high.

4.3.2 Effects of defoliation of outer plants on acquisition of radioactive phosphorus isotopes by central indicator plants

The effects of defoliation of outer species on P isotope uptake were presented as total isotope uptake/g shoots from both sides of the central indicator row of plants (Figure 4.3). None of the possible treatment interactions were significant.

There were no effects of the defoliation treatments on the total P isotope per g of shoots in central indicator plants of browntop (Figure 4.3a). The results suggest that there may have been an increase in the acquisition of isotope by central indicator browntop plants with the defoliation of outer browntop plants at 68 mg P applied/kg soil, however this was not significant.

At 68 mg P applied/kg topsoil, but not at 344 mg P applied/kg topsoil, the total P isotope concentration in shoots of central indicator white clover plants was greater when outer browntop plants were defoliated compared with the other two defoliation treaments (i.e., when outer white clover plants were defoliated and with no defoliation treatment) (Figure 4.3b).

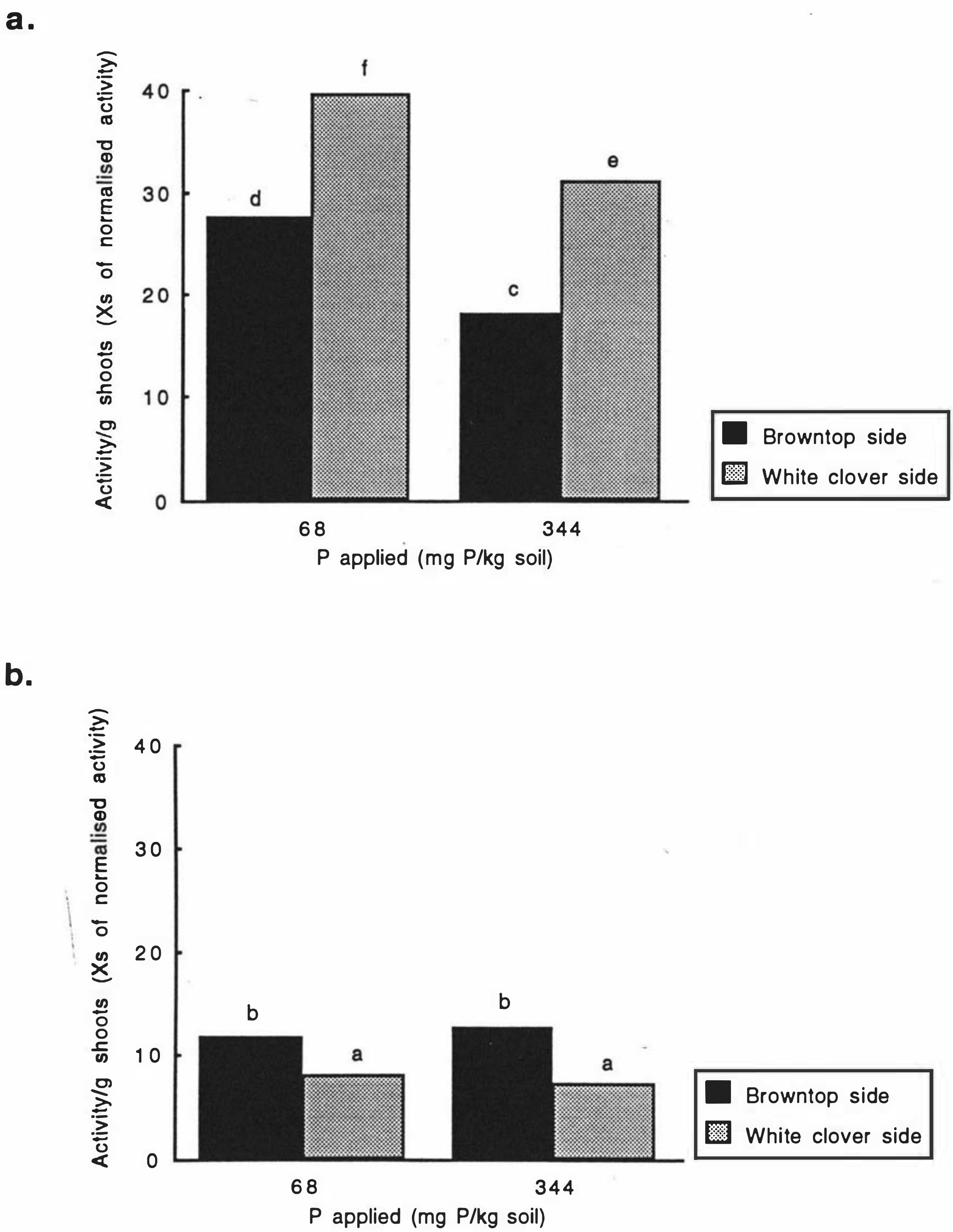


Figure 4.2 Effect of outer simulated sward species on P isotope acquisition (normalised activityx10^{-5}/g) by central indicator (a) browntop, and (b) white clover plants, 4 days following the imposition of defoliation treatments and isotope injection. Means with different letters differ significantly ($p < 0.05$) as indicated by the standard error (calculated from V_{X_S}) of Sichel's estimator (X_S). Comparisons can be made between the two graphs.

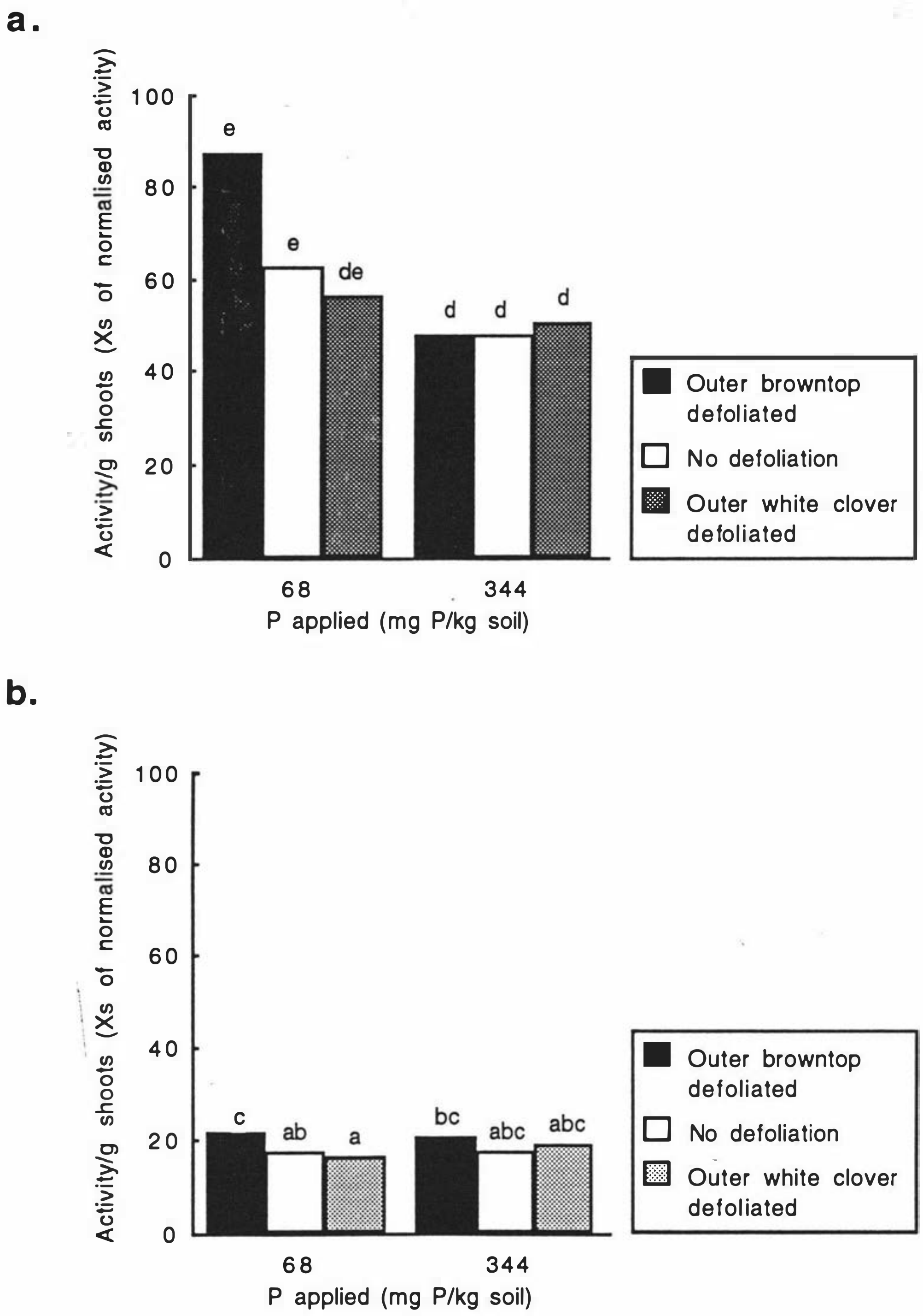


Figure 4.3 Effect of defoliation of outer simulated swards on the total P isotope uptake (normalised activityx10^{-5}/g) from both sides by central indicator (a) browntop, and (b) white clover plants, 4 days following the imposition of defoliation treatments and isotope injection. Means with different letters were significantly different ($p < 0.05$) as indicated by the standard error (calculated from V_{X_s}) of Sichel's estimator (X_s). Comparisons can be made between the two graphs.

4.3.3 Dry weights and phosphorus concentration of shoots of central indicator plants

There was no significant response of shoot dry weight to increasing P supply from 68 to 344 mg P/kg soil for either central indicator browntop or white clover plants (Table 4.1). The shoot dry weights were greater for browntop than for white clover (Table 4.1). There was no significant interaction between species and P level for shoot dry weight.

The P concentrations were high, even at 68 mg P applied/kg topsoil; 0.34 and 0.33% for white clover and browntop, respectively (Table 4.1). The phosphorus concentrations in shoots increased significantly with P application (Table 4.1), but there was no corresponding increase in shoot weight. There was a significant interaction between species and level of P supply for the shoot phosphorus concentration. At 68 mg P applied/kg topsoil, the shoot phosphorus concentrations were similar for central indicator browntop and white clover plants, but at 344 mg P applied/kg soil the phosphorus concentration in browntop was greater than in white clover. Browntop was absorbing P to concentrations beyond it's requirement to achieve optimum shoot yield.

As expected, due to the short time of only four days between the defoliation of outer simulated swards and harvest of central indicator plants, there was no effect of the defoliation treatments on the shoot dry weights or on the P concentration of the central indicator plants.

4.3.4 Nitrogen concentration in shoots

The nitrogen concentration in the shoots of white clover was greater than in browntop (Table 4.1). Increasing the phosphorus supply led to a significant reduction in the N concentration of browntop shoots, while the N concentration in white clover shoots increased by 0.01%. The decrease in %N in browntop with increasing P supply was surprising in view of the lack of shoot response to P supply which might have caused a dilution of the nitrogen in the shoots.

4.3.5 Dry weights, and phosphorus and nitrogen concentration of root sub-samples

There was no effect of P application on the root sub-sample dry weights or N concentration in root sub-samples of browntop or white clover (Table 4.2). The root sub-sample weight of browntop was around 3.5 times that of white clover, while the N concentration in white clover roots was about double that in browntop roots.

There was no significant difference in P concentration in roots between the two species, and the P concentration increased by approximately 40% at the higher P rate (Table 4.2).

4.3.6 P depletion zones around roots

Root length measurements were not made in this trial, however the estimated time taken for depletion zones between roots to overlap were considered, with calculations made based on specific root lengths (SRL) of 30 and 20 m per g root for browntop and white clover, respectively. These SRL values were estimated from the results presented in Figure 3.8b from the previous dual labelling trial (Chapter Three). The estimated times for P depletion zones around roots of browntop to overlap were 490 and 110 days at 68 and 344 mg P applied/kg soil, respectively. For white clover, the estimated times were 3000 and 500 days at 68 and 344 mg P applied/kg soil, respectively. Therefore the estimated times for P depletion zones to overlap were longer at the lower level of P supply due to the smaller diffusion coefficient of P in soil (Appendix 1), i.e., a slower rate of diffusion of P at a lower level of soil P supply.

Table 4.1 Effect of P application as monocalcium phosphate on shoot dry weight, and P and N concentrations in shoots of central indicator browntop and white clover plants. Means with different letters were significantly different, tested using LSDs ($p < 0.05$).

Central species	P applied (mg P/kg soil)	Shoot dry weight (g)	%P in shoots	%N in shoots
Browntop	68	2.20 bc	0.33 a	2.6 b
Browntop	344	2.26 c	0.44 c	2.3 a
White clover	68	1.76 a	0.34 a	3.5 c
White clover	344	1.92 ab	0.37 b	3.6 d

Table 4.2 Effect of P application as monocalcium phosphate on root sub-sample dry weight, and P and N concentrations in root sub-samples of central indicator browntop and white clover plants. Means with different letters were significantly different ($p < 0.05$).

Central species	P applied (mg P/kg soil)	Root dry weight (g)	%P in roots	%N in roots
Browntop	68	0.24 b	0.25 a	1.2 a
Browntop	344	0.21 b	0.36 b	1.1 a
White clover	68	0.06 a	0.27 a	2.5 b
White clover	344	0.07 a	0.36 b	2.4 b

4.4 Discussion

4.4.1 Evidence of root competition for P

The competitiveness of browntop can be explained by the ability of browntop to acquire more radioactive P from the soil than white clover, which would have been reduced by defoliation (Figure 3.5). Defoliation of outer browntop plants reduced their root competitive ability for soil P with central indicator white clover plants at 68 mg P applied/kg topsoil (Figure 4.3 b). Browntop acquired more P isotope from the white clover side than the browntop side (Figure 4.2 a), supporting the suggestion that browntop was more competitive than white clover for soil P. However, the net effect of outer white clover plants on P isotope acquisition by central indicator white clover plants was more harmful than that of outer browntop plants (Figure 4.2 b). Some non-competitive interference was also occurring, possibly autotoxicity of white clover, masking the competitive effect of browntop on white clover's P isotope acquisition (Figure 4.2 b).

The effect of defoliation reducing the root competitive ability of browntop was rapid (four days), and it is interesting that this reduction was not evident in the first dual labelling trial which was harvested seven and fourteen days following the defoliation of outer plants. It is possible that the reduction of the competitive effects of browntop by defoliation on white clover may be short lived because browntop may quickly regain its root competitiveness. This may be through rapid root growth, or physiological increases in the rate of P uptake (Clarkson 1985), as suggested by the ability of browntop to continue to acquire P to higher concentrations into the shoots following defoliation (Figure 3.4).

4.4.2 Non-competitive interference

Overall, both browntop and white clover appeared to gain mutual benefit for P isotope acquisition from the presence of the other species (Figure 4.2), as found in the first dual-labelling trial at 100 mg P applied/kg soil (Figure 3.2). Therefore, the competitive effects of browntop on white clover were not of overriding importance at adequate to luxury levels of P supply, or at intermediate levels of P supply (Chapter Three).

Because central white clover plants acquired more P isotope from the outer browntop side than from the outer white clover side at luxury levels of P supply it makes it possible to eliminate some factors (other than autotoxicity of white clover upon its own root growth (Section 3.4.2)) that may have contributed to this apparent beneficence. Phosphatase activity is inhibited at high levels of soil P supply (Dracup *et al.* 1984), therefore it was unlikely that white clover roots growing in association with browntop roots could have gained any benefit from any phosphatase activity associated with roots of browntop. Similarly, it was not likely that white clover had an increased P uptake from rhizosphere effects at adequate levels of P supply. Mycorrhizal infection would also be low at such high levels of soil P supply, as was the case even in the previous dual labelling trial at lowest level P supply, i.e., grown on unamended Ramiha subsoil. Also, V.A. mycorrhizal transfer of P between plants would be too slow to explain the results (Newman and Eason 1993). Therefore, the greater P isotope acquisition by central indicator white clover plants associated with outer browntop plants compared with those associated with outer white clover plants was probably due to greater soil exploration by roots of white clover the closer they were to the outer browntop simulated sward, as discussed in Section 3.4.2.

4.4.3 Relative importance of interferences occurring between browntop and white clover in the field

Recent discussion of the role of competition in community ecology by Hunter and Aarssen (1988) has revealed that some researchers have become disillusioned with the importance placed on interspecific competition to explain community structures. They concluded that there is substantial evidence to indicate that other types of plant interference my occur simultaneously with interspecific competition.

The significance of competition for soil P between browntop and white clover may have been over-emphasized. Other factors contributing to the success or failure of these two species in hill country pastures in New Zealand have been considered by some researchers (Jackman and Mouat 1972a; Harris 1974). However, competition for soil P from browntop has often been considered to be of utmost importance in contributing to the lack of white clover in hill country pastures.

The results of the dual labelling trials (Chapters Three and Four) provide a "snap-shot" picture of the net effect of interferences that were occurring between roots of browntop and white clover in an established simulated sward at the time of isotope injection. They do not give any indication of the effect the two species have on the total P acquisition of each other over their life span, which can be determined by field trials and by the more traditional types of plant competition trials (additive and replacement series experiments). From traditional plant competition trials the mechanisms of root competition between plants can only be inferred from the physiological performance of plants (Caldwell *et al.* 1985), while the dual P isotope labelling trial gives a realistic observation of the effect of two plant species on each others P acquisition, at a particular chosen point in time.

From the P response trial (Chapter Two) and from field observations it is clear that browntop is better adapted than white clover to survive under conditions of low soil P fertility. The lower growth rate of browntop compared with white clover may be the most important factor contributing to the dominance of browntop in hill country pastures. Also of precedence is the mat forming nature of browntop (Jackman and Mouat 1972a; Harris 1974). Jackman and Mouat (1972a) found in a field trial that the severity of competition for P between browntop and white clover decreased in parallel with the increase with time in the build up of browntop mat. This reduction in competition for P from browntop with white clover would be expected as a result of shading of white clover stolons and physical impedence by the browntop mat, leading to niche differentiation and thus, a negative association of browntop and white clover.

Grown on unamended subsoil, browntop displayed the ability to affect the P acquisition by white clover roots (Figure 3.2b). However, in the field white clover would gradually be physically impeded by the browntop mat which tends to be more dense under poor nutritional conditions (Harris 1974). Thus the importance of competition for soil P from browntop may not be as important as the ousting of white clover from areas of browntop dominance, particularly in view of the low mobility of P in soil. However in the long term browntop would have a major impact on soil P supply (Section 2.4), especially if P fertilisers are not applied.

The apparent beneficence gained by white clover from growing with browntop at intermediate to luxury levels of P supply was a product of species diversity, because in terms of root growth, white clover would have performed worse in monoculture than in mixture with browntop. Again, the exclusion of white clover by browntop mat when grown in the field would reduce any possible impact of apparent beneficence between the two species. If this apparent beneficence from browntop was actually due to autotoxic effects of white clover on its own root growth, white clover may benefit from growing with other pasture species, and this may help to explain the positive association found between white clover and ryegrass (Harris 1974; Simpson *et al.* 1987).

Autotoxicity is an evolutionary paradox. The advantages of autotoxicity are not clear, and as Grime (1979) points out, must be explained before allelopathy will find acceptance. A further difficulty with accepting autoallelopathy as an adaptive plant interference is that phytotoxins are often compounds or breakdown compounds essential to plant growth, or are secondary chemicals that provide protection to plants from predators or pathogens (Grime 1979). However, some researchers have argued that autoallelopathy plays an important role in regulation of population size by self thinning (Whittaker and Feeny 1971; Chou 1987) and controls species diversity (Lieth 1960). Whittaker and Feeny (1971) pointed out that some cases of autotoxicity would be of no selective advantage to the plant, and suggested that in other cases, "adaptive auto-inhibitors" would control population numbers.

Many successional and seasonal plant species, and competitive ruderals (Grime 1979) have been suggested to be autoallelopathic: white clover migrates from year to year (Lieth 1960); alfalfa autotoxicity has been associated with autumn dormancy (Miller 1983); *Calluna vulgaris* opens way to succession to Birch through self intoxication (Robinson 1971). Grime suggested that if the highest concentration of phytotoxins occurs after the main growth period of the plant species producing the phytotoxin, and acts on another species with complementary phenology, and breaks down before the next main growth period of the producer, then autoallelopathy may be of advantage. However, evidence of seasonal allelopathy is only circumstantial (Grime 1979) and the cause of cyclical phenology, migration and/or succession of some plant species may be caused by autotoxicity and of no real advantage.

Jackman and Mouat (1972a) claimed that shading of white clover stolons by browntop was a most important component affecting white clover production in browntop pastures, along with competition for soil P. The results of the dual labelling trial suggest that competition for P may not be as important as shading. The dramatic effects of grazing management practises on the botanical composition of hill country pastures (Harris 1974; Lambert *et al.* 1986) support this view.

In the dual labelling trials, the only evidence of direct competition for soil P between browntop and white clover was observed at adequate levels of P supply (Figure 4.3 b) where apparent beneficence had an overriding effect (Figure 4.2). Also, the N supply in the second dual labelling trial was adequate (Table 4.1), suggesting that in the field (where N supply would most likely be lower) browntop would be even less competitive.

4.4.4 Summarising comments

Browntop was more competitive for soil P, and acquired more P isotope than white clover. The competitiveness of browntop was reduced by defoliation, and this reduction was evident only four days following defoliation. At adequate to luxury levels of soil P supply, however, both browntop and white clover appeared to gain mutual benefit for P isotope acquisition from the presence of roots of each other (apparent beneficence). Therefore, the competitive effects of browntop roots on P acquisition by white clover were not of overriding importance.

The dual P isotope labelling technique has proven to be useful in elucidating more than one type of plant interference occurring within browntop-white clover simulated swards at the same time. The technique could also be used in the field. The dual labelling technique demonstrated plant interferences that the more traditional types of plant competition (interference) trials have failed to elucidate. It may be useful to use more than one type of experimental design to gain a clearer picture of the nature of interactions occurring between roots of different species. The more traditional types of competition (interference) trials may be useful to give an indication of the net result of plant interferences on the growth of plants in mixtures by measuring the total P uptake and growth of plants over their life span.

CHAPTER FIVE

THE EFFECTS OF ADEQUATE TO LUXURY LEVELS OF SOIL P SUPPLY ON THE INTER- AND INTRASPECIFIC ROOT INTERFERENCES OF INDIVIDUAL PLANTS OF BROWNTOP AND WHITE CLOVER GROWN IN POTS

5.1 Introduction

There is evidence that apparent beneficence for soil P acquisition may occur between roots of established plants of browntop and white clover at intermediate to luxury levels of soil P supply (Chapters Three and Four). This apparent beneficence has not been demonstrated previously, possibly because unsuitable techniques have been used to study root competition between browntop and white clover.

There has been much debate over many years about the shortfalls of the many types of experimental designs used to study root competition between plants (Harper 1977; Snaydon 1979, 1991; Joliffe *et al.* 1984; Firbank and Watkinson 1985, 1990; Taylor and Aarssen 1989; also see Chapter One). Neither the additive nor replacement series types of experiments have been shown to be completely adequate for studying plant competition between two species. The inadequacies of these designs need to be considered when interpreting the results of such experiments.

The results of Chapters Three and Four contrast with the results from competitive settings types of designs used in previous research in that apparent beneficence for soil P acquisition between browntop and white clover had not previously been demonstrated (Mouat and Walker 1959a; Jackman and Mouat 1972a, 1974). This may have been because root interferences between browntop and white clover have not previously been independently examined. The effects of shoot interference (Harris 1974) and, possibly, soil moisture (Jackman and Mouat 1972b) may have had an overriding influence over the effects of root interferences between browntop and white clover. There is also the possibility that the effects of plant density may have confounded the results. However, it is difficult to draw a conclusion on this matter, as the papers of Mouat and Walker (1959a) and Jackman and Mouat (1972a, 1974) did not give sufficient details on the plant densities used.

Another possibility may be that apparent beneficence only occurs in established swards of older plants. Many root interference studies either examine young plants or the final measurements are of the cumulative effects of the two plants on each other over their whole life span, and therefore, may fail to elucidate the interferences that are occurring between two plants at the time of their harvest.

In the present trial, a more traditional competitive settings type of experiment was conducted, at adequate to luxury levels of soil P supply, to determine whether the apparent beneficent effects of browntop roots on soil P acquisition by white clover roots observed using the dual labelling technique could be observed. The design of the competitive settings experiment attempted to overcome some of the problems encountered with previously used designs. As competition operates largely at the individual plant level (Rhodes 1970), the effects of neighbouring plants on the growth and P nutritional status of a single plant was studied. The approach enabled the effects of shading between plants to be removed by the use of an aerial partition that isolated the shoots of a single plant from shoots of neighbouring plants, whilst allowing roots to interact.

The objective of this work was to compare the findings of the dual labelling trial with a competitive settings trial, to determine whether the interferences occurring between the roots of browntop and white clover were dependent on the experimental methods used.

5.2 Materials and methods

The design of the experiment incorporated aspects of both additive and replacement series types of experiments. As plant density is known to affect the severity of root competition (Harper 1977), the soil weight:plant density ratio was held constant at 5 plants per kg of soil (i.e., one plant per 200 g soil). The experiment was designed to enable the effects of both inter- and intraspecific root interferences to be examined. This involved the comparison of the growth and P nutritional status of a single central indicator plant in a small pot (no interference with other plants) with the growth of a single central indicator plant in a larger pot with its roots associated with roots of plants of the same species (intraspecific root interference) or of different species (interspecific root interference) (Figure 5.1).

The effects of three treatments were examined. The treatments imposed were; planting combination: roots of central indicator plant associated with roots of outer plants of the other species (combinations 1 and 2) or with roots of the same species (combinations 3 and 4), or no association (combinations 5 and 6) (Figure 5.1); central indicator plant species: browntop or white clover; and level of P applied as monocalcium phosphate: 0, 100, 200 and 400 mg P/kg soil. There were five replicates, giving a total of 120 pots. The experimental design was a randomised complete block with a factorial combination of treatments.

The central indicator plant was surrounded by an aerial partition constructed from a waxed cardboard cup of 130 mm height and covered in reflective aluminium foil (Figure 5.2 and Plate 5.1). The purpose of the aerial partition was to ensure that the shoots of the central indicator plants in all planting combinations received the same amount of light, and that there was no shading (shoot interference) of the central indicator plant by the outer plants.

5.2.1 Soil

Air dry Ramiha topsoil was passed through a 5 mm sieve. After mixing thoroughly with the appropriate amount of monocalcium phosphate, 200 g of air dry soil was weighed into the smaller inner pot. This pot was placed inside the larger pot and then 800 g of air dry soil mixed with the appropriate amount

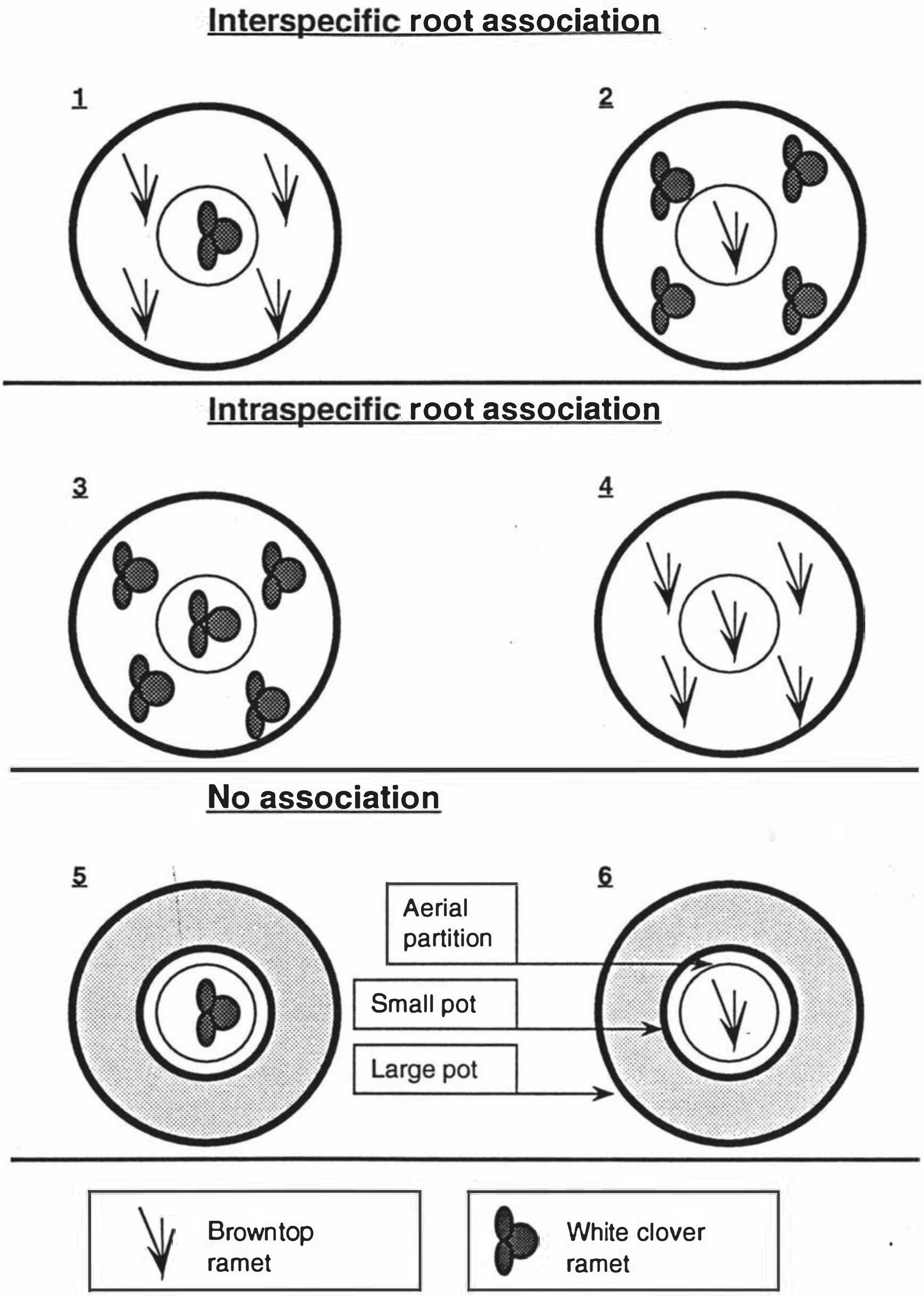


Figure 5.1 Planting combinations of ramets of browntop and white clover, showing the three types of root associations between the central indicator plant and outer plants, and the positioning of the aerial partition.

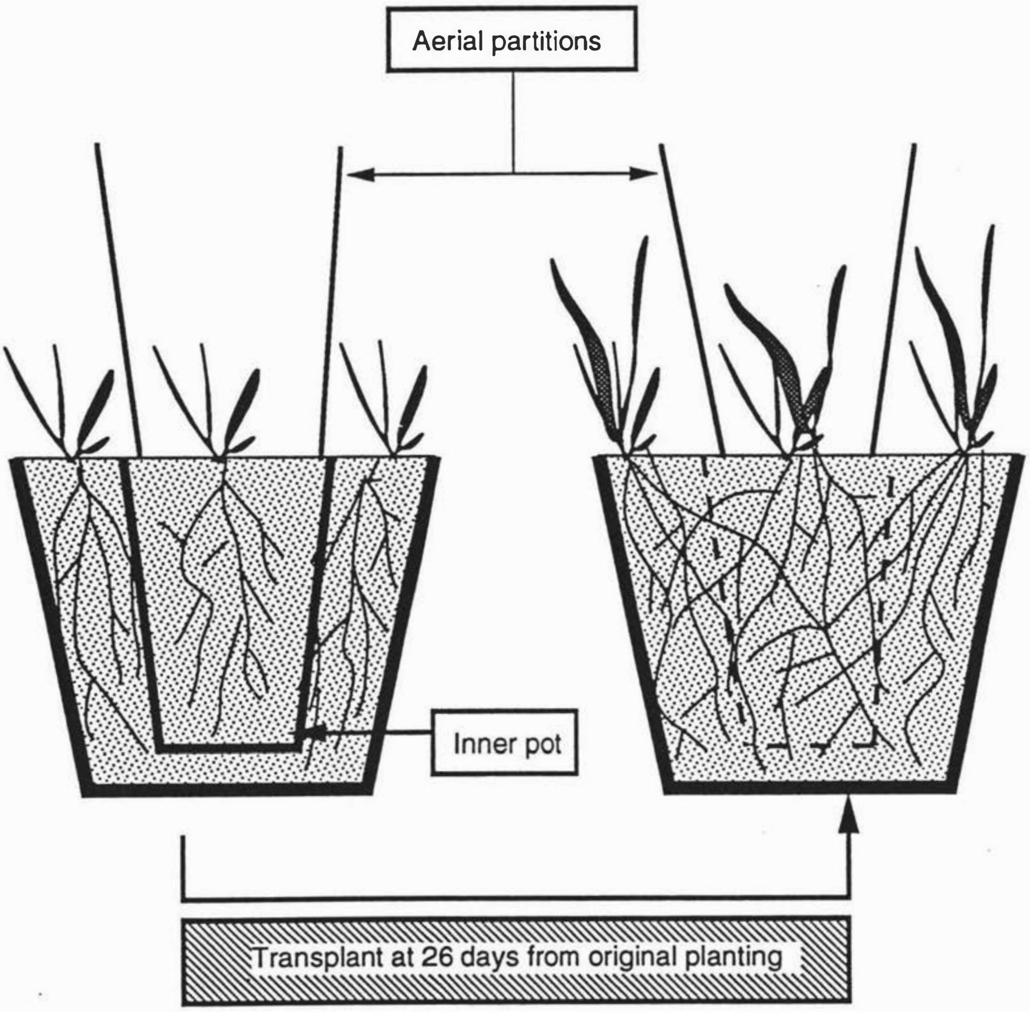


Figure 5.2 Side view of pots for the planting combination 4 (Figure 5.1) allowing intraspecific root interferences to occur between a central indicator browntop plant and four outer browntop plants, showing the transplantation of the central indicator plant 26 days after planting.

Plate 5.1 Photograph showing the positioning of aerial partitions in pots

of monocalcium phosphate was weighed into the outer pot (Figures 5.1 and 5.2).

5.2.2 Measurement of nitrate and ammonium ions in the soil

Nitrogen mineralisation on rewetting was measured to assess NO_3^- and NH_4^+ levels in the soil. Four replicates of 20 g of Ramiha topsoil were weighed into sealable plastic bags and moistened to a gravimetric water content of 38%. The moistened soil was incubated at 25°C for 72 hours. 6.5 g of moist soil (equivalent to 5 g of dry soil) was sub-sampled and extracted for 20 minutes in 25 ml of 1M KCl and filtered through a Whatman 40 filter paper, similar to the method of Adamsen *et al.* (1985).

NO_3^- was measured colourimetrically on a Technicon Series II auto-analyzer, using N-1-Naphthylethyl-enediamine dihydrochloride/Sulphanilamide indicator (Litchfield 1967).

NH_4^+ was measured colourimetrically by titration using a phenolphthalein indicator. 10 ml of 1M KCl filtrate extract was pipetted into a 50 ml conical flask and 3 drops of phenolphthalein indicator was added. 0.002M NaOH was added dropwise until the solution was just alkaline, producing a pink colour. 2.5 ml of 40% neutralised formalin was added, producing a colourless solution. After standing for 5 minutes, the colourless solution was titrated with 0.002M NaOH, with the end point being a pink colour (Harwood and Kuhn 1970). The titration reaction is given in equations 5.1 and 5.2.

$$4NH_4^+ + 6CH_2O \longleftrightarrow C_6H_{12}N_4 + 4H^+ + 6H_2O \text{................} [5.1]$$

$$4[H^+ + NaOH \longleftrightarrow H_2O + Na^+] \text{..} [5.2]$$

The level of N in the soil and N mineralisation were high (Table 5.4), so it was not necessary to apply nitrogen in the basal nutrient solution.

5.2.3 Planting

Clones from five parent plants were selected for the five replicates. The more stoloniferous types of white clover were selected for cloning in preference to the large leaved types. One ramet was planted into the small central pot in each case. Five ramets were planted into the outer pots in planting combinations 1, 2, 3 and 4, and were thinned to 4 plants per outer pot 14 days after planting. No plants were grown in the outer pots in planting combinations 5 and 6, but soil was placed in them. The purpose of having soil in the outer pots in combinations 5 and 6 was to maintain the soil temperature at the same level as combinations 1, 2, 3 and 4. To achieve this, the soil in the outer larger pot in combinations 5 and 6 was also watered. White clover plants were inoculated with *Rhizobium trifolii* to ensure good nodulation of roots.

To encourage good establishment of the central indicator plants, they were originally grown without any root association with outer plants until roots were visible through the transparent small central pots. Twenty six days after planting, the contents of the small central pots, in combinations 1, 2, 3 and 4, were transplanted out of the small pot, and back into the centre of the larger pot (Figure 5.2). This allowed roots of the central indicator plant to associate with roots of plants growing in the outer pot (Figure 5.2).

5.2.4 Watering

Initially the soil was watered up to a gravimetric water content of 30%. Two weeks after planting, the soil water content was increased to and held at 38% gravimetric water content, i.e., 75% of the gravimetric water content at 50 cm suction.

5.2.5 Basal nutrients

Basal nutrients, excluding N and P, were applied as required. A stock solution of 60 g/l $MgSO_4.7H_20$, 80 g/l KCl, 0.192 g/l $Na_2MoO_4.2H_2O$, 1.3 g/l $MnSO_4.4H_2O$, 1.g/l $CuSO_4.5H_2O$, 1 g/l $ZnSO_4.7H_2O$ and 0.68 g/l $Na_2B_4O_7.10H_2O$ was used. 200 ml of stock solution was diluted to 5 l before application to the pots.

5.2.6 Spraying

During the course of the experiment the plants were sprayed with Benlate (active ingredient: Benomyl) to treat a black spored fungus, and with a miticide, Neoron 500 (active ingredient: Bromopropylate).

5.2.7 Harvesting

All plant shoots and roots were harvested 69 days after planting. Fresh weights of tissue-dried roots were recorded and the roots were then preserved in FAA (i.e., 13 ml Formalin, 5 ml glacial acetic acid and 200 ml 50 % ethanol). Shoots were dried in an oven at 65°C to constant weight.

5.2.8 Measurements

Dry weights of shoots and fresh weights of roots were recorded. Concentrations of N and P were measured using a Kjeldahl-type digestion and measured colourimetrically on an auto-analyser, (see Section 2.2.3). Roots were assessed for V.A.mycorrhizal infection using the method outlined in Appendix 3. Root lengths were not measured as the samples were accidently discarded.

5.2.9 Analysis of data

Many indices, (e.g., relative yield total, aggressivity, relative crowding coefficient), have been used to study plant interference (Snaydon 1991). However, due to inconsistencies in their use, and confusion about their real significance (Snaydon 1991), they were avoided in the examination of the results of the present trial. Some indices can only be used under specific experimental conditions (Snaydon 1991). Instead, shoot dry weight, P content of shoots, and P and N concentrations in shoots data were used to examine the effects of the root association treatments.

Curves were fitted to the responses of shoot dry weights to P application using an iterative procedure with a computer programme, Curvefit (Kolb 1979). Curvefit uses a maximum likelihood method, rather than the least squares method to determine the best fit of a curve. The curve of best fit was linear where:

$$Y = (m.x) + b$$

Y = dry weight of shoots of central indicator plant,
m = slope of line,
x = level of P applied in mg P/kg soil,

and,

b = constant (Y on unamended soil).

The slopes of the fitted lines were compared using t-tests (Zar 1984).

An attempt was made to compare slopes of the responses of the P concentration in shoots of central indicator white clover plants to P application under the different root association treatments. However, comparisons could not be made due to differences in the types of curves that best fitted the data for different root-association treatments.

5.3 Results

The results have been presented under the sections of P response of central plant, inter- and intraspecific associations, P response of monocultures, and N concentrations in the soil. It was difficult to present figures and tables relating to these section topics in the same order because the relationships between the parameters measured and observations made were considered.

5.3.1 Response of central indicator plant to P application

Both browntop and white clover shoot dry weights (Figure 5.3), and the P content of shoots (Figure 5.5), increased with P application. N was not limiting (Figure 5.6). The slope of the linear response to P level of the shoot dry weight of the central indicator plant, grown with roots associated with outer plants, was greater for browntop than for white clover (Figure 5.7, Tables 5.2 and 5.3). For the central indicator plants, the P concentration in shoots was 0.3 % or greater in all treatments, except for white clover grown alone on unamended soil (Figure 5.4). P concentration in shoots increased with P application for central indicator browntop plants in all species-association treatments (outer species), and for the central indicator white clover plants grown alone. At 200 and 400 mg P applied/kg soil, the P concentration in shoots of browntop was greater than in shoots of white clover (Figure 5.4).

5.3.2 Inter- and intraspecific associations

5.3.2.1 *Shoot dry weight of central indicator plant*

For the central indicator browntop plant grown on unamended soil, there was no effect of the root association treatments (i.e., outer plants) on the shoot dry weight (Figure 5.3a). However, at 100 mg P applied/kg soil, the dry weight of shoots of the central indicator browntop plant grown alone was less than for the central indicator browntop plant grown with roots associated with either outer browntop, or outer white clover plants (Figure 5.3a). At 200 and 400 mg P applied/kg soil, the shoot dry weight of the central indicator browntop plant, either grown alone, or with roots associated with outer browntop plants, was less than for the central indicator browntop plant grown with roots associated with outer plants of white clover (Figure 5.3a).

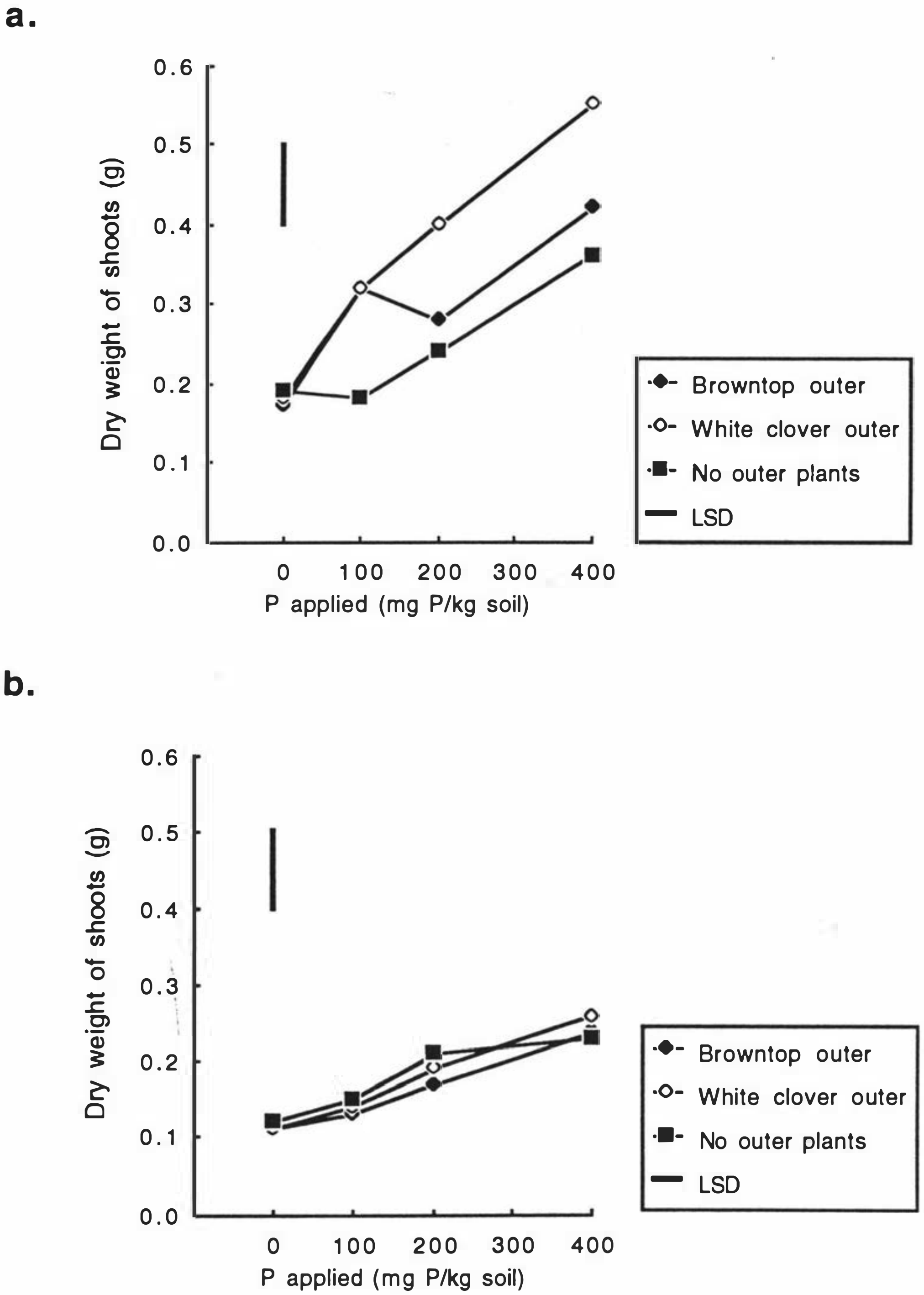


Figure 5.3 Effect of root associations with outer plants on the response of shoot dry weight of the central indicator (a) browntop, and (b) white clover plant to P application as monocalcium phosphate. The LSD of 0.1 g allows comparisons to be made between means for browntop and white clover ($p < 0.05$).

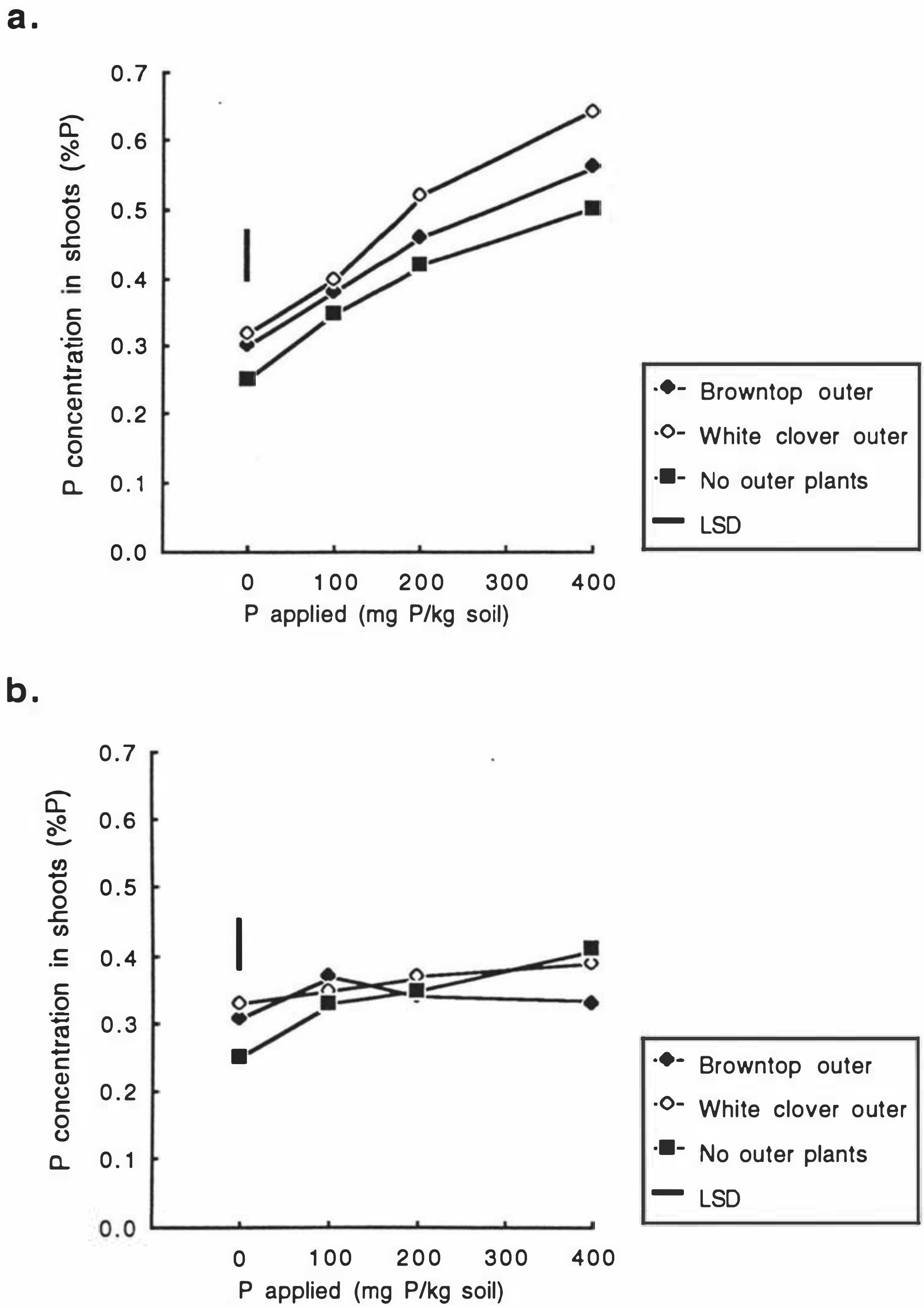


Figure 5.4 Effect of root associations with outer plants on the response of P concentration in shoots of the central indicator (a) browntop, and (b) white clover plant to P application as monocalcium phosphate. The LSD of 0.07% allows comparisons to be made between means for browntop and white clover.

The slope of the linear response of shoot dry weight of the central indicator browntop plant grown with roots associated with outer white clover plants was greater than when they were associated with outer browntop plants, or when grown alone (Figure 5.7a, Tables 5.2 and 5.3).

There was no effect of the root association treatments on the shoot dry weight of the central indicator white clover plant (Figure 5.3b), or on the slope of the linear response curve fitted to the data (Figure 5.7b, Tables 5.2 and 5.5).

5.3.2.2 *Root weights of central indicator plants*

The fresh weight of roots of central browntop was greater when grown with outer browntop plants than when white clover was the outer species at 100 and 400 mg P applied/kg soil (Table 5.1). There were no other significant effects of any of the species association treatments on the root fresh weights of central indicator white clover or browntop plants (Table 5.1).

5.3.2.3 *P concentration in shoots of central indicator plant*

There were no statistically significant effects of the root-association treatments on the P concentration in shoots of central indicator browntop plants grown either on unamended soil, or at 100 mg P applied/kg soil (Figure 5.4a). However, there was an overall trend for shoots of the central browntop plant to have a higher P concentration when grown with roots associated with those of outer white clover plants than when grown with roots associated with outer browntop plants (Figure 5.4a). Also, P concentrations in shoots of central browntop plants were lowest when grown alone (Figure 5.4a). The P concentration in shoots of the central indicator browntop plant was significantly greater when roots were associated with outer white clover plants than when grown alone at 200 mg P applied/kg soil, and when grown alone or with roots associated with outer browntop plants at 400 mg P applied/kg soil (Figure 5.4a).

The P concentration in shoots of the central indicator white clover plant grown alone, was less than when grown with roots associated with outer white clover plants when grown on unamended soil (Figure 5.4b). There were no other effects of root-association treatments on the P concentration of shoots of the central indicator white clover plant.

Table 5.1 Effect of root association with outer species and P supply on the fresh weights of roots (g) of central indicator plants.

P applied (mg P/kg soil)	Root association	Root fresh weight (g), with standard errors in brackets	
		Browntop central	White clover central
0	alone	0.62 (0.2)	0.26 (0.03)
0	with outer white clover	0.36 (0.09)	0.23 (0.05)
0	with outer browntop	0.52 (0.08)	0.25 (0.04)
100	alone	0.40 (0.11)	0.29 (0.02)
100	with outer white clover	0.60 (0.10)	0.27 (0.03)
100	with outer browntop	0.95 (0.12)	0.28 (0.02)
200	alone	0.54 (0.13)	0.31 (0.03)
200	with outer white clover	0.86 (0.19)	0.34 (0.06)
200	with outer browntop	0.77 (0.11)	0.43 (0.14)
400	alone	0.61 (0.12)	0.34 (0.02)
400	with outer white clover	0.86 (0.09)	0.46 (0.06)
400	with outer browntop	1.16 (0.18)	0.42 (0.08)

5.3.2.4 *P content in shoots of the central indicator plant*

The effect of the root-association treatments on the P content of the central indicator browntop plant (Figure 5.5a) was similar to that found for shoot dry weight (Figure 5.3a). There was no effect of the root-association treatments on the P content of shoots of the central indicator browntop plant grown on unamended soil. At 100 mg P applied/kg soil, the P content of shoots of the central indicator browntop plant grown alone was less than when grown with roots associated with either outer browntop, or outer white clover plants (Figure 5.5a). At 200 and 400 mg P applied/kg soil, P content of shoots of the central indicator browntop plant grown alone or with roots associated with outer browntop plants, was less than when grown with roots associated with outer plants of white clover (Figure 5.5a).

For the central indicator white clover plant, there was no effect of the root-association treatments on the P content of shoots (Figure 5.5b).

5.3.2.5 *N concentration in shoots of central indicator plant*

There were no effects of the root association treatments or soil P supply on the N concentration in shoots of either browntop or white clover,and the N concentration in shoots of browntop and white clover were similar (Figure 5.6). Nitrogen was not likely to be limiting the growth of browntop (Andrew and Johansen 1978) or white clover (McNaught 1970).

5.3.2.6 *Outer plants*

The effects of central indicator plant species on the growth and P status of outer plants was similar to the effects of root associations with outer species on the central indicator plant. The replacement of a central indicator browntop plant with a white clover plant led to an increase in the P concentration in shoots of the four outer browntop plants grown on unamended soil and at 400 mg P applied/kg soil (Figure 5.9). Also, the shoot dry weight (Figure 5.8) and total P content in shoots (Figure 5.10) of outer browntop plants was greater, at 200 mg P applied/kg soil, when white clover was the central indicator plant species rather than browntop.

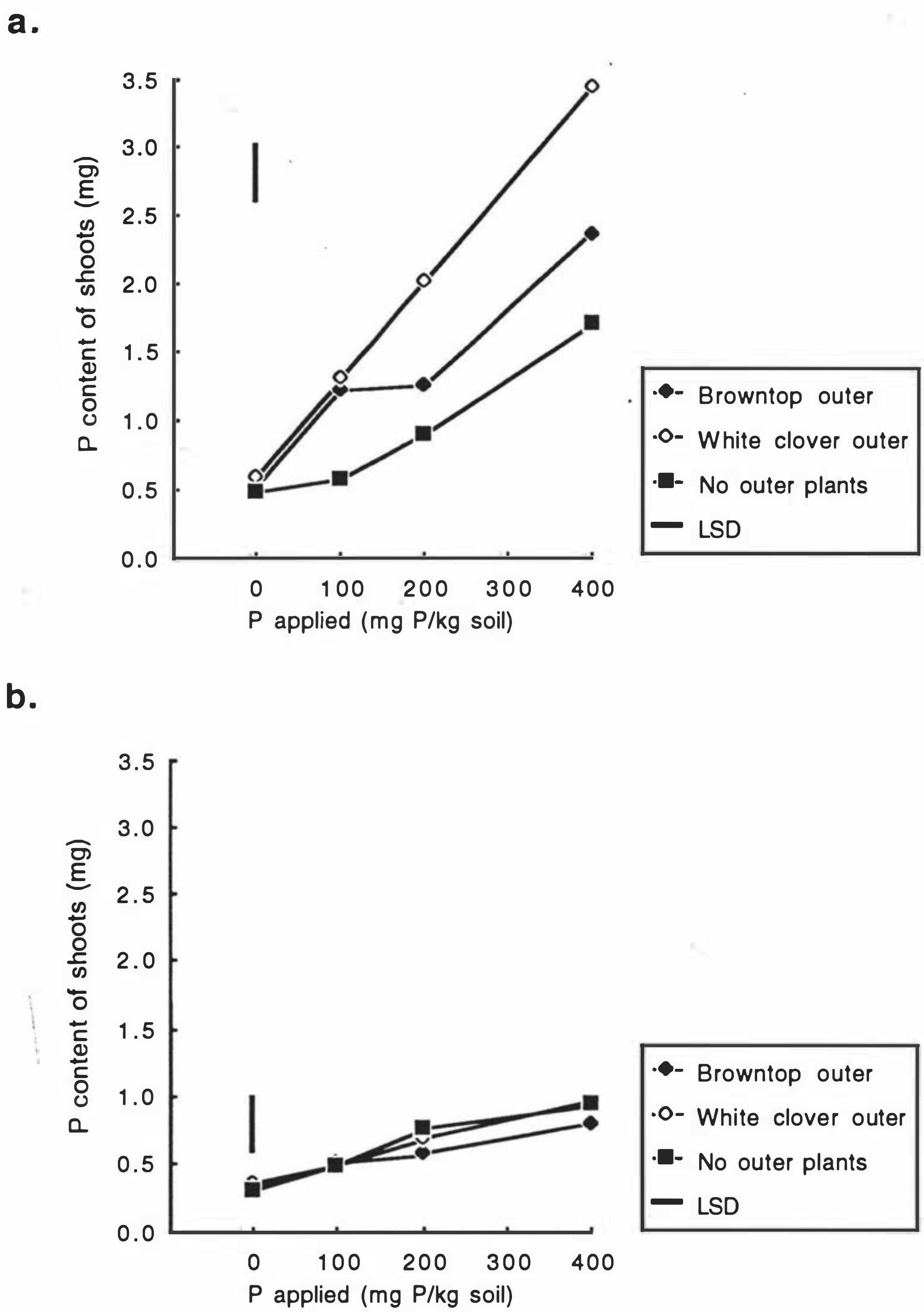


Figure 5.5 Effect of root associations with outer plants on the response of P content in shoots of the central indicator (a) browntop, and (b) white clover plant to P application as monocalcium phosphate. The LSD of 0.4 mg allows comparisons to be made between means for browntop and white clover.

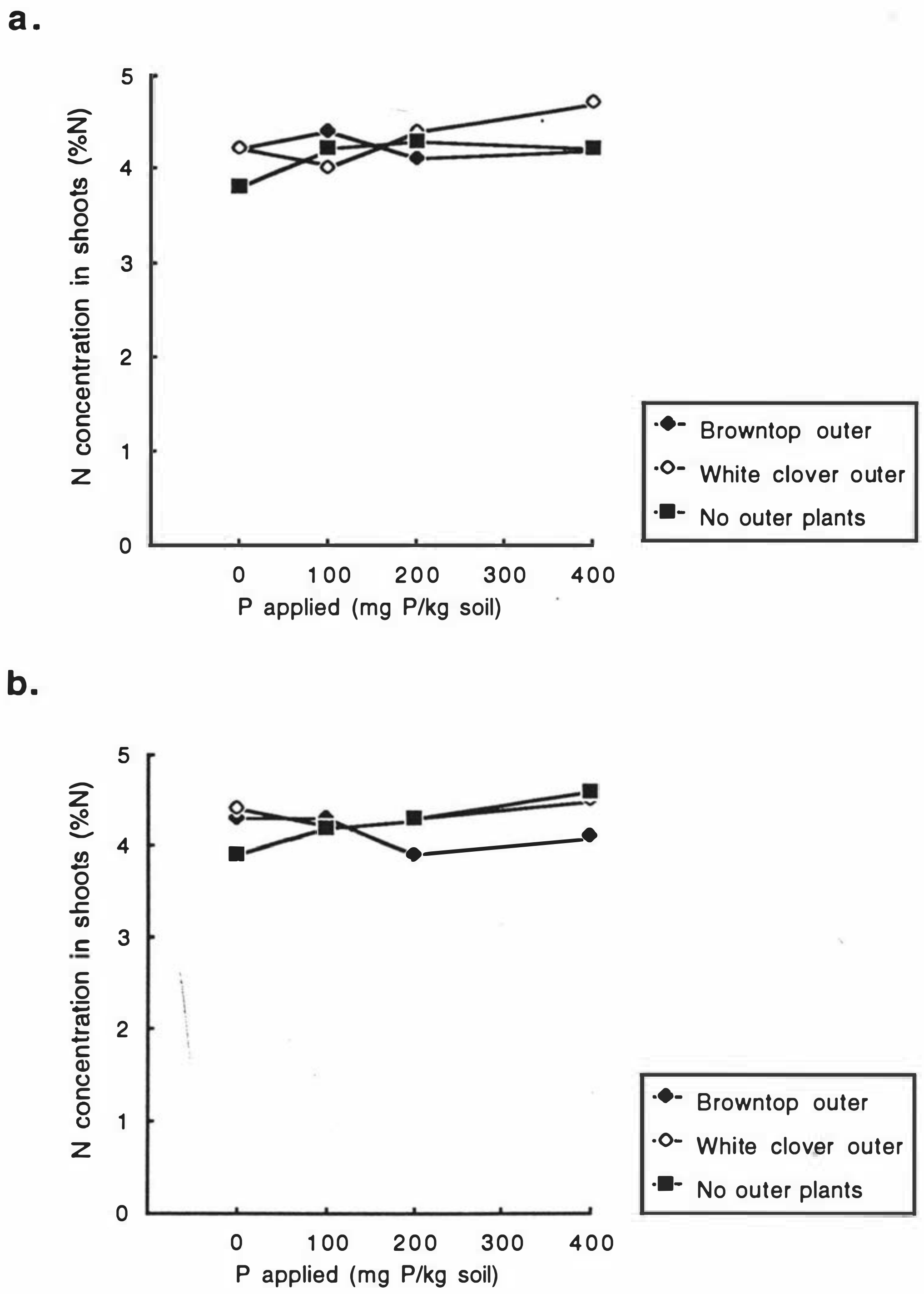


Figure 5.6 Effect of root associations with outer plants on the response of N concentration in shoots of the central indicator (a) browntop, and (b) white clover plant to P application as monocalcium phosphate.

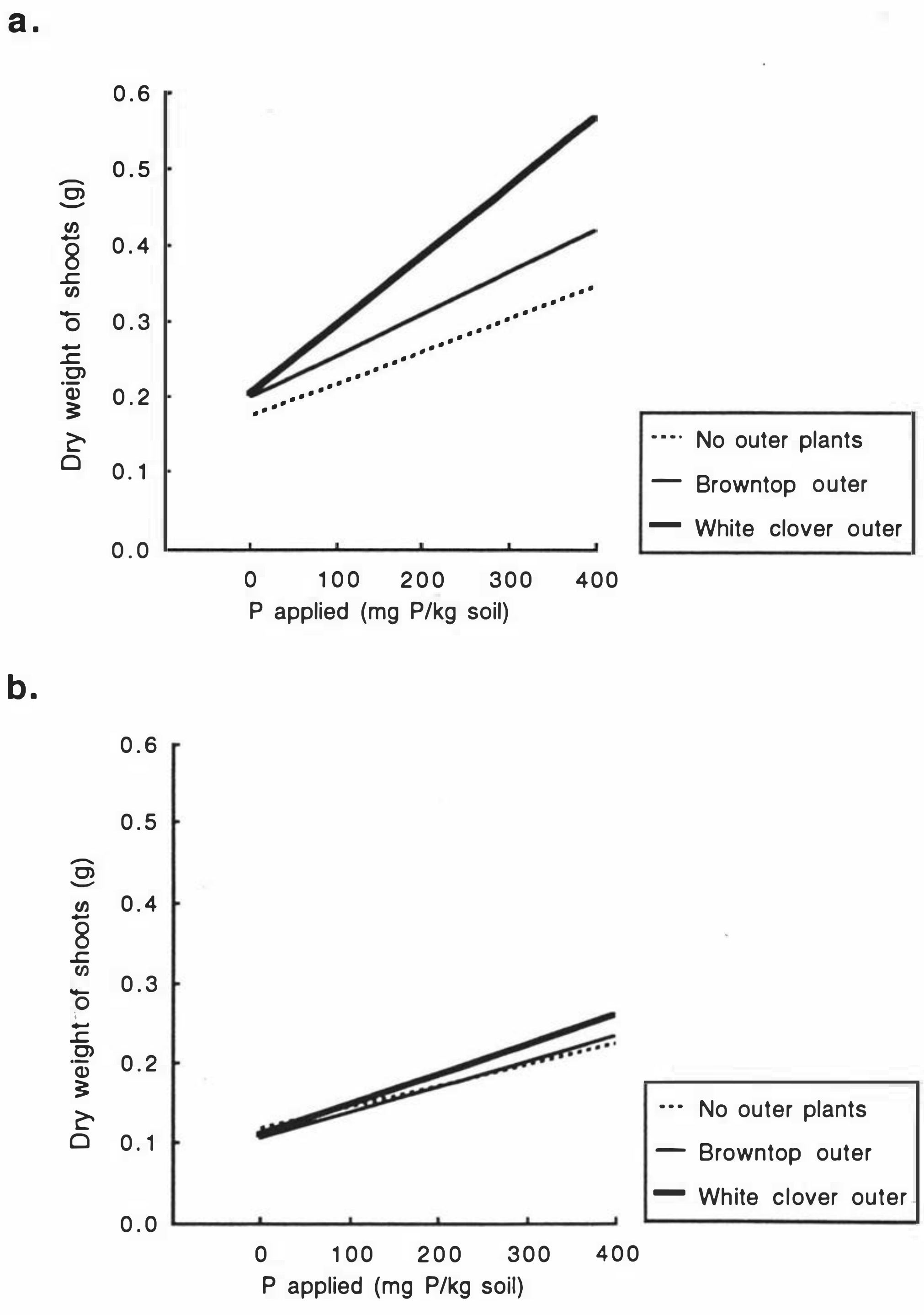


Figure 5.7 Lines of best fit to describe responses of the shoot dry weight of the central indicator (a) browntop, and (b) white clover plant to P applied as monocalcium phosphate to Ramiha topsoil. Details of slopes of lines and comparisons of slopes are given in Tables 5.1 and 5.2, respectively.

Table 5.2 Coefficients for equations of lines of best fit for responses of dry weight of shoots of central indicator browntop and white clover plants, grown on Ramiha topsoil, to P applied as monocalcium phosphate.

Central indicator species	**Outer species**	**Slope of line (*m*)**	**Constant (*b*)**	**Correlation coefficient (r^2)**
Browntop	none	0.00043	0.18	0.60 **
Browntop	browntop	0.00055	0.20	0.40 *
Browntop	white clover	0.00090	0.20	0.59 **
White Clover	none	0.00027	0.12	0.62 **
White Clover	white clover	0.00038	0.11	0.62 **
White Clover	browntop	0.00033	0.11	0.67 **

Level of significance
** $p \leq 0.001$ * $p \leq 0.005$

Table 5.3 t-tests to compare whether or not slopes (*m*) of lines of best fit are the same for responses of dry weights of shoots of central indicator plants, grown in the three root association treatments, to P applied as monocalcium phosphate to Ramiha topsoil.

Treatments compared		**Calculated t**	**p**
White clover alone	Browntop alone	$t_{35} = 1.14$	ns
White clover alone	White clover with WC	$t_{35} = 1.96$	0.10
White clover alone	White clover with BT	$t_{35} = 1.22$	ns
Browntop alone	Browntop with BT	$t_{36} = 0.71$	ns
Browntop alone	Browntop with WC	$t_{36} = 2.70$	0.02
White clover with WC	Browntop with BT	$t_{36} = 1.67$	ns
White clover with WC	Browntop with WC	$t_{36} = 4.49$	0.001
White clover with BT	Browntop with BT	$t_{36} = 2.20$	0.10
White clover with WC	White clover with BT	$t_{36} = 0.89$	ns
Browntop with BT	Browntop with WC	$t_{36} = 2.43$	0.05

Where BT = outer browntop plants, and
WC= outer white clover plants.

There were no effects of root associations with central indicator plant species on the shoot dry weight or P status of shoots of outer white clover plants (Figures 5.10, 5.11, and 5.12).

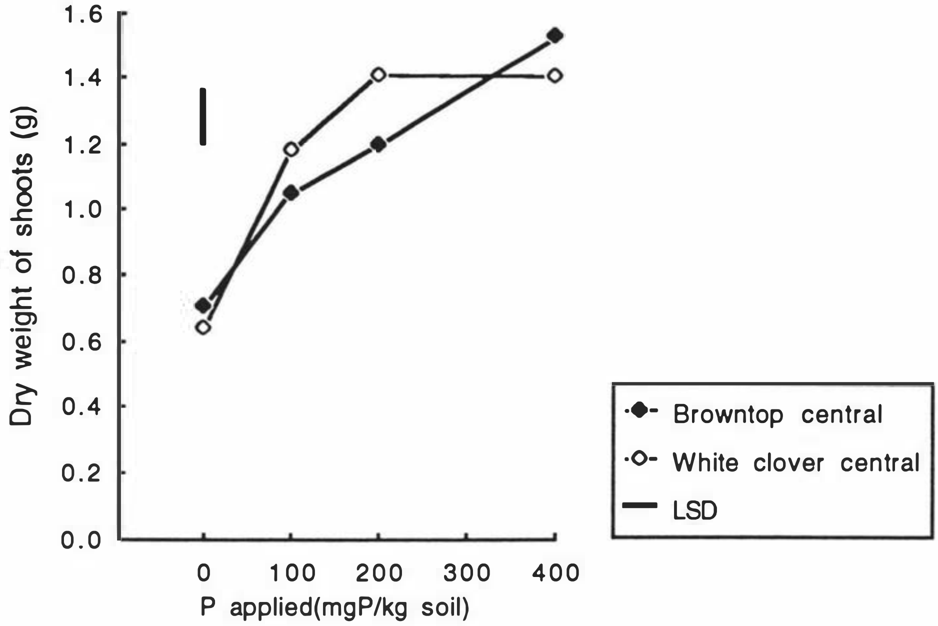
5.3.3 P responses of monocultures: effect of position in pot

There was no effect of position in pot (central or outer position), on the shoot dry weight per plant of either browntop or white clover plants (Figure 5.11). However, the P and N concentration in shoots of outer plants was consistently less than in the central indicator plant at all levels of P applied, for both browntop and white clover monocultures (Figures 5.12 and 5.13).

5.3.4 N concentrations in the soil

Nitrate levels in the soil increased following wetting. After 18 days, soil nitrate N levels had increased substantially from 0.6 mM at the start of the experiment, to 1.5-2.0 mM (Table 5.4), and were unlikely to decrease significantly due to plant uptake before harvest (69 days from planting) (pers. comm. R. Bramley). The concentration of soil nitrate was high enough not to hinder plant growth (Russell 1973; Mengel and Kirkby 1982) and therefore, would not have affected the plants response to applied P. There were nodules on the roots of white clover plants. It is also unlikely that plants would have competed for soil N.

a.



b.

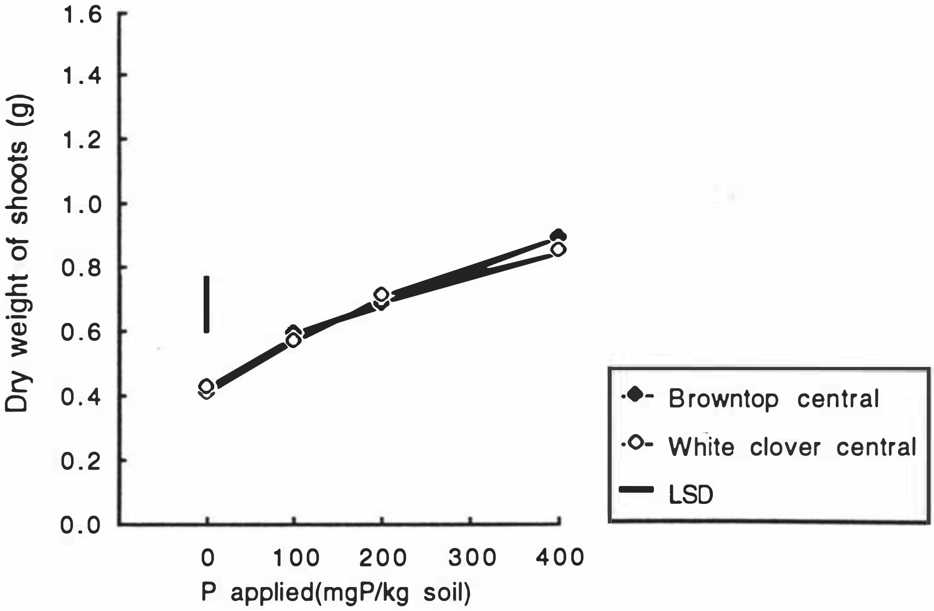
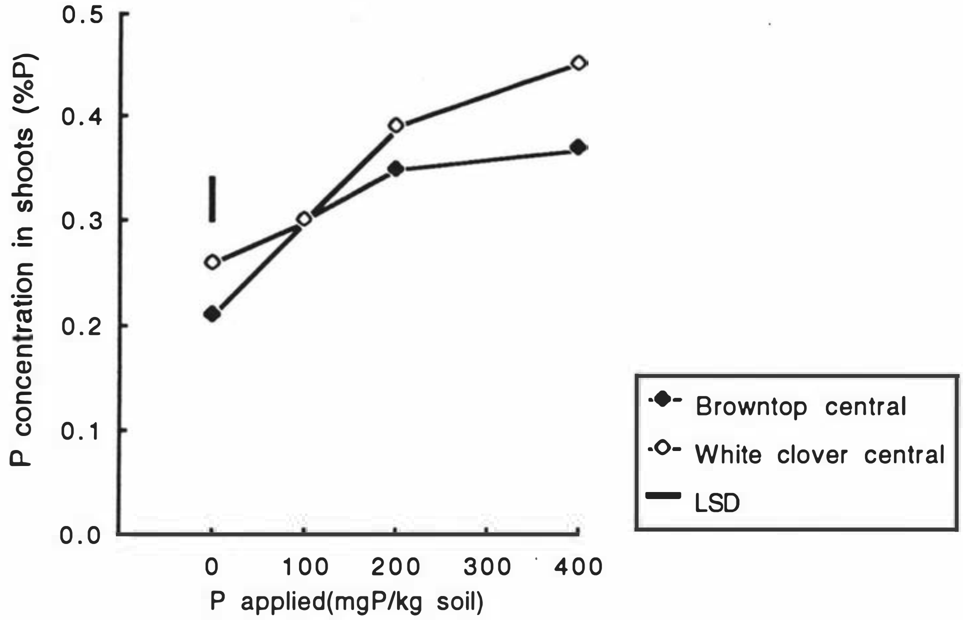


Figure 5.8 Effect of root associations with the central indicator plant, either browntop or white clover, on the response of shoot dry weights of the four outer plants of (a) browntop, and (b) white clover to P application as monocalcium phosphate. The LSD of 0.16 g allows comparisons to be made between the means for browntop outer and white clover outer plants.

a.



b.

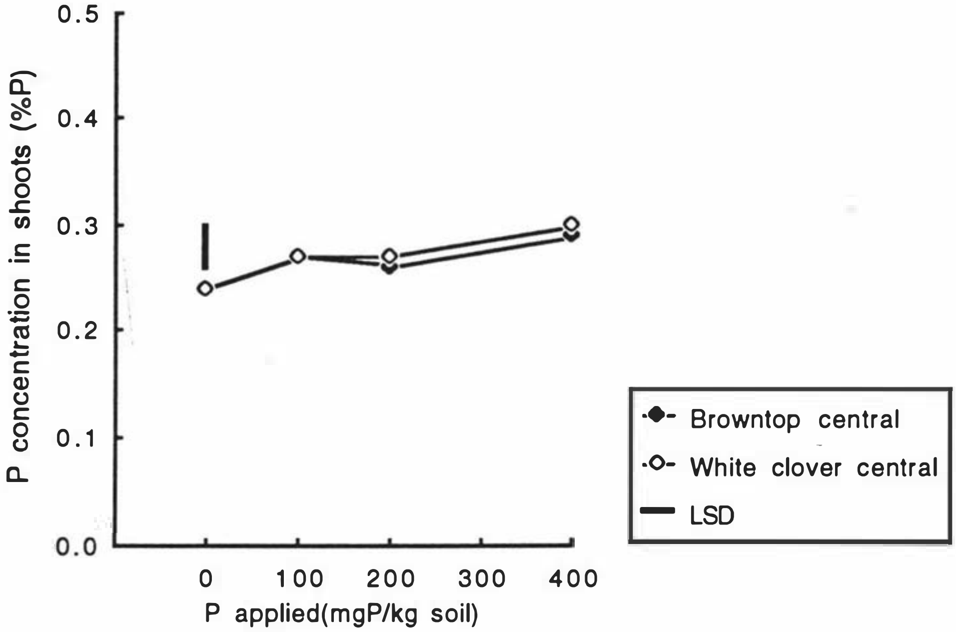


Figure 5.9 Effect of root associations with the central plant, either browntop or white clover, on the response of the P concentration in shoots of the four outer plants of (a) browntop, and (b) white clover to P application as monocalcium phosphate. The LSD of 0.04 % allows comparisons to be made between the means for browntop outer and white clover outer plants.

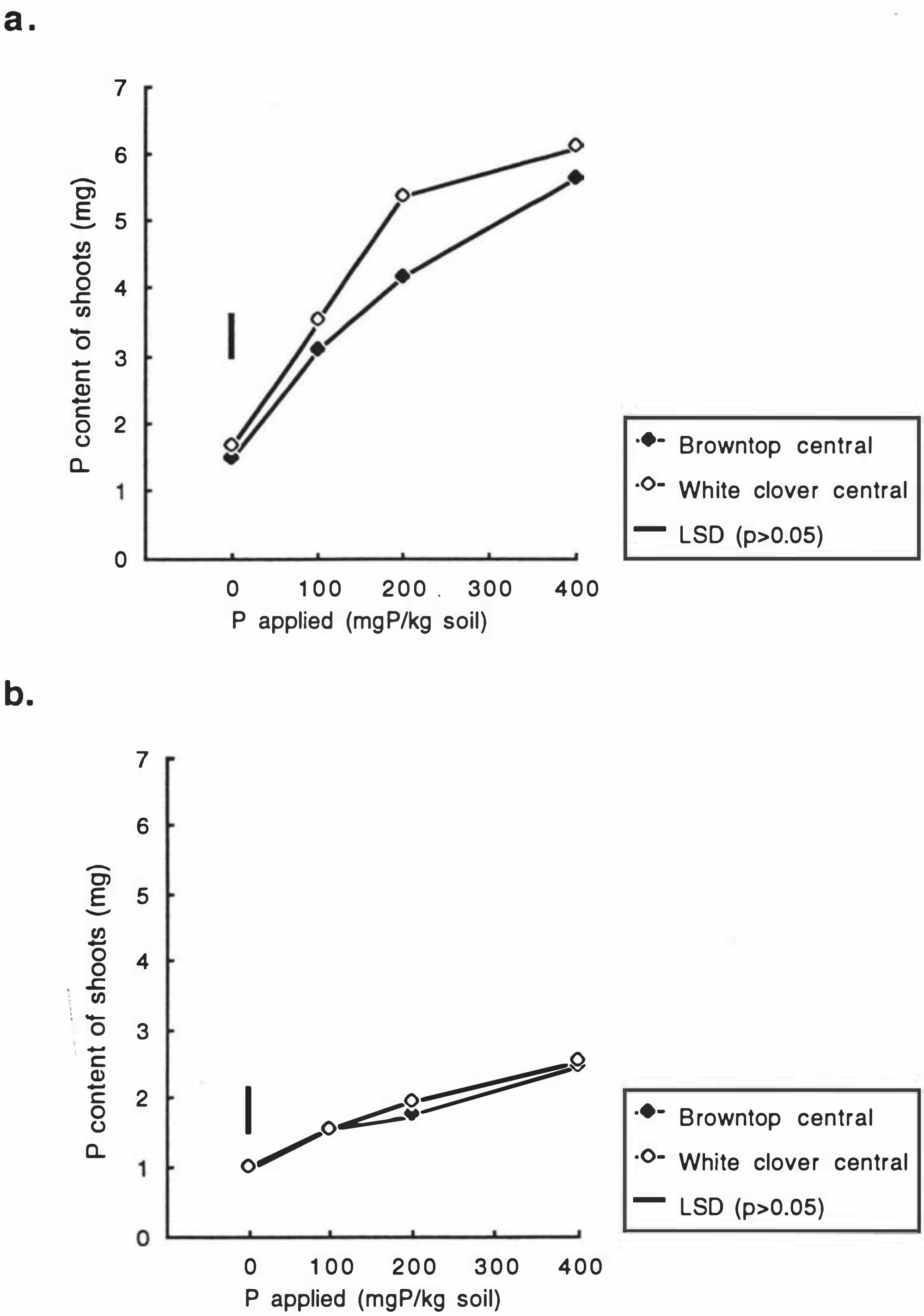


Figure 5.10 Effect of root associations with the central indicator plant, either browntop or white clover, on the response of the P content of shoots of the four outer plants of (a) browntop, and (b) white clover to P application as monocalcium phosphate. The LSD of 0.62 mg allows comparisons to be made between the means for browntop outer and white clover outer plants.

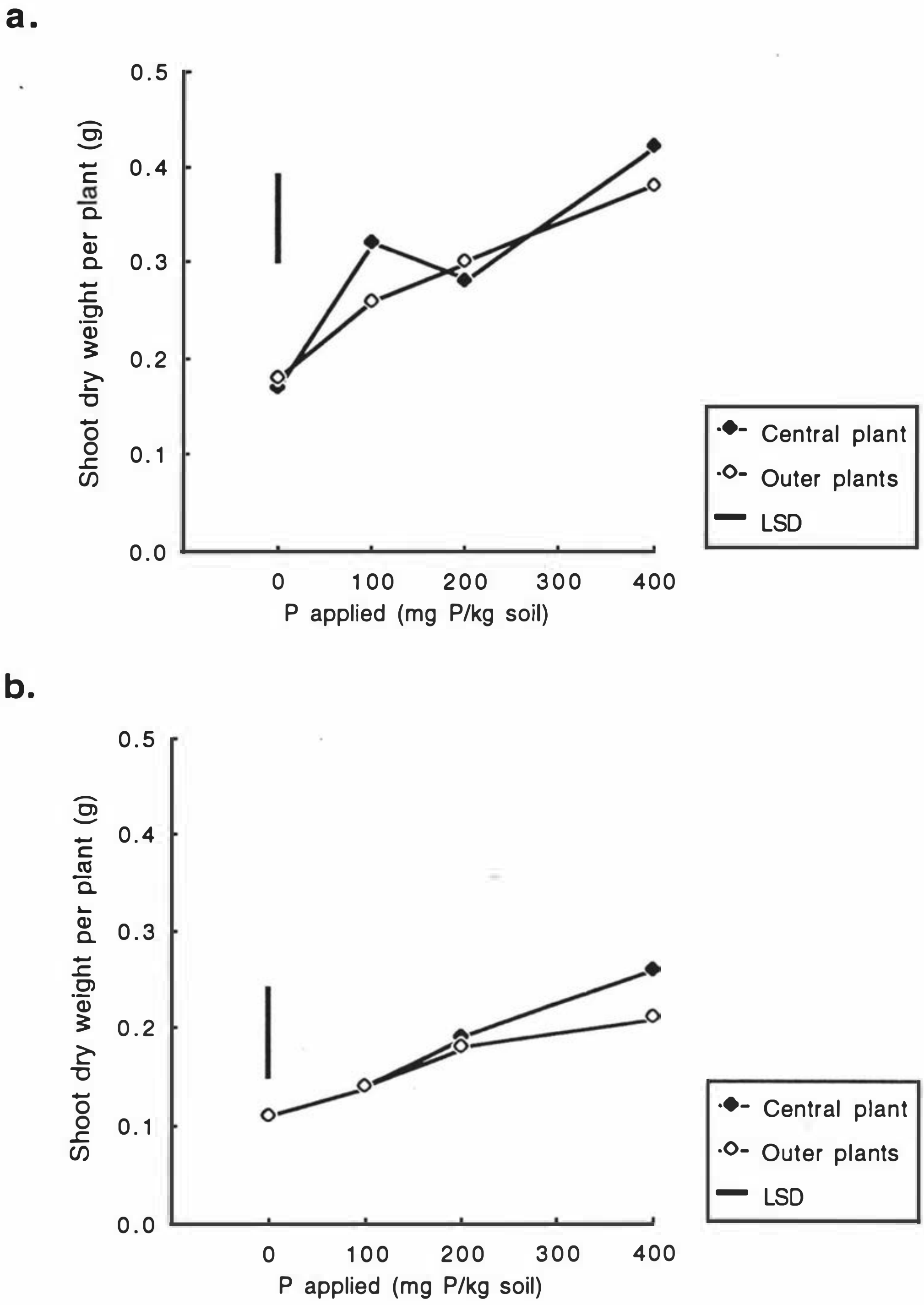


Figure 5.11 Effect of plant position on the response of shoot dry weight per plant of (a) browntop, and (b) white clover plants grown in monocultures, to P application as monocalcium phosphate. The LSD of 0.09 g allows comparisons to be made between means for browntop and white clover.

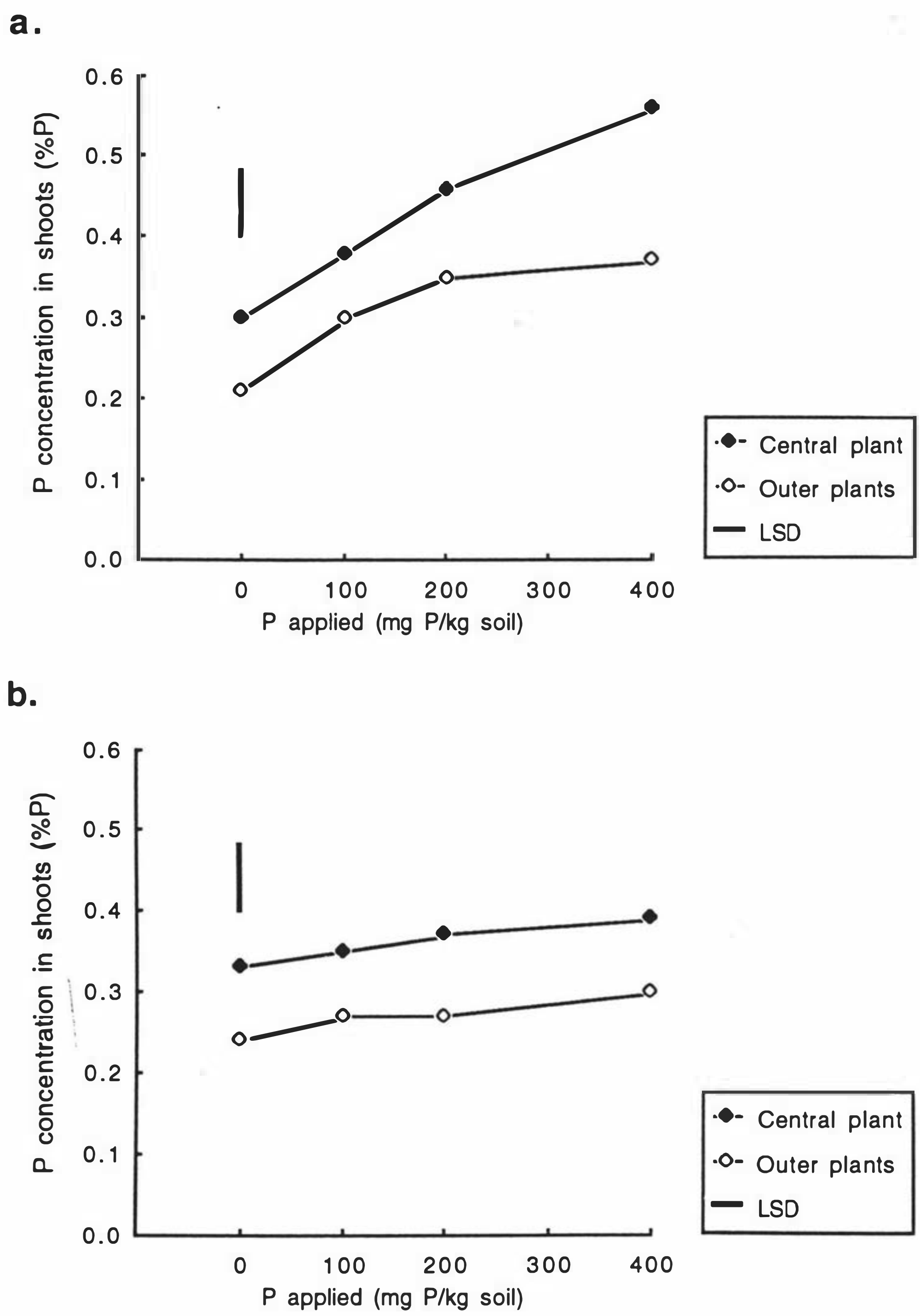
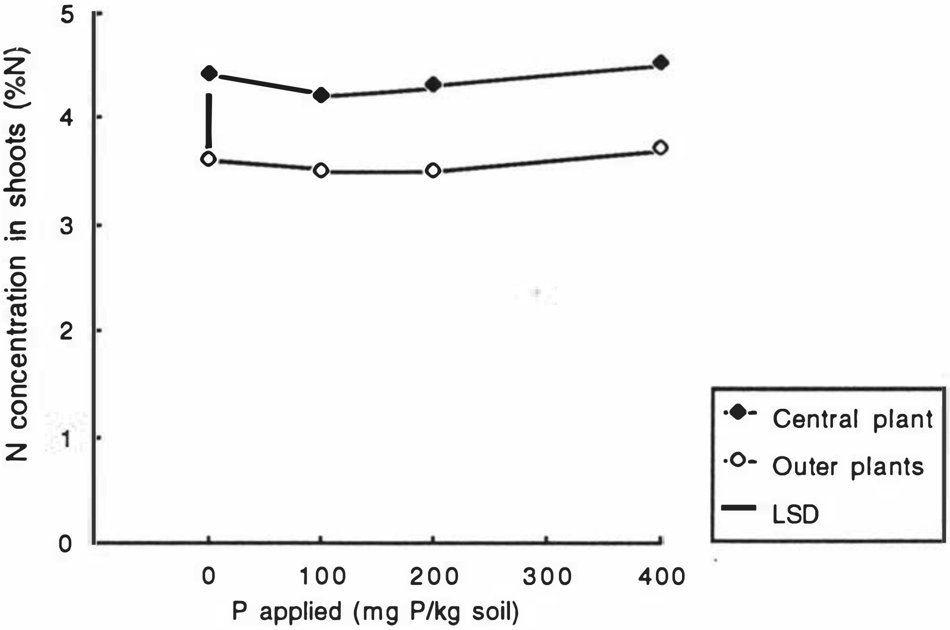


Figure 5.12 Effect of plant position on the response of P concentration in shoots of (a) browntop, and (b) white clover plants grown in monocultures, to P application as monocalcium phosphate. The LSD of 0.08 % allows comparisons to be made between means for browntop and white clover.

a.



b.

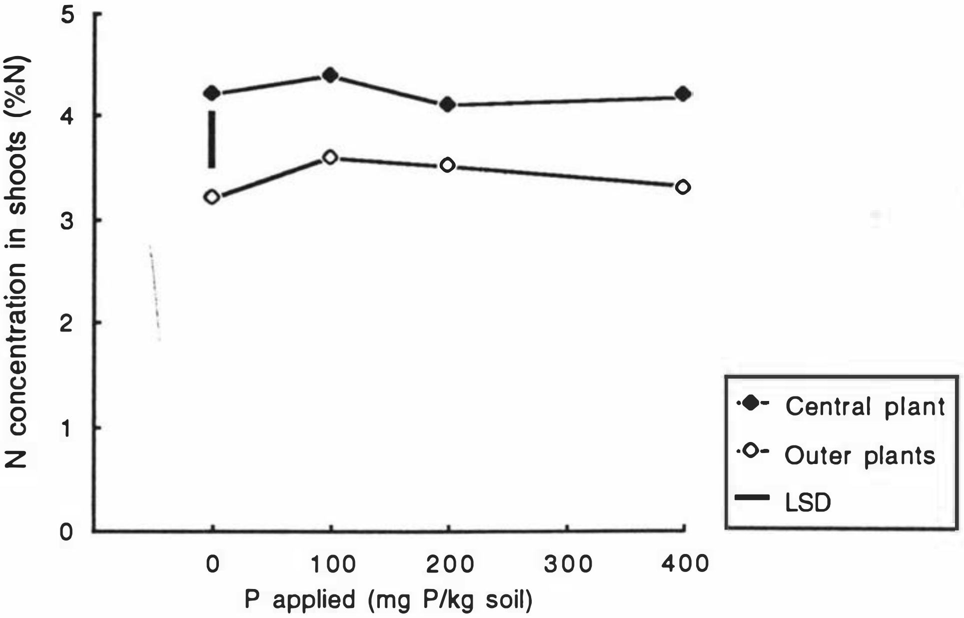


Figure 5.13 Effect of plant position on the response of N concentration in shoots of (a) browntop, and (b) white clover plants grown in monocultures, to P application as monocalcium phosphate. The LSD of 0.5 % allows comparisons to be made between means for browntop and white clover.

Table 5.4 Nitrate levels measured in Ramiha topsoil, either following incubation of wet soil for 0 and 3 days, or sampled after 18 days from pots with browntop or white clover plants growing in the soil.

Days incubated	**NO_3^- (μg N/g soil)**	**NO_3^- (mM N)**
0 days	20.2 (0.3)	0.6
3 days	26.4 (0.7)	0.7
18 days **White clover monoculture (mg P/kg soil)**		
0	52.7 (0.7)	1.4
400	53.3 (0.9)	1.4
18 days **Browntop Monoculture (mg P/kg soil)**		
0	76.0 (2.1)	2.1
100	83.7 (3.5)	2.3
200	52.0 (2.3)	1.4
400	56.7 (3.4)	1.5

5.4 Discussion

5.4.1 Interference between browntop and white clover

The soil P acquisition (Figures 5.4a and 5.5a) and shoot growth of browntop plants (Figure 5.3a) benefitted when grown with roots associated with outer white clover plants, compared with when grown with outer browntop plants, or alone, at luxury levels of soil P supply. Browntops' better performance in the presence of roots of white clover compared with in the presence of other browntop roots was not due to an N sparing effect, as there was no effect of the root association treatments on the N concentration measured in shoots of browntop (Figure 5.6). The high levels of soil N in the Ramiha topsoil used in the pot trial would have given browntop an obvious competitive advantage over white clover. The high growth of browntop at a high N supply would have given browntop a high demand for P, and therefore a high level of P uptake. Similarly, Donald (1963) discussed the effect of N on grass growth in relation to increasing competition for soil K.

Browntop was more responsive to P application than white clover, as indicated by the slopes of the response lines and the greater shoot dry weights measured. Although browntop is a low fertility tolerant grass, with a low growth rate at deficient levels of soil P and N supply, this grass has demonstrated the ability to produce a greater shoot dry weight than white clover when the soil N supply was not limiting and large quantities of P were applied. However, in the field situation, particularly in hill country pastures of New Zealand, it is unlikely that soil P and N levels would ever be found at the luxury levels used in this trial.

The results for the central indicator browntop plant suggest that white clover was not as competitive as browntop for soil P at luxury levels of soil P supply. However, this was not apparent in the findings for central indicator white clover plants. White clover did not appear to suffer from growing with outer browntop plants compared with when grown with outer white clover plants (Figures 5.3b and 5.7b), despite the greater P uptake (content) of browntop compared with white clover (Figure 5.5).

The results of the P isotope dual-labelling trials suggested that it is possible that browntop was competing with white clover for soil P, and that autotoxic effects of white clover upon its own root growth may have masked the effect of competition for P from browntop when comparing the two species association treatments: central white clover with outer browntop; and central white clover with outer white clover. That is, autotoxic effects of white clover may have been of a similar severity to competitive effects of browntop for soil P. However, there was no evidence of autotoxic effects of white clover upon its own root growth in the present competitive settings trial (Table 5.1). However, browntop responded to being with other browntop plants by increasing root fresh weight compared with when white clover was the outer species at 100 and 400 mg P applied/kg soil (Table 5.1), possibly due to toxicity in rhizosphere of white clover plants.

5.4.2 The competitive settings design

Idealistically, the competitive settings experiment could have been interpreted as a replacement series, by ignoring the data for the single central plant with no associations with roots of other plants, using the de Wit model (Hall 1974). However, the P concentration in shoots of central and outer plants was not similar (Figure 5.12), therefore confounding the results. Presumably the outer plants suffered from intraspecific competition while the central plant was still enclosed in its small central pot before transplanting (Figure 5.2).

Another problem with interpreting the results of the trial was the poorer growth and P uptake of the central indicator browntop plants grown alone in the small pot compared with the plants grown in the outer section of the pots (Figures 5.3a and 5.4a). The reason for the poorer performance of the single browntop plant is not clear, and has been discussed below. Therefore, interpretation of the comparison of the single central plant with a central plant grown with species root associations difficult.

There are two possible mechanisms that may explain the better P acquisition by central indicator browntop plants grown in association with roots of outer plants, compared with when grown alone in the small central pot. Firstly, there may have been an effect of pot size, or secondly, the effect may have been due to poorer growth of outer plants compared with with central indicator plants. The poorer growth of outer plants may have been due to an effect of the aerial partition or due to shading between outer plants. Alternatively, there may have been a difference in root distribution through the soil across the pot.

Although the total amount of soil resource per plant was held constant, there may have been a restriction in the exploration of the soil by roots in the smaller pots, particularly as the roots tended to be more concentrated at the bottom of the small pots in comparison with the root distribution in the larger pots.

Rennie (1974) suggested that the intermingling of roots with adjacent plants may have resulted in a more efficient use of soil resources. Rennie did not offer any suggestions as to how this may occur, but in this case, it can be speculated that exploration of previously unexplored soil could be better in the larger pot than in the small central pot. Examination of the findings for monocultures grown in the larger pots, comparing central indicator plants with outer plants, provide evidence that central indicator plants may perform better in root associations than alone due to the poorer acquisition of soil P by outer plants than by central indicator plants. Although the soil weight allocation per plant was held constant, the lower P acquisition by outer plants compared with central indicator plants, effectively means that the central indicator plants grown in root associations with outer plants may have access to more soil resources than the central indicator plant grown alone.

Like previous designs used by other researchers involving different competitive settings (i.e., species associations in the present experiment) to compare inter- and intraspecific interferences between plants, the present design was difficult to interpret, in view of the differences in performance between outer and central indicator plants. The legitimacy of comparing the central indicator plant with the no root association treatment with the central indicator plant grown with outer species root associations is questionable.

The effects due to changes in environmental conditions brought about by the different competitive settings could not be separated from the effects due to the different species root associations. The effect of intraspecific root association, determined from the comparison between the single central indicator plant grown alone and the central indicator plant grown in monoculture was confounded by changes in environmental conditions. The change in environmental conditions caused by the change in competitive setting was the due to the effect of the presence of outer plants on the resource allocation per plant of the central indicator plant or due to effect of pot size. The level of interspecific root interference was also confounded in the same manner, and the comparison between inter- and intraspecific root interferences was similar to a replacement series, and would be density dependent (Snaydon 1991). Keeping this in mind, a conclusion can be drawn that when grown at a density of 5 plants per kg of soil, at luxury levels of soil P supply, young browntop plants (69 days old), grew better and acquired more soil phosphorus in root associations with white clover than in root associations with browntop plants. The dual labelling trial also found that browntop plants acquired more soil P when grown with roots associated with outer white clover plants than when grown with roots associated with outer browntop plants (Figures 3.2 and 4.2a).

Clearly, the acquisition of soil P by browntop was greater in the presence of white clover roots than in the presence of roots of other browntop plants.

White clover did not seem to be greatly affected by the root association treatments. White clover was not affected more from growing in the presence of roots of outer browntop plants than with roots of other white clover plants. This suggested that browntop did not benefit from competition with white clover roots as white clover did not appear to suffer from competition for soil P from roots of browntop. However, if white clover was autotoxic then this would have disguised the competitive effects of browntop in this competitive settings trial. A more feasible explanation may be that browntop roots were competing with other browntop roots, and this competition was lessened when the browntop plants were growing in association with white clover roots.

Had the response of outer plants to P application been the same as for central indicator plants, the results of this trial would have been more conclusive in determining the nature of the inter- and intraspecific interference occurring between roots of browntop and white clover plants, and allowed interpretation as a replacement series. To improve the design of this experiment, the reason for the better P and N acquisition by central indicator plants compared with outer plants needs to be determined. The most obvious reason is that there was an effect of the plant position, particularly in relation to the aerial partition. Aerial partitions are known to influence the growth rate and growing environment of plants by altering the radiation climate and airflow (Warren and Lill 1975). The plants in the centre of the aerial partition would possibly have been subjected to a different radiation climate and air flow from the plants in the outer section of the pot. The plants in the outer section of the pot would also be shading each other. Air movement and shading was most likely to be greater outside the aerial partition which would have affected plant growth and nutrient uptake (Donald 1958).

5.4.3 Summarising comments

The findings of this trial generally support those of the dual labelling trial. At adequate to luxury soil P supply browntop was found to acquire more soil P in the presence of roots of white clover plants than in the presence of roots of other browntop plants. There was no corollary evidence in the competitive settings trial to support that this was due to competition for soil P because white clover did not suffer from the presence of browntop roots, and in fact performed as well in the presence of browntop roots as in the presence of roots of other white clover plants or when grown alone. However, intraspecific competition for soil P between roots of browntop plants was most likely occurring, considering the absence of other evidence to suggest otherwise, and this competition was alleviated by the substitution of outer browntop plants for white clover plants. Autotoxicity of white clover may have been the factor preventing the competitive effects of browntop on P acquisition by white clover from being elucidated.

It would be expected that if intraspecific root competition between browntop plants was occurring, then the single plants grown alone would be expected to perform better than when grown with other browntop plants. However, the single central browntop plants grown with no root associations with outer plants grew poorer than central browntop plants grown with roots associated with outer plants. If the poorer growth of single central browntop plants was due to less soil resource available per plant or pot size, then the comparison with central plants grown with outer plants is not valid. In order to assess the experimental design a single plant grown in a large pot was needed, but unfortunately, this was not done.

Although the soil P supply was high, the high total P uptake by browntop suggests that there was a large demand for soil P by browntop roots. With the lower demand for soil P by white clover plants and high soil P supply, they may have avoided competition. No evidence of white clover roots benefitting from the presence of browntop roots for soil P acquisition was found. However, the plants in the present trial were younger and the plant density was lower than in the dual labelling trial. Also, the measurements made were the cumulative effects of the whole life of the plants, which may have masked any positive interferences that were found to occur between roots of white clover and browntop in a mature sward in the dual labelling trial.

In the following, and final chapter, the results of the preceding chapters are summarised and compared to what is already known about root interference, particularly root competition for P between browntop and white clover. Suggestions have been made for areas of research where further work is required in order to fully understand the nature of root interference between plants in browntop-dominated hill country pastures.

CHAPTER SIX

SUMMARY AND CONCLUSIONS

6.1 Overview

A review of literature (Chapter One) indicated the need to investigate the mechanisms of root interference occurring between roots of browntop and white clover. Evidence was presented to show that the claim that browntop competes strongly with white clover for soil P (Mouat and Walker 1959a; Jackman and Mouat 1970, 1972a) has not been conclusive. Researchers have found that application of P increases white clover production when grown in association with browntop (Section 1.6.3; Mouat and Walker 1959a; Jackman and Mouat 1970, 1972a)), however this does not prove that competition between roots for P was occurring.

The effect of defoliation on root interference between browntop and white clover was shown to be an important interacting factor worthy of investigation in relation to the effect of browntop on white clover, because grazing management is known to be an important determinant in controlling species composition in hill country pastures.

Experiments were designed to examine the nature of root interferences between browntop and white clover by employing three different techniques: plant strategies; dual P isotope labelling (Caldwell *et al.* 1985, 1987); and a competitive settings trial employing aerial partitions. Plant growth and root interferences were studied at a range of levels of soil P supply. The techniques used were assessed and compared for their usefulness and ability to elucidate plant root interferences.

The main findings have been summarised below in terms of plant growth strategies, and competitive and non-competitive interferences, and other factors that may contribute to the success or failure of browntop and white clover in hill country pastures have been considered in terms of their relative importance to root interferences.

The growth of browntop and white clover and the nature of root interferences occurring within and between the two species was dependent on the level of soil P supply. However, the higher root density of, and therefore greater soil exploration and exploitation by browntop compared with white clover appeared to be the most important factor determining the success of browntop at all levels of soil P supply regardless of whether or not roots of browntop were competing directly with roots of white clover. The greater root density of white clover plants grown with roots adjacent to roots of browntop compared to when grown with roots adjacent to other white clover plants (Figures 3.7b and 3.11b) was associated with greater P isotope acquisition (Figure 3.3). For immobile ions, such as phosphate, increasing local root density is effective in increasing uptake (Fitter 1976; Bolan 1991).

6.2 Plant strategy

From the P response trial and field observations, the conclusion was made that browntop is better adapted than white clover to survive under conditions of low P supply. White clover required a higher P application to attain the same relative yield as browntop (Chapter Two). The lower growth rate of browntop at low levels of P supply compared with white clover may be one of the most important factors contributing to the dominance of browntop in hill country pastures. Browntop's lower growth rate when growing under conditions of low nutrient supply gives it the characteristics of a stress tolerant plant (Grime 1979).

The possibility that browntop may be capable of locking up large quantities of P, depending on the rate of recycling (decomposition) was discussed. Browntop had a greater P content than white clover which is important when the rate of P recycling in the field is considered (Chapter 3, Hobbie 1992). The importance of a slow rate of recycling of P in browntop dominated pastures may be significant considering that roots of browntop were finer than white clover because fine roots are known to have a low rate of turn over (Boot 1989).

According to Kemp (1989), there is evidence that differences in the productivity and persistence of white clover selections grown on low P soils are related to root morphology rather than the commonly used micro definitions of P efficiency (Williams and Caradus 1979; Simpson *et al.* 1987). Bolan *et al.* (1987) found that the greater uptake of P by ryegrass over white clover was only due to a larger root system and not due to efficiency of P absorption, which supports Kemp's (1989) view.

The main finding of the dual P isotope labelling trial was that root density was a very important factor in determining the performance of plants in mixtures. Root size expressed in terms of root length is an important factor contributing to root competitive ability (Boot 1989). Large total root length, either due to a large root dry matter, or a high SRL (specific root length) and a moderate root/shoot ratio favours uptake capacity and competitive ability (Boot 1989). The root morphology of white clover contributes to its poor competitive ability. Although small leaved types of white clover were selected for this study (small leaved types of white clover have more fibrous root systems than large leaved types of white clover (Caradus 1981a)), the SRL of browntop was greater than that of white clover. Also the SRL of browntop decreased with P application, therefore the SRL of browntop was more plastic than that of white clover (Chapter Three). Browntop had a more efficient use of biomass allocation for soil exploration. SRL is particularly important for access to low mobility nutrients like P.

Browntop is able to increase root growth relative to shoot growth more rapidly in response to a developing nutrient deficiency than white clover. Mouat (1983a) found that under P deficiency the root/shoot ratio of browntop was increased more than for white clover when compared with at higher levels of soil P supply. Similarly, the findings of this thesis were that browntop had a higher root/shoot ratio than white clover at deficient levels of soil P supply (Table 2.3). Increasing root/shoot ratio is a common adaptation to nutrient stress. However, absolute size of the root system is more important for phosphate uptake than root/shoot ratio. Generally, browntop acquired more phosphate (Figure 3.15b and Table 4.1) and phosphate isotope (Figures 3.2, 3.3, and 4.2) than white clover due to its greater root density and root weight (Figures 3.6 and 3.7, and Table 4.2).

Browntop was more responsive to P than white clover, even though browntop is known to perform better at P deficiency due to a low growth rate. At adequate to luxury levels of soil P supply there was a large demand for soil P by browntop roots. Browntop had the ability to acquire P to tissue concentrations greater than was required to achieve maximum growth (luxury consumption). The ability of browntop to grow fast and have a large demand for P at adequate levels of soil P supply gives browntop a high competitiveness (Grime 1979). In particular, a high rate of root growth would be important in terms of the rate at which unexplored and unexploited soil is entered, i.e., soil volumes of a relatively higher P concentration (Clarkson 1985). However, it should be taken into account that the lower demand for soil P by white clover and the high P supply may have enabled white clover to avoid competition from browntop.

6.2.1 Evidence of root competition between browntop and white clover

According to the plant strategy theory of Grime (1979) white clover is a competitor. Competitors grow well under conditions of low stress and disturbance. White clover had a high dry matter production and was responsive to P at low N supply (Figure 2.1). White clover is not a stress tolerator. It does not have a low growth rate when grown under conditions of high nutrient stress (low nutrient supply) (Figure 2.1).

At high P and N supply, but not at low P and N supply, browntop is a competitor. Under these conditions, browntop had a greater dry matter production than white clover (Table 4.1). The competitiveness of browntop was alleviated by defoliation at high P supply (Figure 4.3, at 68 mg P applied/kg soil). Browntop's P acquisition was opportunistic. Luxury consumption of P led to extremely high P concentrations in shoots (Figure 5.4). Using Grime's theory, the greater competitiveness of browntop was reflected by browntop's ability to acquire more total radioactive P from the soil than white clover (Figure 4.2), and was due to its greater root length and weight (Table 4.2).

On unamended subsoil, browntop reduced P acquisition by white clover, and had a greater P uptake in the presence of white clover than with other browntop plants (Figure 3.3), providing evidence of competition from browntop. According to the depletion zone calculations, the root densities were too low to prove that P depletion zones overlapped (Section 3.4.1), and therefore did not support the argument that direct competition between roots for P was occurring. The findings of this thesis suggest that there may be shortcomings with using the overlapping depletion zone theory to assess competition between roots. The results suggest that browntop may compete with white clover by decreasing the availability of P in the soil, explained by browntop's ability to acquire more total radioactive P from the soil than white clover. This was supported by the finding that application of P removed deleterious effect of browntop on white clover (Figure 3.3). The competitive effects of browntop were alleviated at low levels of P application. Defoliation of browntop did not alleviate its competitive effect, probably because browntop had already decreased the P content in the soil, or because the defoliation effects upon browntop were not severe.

Evidence of direct root competition between browntop and white clover was found at adequate levels of soil P supply. Defoliation decreased the root competitiveness of browntop although browntop acquired more P isotope than white clover. However, the net effect of outer browntop plants on P acquisition of central white clover plants was less harmful than that of outer white clover. Some non-competitive interference, possibly autotoxicity, was masking the competitive effects of browntop on white clover's P acquisition (discussed below in Section 6.2.3).

The effect of defoliation reducing the root competitive ability of browntop was rapid (four days), and this reduction was not evident in the first dual labelling trial, which was harvested seven and fourteen days following defoliation of outer species. Possibly the reduction of root competitiveness by defoliation may be short lived. This may be through rapid root growth, or physiological increases in the rate of P uptake. Browntop was able to acquire P isotope to higher concentrations in shoots than when undefoliated (Figure 3.4).

In the competitive settings trial (Chapter Five) browntop benefitted in terms of soil P acquisition and shoot growth by growing with white clover, compared with when grown alone and with browntop at luxury P supply (Figures 5.3a, 5.4a, and 5.5a). This was not due to an N sparing effect (Figure 5.6). Browntop appeared to compete with white clover, and therefore benefitted from growing with roots associated with white clover, however competitive effects of browntop on white clover were not apparent at adequate to luxury P supply. Some other factor, possibly autotoxic effects of white clover, may have masked the competitive effects of browntop.

6.2.2 Evidence of non-competitive root interference

At 100 mg P applied/kg subsoil browntop did not benefit from growing with white clover (Figure 3.2), however, surprisingly white clover did benefit from growing with browntop, in relation to with growing with other white clover plants (Figure 3.3). This was found to be due to greater local root density leading to increased uptake of phosphate (Fitter 1976; Bolan 1991). The increased root density of white clover was due to increases in root length and weight, but no effect on SRL.

No definite conclusion could be drawn as to why white clover roots had greater density when grown with browntop, compared with when grown with other white clover plants. The suggestion that rhizosphere acidity around roots of N fixing white clover may lead to increased toxicity of phenolics from decomposition of organic substances (Marschner 1986) was considered. The results suggest an autotoxic effect of white clover on its own growth. The possibility that browntop may be stimulative to white clover root growth should also be considered. However, nothing was proven in this area as no toxic compound was identified.

The harmful effects of competition for P from browntop on white clover would be in balance with autotoxic effects of white clover on its own growth if the competition and autotoxicity were occurring simultaneously.

Overall, both browntop and white clover appeared to gain mutual benefit for P acquisition from the presence of roots of the other species (apparent beneficence), except when grown at very deficient levels of nutrient supply (unamended subsoil). Therefore, competitive effects of browntop were not of overriding importance at intermediate, adequate, or luxury levels of soil P supply.

Apparent beneficence (where neither species is at a disadvantage from growing in association with each other) of browntop and white clover was not due to phosphatase activity on browntop roots, or from rhizosphere effects because it is unlikely that white clover would have had an increased P uptake from rhizosphere effects, nor would V.A. mycorrhizal effects be important at such high levels of soil P supply. The advantage for P acquisition gained by white clover from growing with browntop was simply due to increased root growth of white clover associated with browntop. Direct competition between roots of browntop and white clover was evident at adequate P, as demonstrated by the defoliation treatment, however, apparent beneficence had an overriding effect on the final outcome.

6.3 Other factors affecting interference between browntop and white clover

White clover is a ruderal species (Grime 1979) capable of tolerating high levels of disturbance, in particular, defoliation (Evans 1973b). Grown at adequate to luxury levels of soil P supply, neither undefoliated browntop nor undefoliated white clover benefitted from defoliation of adjacently growing white clover plants (Figure 4.3), supporting finding of Evans (1973b) that white clover is tolerant of defoliation. However, at lower P supply (100 mg P applied/kg soil, Chapter Three) defoliation of white clover led to an increased P isotope acquisition by nearby browntop plants (Figure 3.5). Therefore, the demand for P isotope by white clover was reduced by defoliation.

Browntop was not as tolerant of defoliation as white clover, as also found by Evans (1973b). White clover benefitted from defoliation of adjacently growing browntop at adequate P supply, 4 days from defoliation (Figure 4.3b), but there was no benefit at luxury P supply because the soil P supply was high enough to meet the demand by roots of white clover (Figure 4.3b). However, browntop does demonstrate some plant growth strategies of ruderal species. Although the effect of defoliation on browntop was short lived, it was associated with an increased physiological rate of P uptake (Figure 3.4). The response of browntop to defoliation was to increase the concentration of P isotope in stubble (Figure 3.4).

Also of importance is the mat forming behaviour of browntop. In the field, white clover would be physically impeded by browntop which tends to be more dense under poor nutritional conditions. Jackman and Mouat (1972a) found that in the field, the severity of competition for P between browntop and white clover decreased as there was an increase in browntop mat build up. The decrease in root competition would have been due to shading of white clover stolons and physical impedance of white clover leading to niche differentiation, and therefore a negative association between the two species. That is, the roots would not have been in close enough proximity to compete directly for P. Browntop has displayed the ability to affect P acquisition by white clover roots (Figure 3.3). However, in the field the importance of competition for soil P would not be as important as the ousting of white clover from areas of browntop dominance by the browntop mat, particularly in view of the low mobility of P in soil.

The effect of browntop on white clover, by reducing the soil P availability, would be more significant following the formation of bare patches of soil from treading and severe grazing, areas which may be colonised by white clover (Lambert *et al.* 1986). The low P supply and P recycling in soils previously dominated by browntop would be deleterious for white clover. In the long term browntop would have a major impact on soil P supply.

The exclusion of white clover from browntop mat in the field would reduce any possible impact of apparent beneficence due to autotoxicity of white clover, and therefore white clover may benefit from growing with other pasture species other than browntop. Autotoxicity of white clover may possibly explain, partly at least, the positive association between white clover and ryegrass in the field (Simpson *et al.* 1987).

Overall, evidence suggests that shading of white clover by browntop is more important than root competition for P, supported by the known dramatic effects of grazing on botanical composition of hill country pastures.

6.4 Usefulness of techniques to investigate the nature of root interferences

Problems of predicting interference in mixtures from their growth in monoculture (Harper 1977) were considered (Section 2.4.4). The monoculture P response trial (Chapter Two) was enlightening when the plant strategy theory of Grime (1979; Grime *et al.* 1988) was used to make comparisons between browntop and white clover.

The mechanisms of interferences occurring between roots of browntop and white clover were not elucidated from studying their growth in monocultures. However, the dual labelling trial was useful in elucidating some possible plant interferences that were not detected by the simple P response trial. Therefore, supporting the claim that the nature of intereference in mixtures cannot be predicted from their growth in monoculture (Harper 1977; Austin *et al.* 1985; Grace 1988).

The nature of interferences occurring between browntop and white clover was shown to be more complex than has been shown by field and competitive settings trials. Previous studies have not attempted to separate the effects of root interactions from shoot interactions, or to separate inter- and intraspecific interference. The present study found that by using several experimental techniques, a clearer picture of the interferences that occur between browntop and white clover was obtained.

The dual labelling method provided a "snapshot" picture of the effects of root interferences occurring between roots of browntop and white clover on P acquisition in an established sward at the time of isotope injection. The dual labelling method was useful for elucidating more than one type of plant interference, occurring in balance with each other simultaneously. However, it did not give any indication of the effect of the two species on the total P acquisition of each other over their whole life-span, which can be determined from the more traditional types of plant competition trials (additive/replacement series), or probably more accurately from field observations and trials.

The competitive settings trial generally supported the findings of the dual labelling trial despite some problems with interpretation. The competitive settings design could not be interpreted as a replacement series, because the growth of central and outer plants was not similar, confounding the results. The effect of plant position, due to the aerial partition affecting air flow and shading and possibly uneven distribution of roots in the pots caused problems. It may have been more useful to conduct a traditional replacement or additive series trial, despite the shortfalls of these designs.

In order to gain a good understanding of the nature of interference between plant species it may be necessary to employ several techniques to obtain an overall picture. It is necessary to consider the level of nutritional supply as plant growth and the nature of interference may change with increasing nutrient supply.

6.5 Suggestions for further work

The dual labelling trial could be applied to a field situation, as used previously by Caldwell *et al.* (1987). Such a trial would be less controlled, however, it would be useful to determine how browntop and white clover roots interfere with each other in a hill country pasture situation. A study of this type would take into consideration the interferences occurring between shoots, and how this affects root interference. The issue of the relative importance of shoot interference and root interference needs to be studied more closely.

It was not clear whether browntop could permanently reduce the P concentration in the soil or if browntop acquired more P by regulating the remaining P supply in the soil solution to a low P concentration as it becomes available, although the latter is more feasible. Measurements of soil P concentrations, and pools also need to be made.

Finally the possibility of autotoxic effects of white clover on its own growth, in particular due to rhizosphere acidity effects on the toxicity of phenolics, needs to be studied. In order to do this the pH of the soil needs to be varied to determine if this has an effect on plant root growth in the absence rhizosphere effects.

6.6 Concluding comments

The total root length of browntop appears to be an overriding factor determining the success of browntop in the field, and therefore the application of P to help promote maintenance of white clover following oversowing will not be very useful. Although it is clear that browntop will reduce the level of soil P supply, and reduce the rate of recycling of P, direct competition for P between browntop and white clover was found not to be as important as previously suggested. Overall, it is probably best to manipulate pasture botanical composition by good grazing management techniques, and to use P fertilisation to enhance total herbage production.

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Appendix 1 P buffer power of Ramiha top- and subsoils

The buffer power or P buffering capacity ($\Delta C/\Delta C_l$) of the soils was found from the inverse of the slope of the desorption isotherms (QI curves) at the appropriate level of desorbed P/g soil, for the level of P applied. The method used to determine the desorption isotherms is described below.

For Ramiha subsoil, with 68% P retention, the level of P desorbed was assumed to be around 0 µg P desorbed per g of soil for unamended subsoil, and 68 µg P desorbed per g of soil for subsoil with 100 µg P applied per g. Similarly, for Ramiha topsoil, with 56% P retention, the level of P desorbed was assumed to be approximately 38 µg P desorbed per g of soil for topsoil with 68 µg P applied per g, and 192 µg P desorbed per g of soil for topsoil with 344 µg P applied per g. Phosphate retention (PR) of the top- and subsoils was measured using the method of Saunders (1965).

The slopes of the desorption isotherms (QI cuves) at the above mentioned levels of P desorbed/g soil (i.e., $\Delta C/\Delta C_l$), and the buffer powers ($\Delta C_l/\Delta C$) are given in the table below.

Soil	**Isotherm slope** $\Delta C/\Delta C_l$	**Diffusion coefficient of solute in soil** D (cm^2/s)
Topsoil (68 µg P applied/g)	3100	$4.07*10^{-10}$
Topsoil (344 µg P applied/g)	615	$2.05*10^{-9}$
Subsoil (0 µg P applied/g)	9300	$1.36*10^{-10}$
Subsoil (100 µg P applied/g)	6200	$2.02*10^{-10}$

The speed of diffusion of phosphate, i.e., the root mean square distance, $x = \sqrt{(2D.t)}$ (Bieleski 1976), was compared with the half the distance between roots to determine whether P depletion zones around roots were overlapping, as described in Section 3.2.8.

Method for determining P desorption isotherms for soils

Air dried soil samples were forced through a 2mm sieve. 2.5 g samples of soil were weighed into 30 ml centrifuge tubes. Samples were shaken with 25 ml of 0.01M $CaCl_2$ (with 40 µg HgCl per ml to inhibit microbial activity) containing varying amounts of P, as KH_2PO_4 on an end over end shaker for 20 hours. The amounts of P in the solutions were equivalent to 0, 4, 10, 40, 100, and 400 µg P/g soil. After shaking the suspensions were centrifuged at 9000 rpm for 10 minutes, and the supernatant solutions were passed through a Whatman 42 filter. Inorganic P was determined in a 4 ml aliquot of the supernatant by the method of Murphy and Riley (1962), and absorbance was measured at 712 nm.

Appendix 2 Dry weights, and P and N contents of ramets used for planting in the P response trial (Chapter Two).

Parent plants of white clover ramets received basal nutrients. Plants were grown on Ramiha topsoil.

Species	Dry weight per ramet (g)	% P	P weight per ramet (mg)	% N
Browntop	0.0064 se (0.0005)	0.25 se (0.01)	0.016 se (0.002)	2.6 se (0.0)
White clover	0.0156 se (0.0001)	0.35 se (0.02)	0.054 se (0.003)	3.6 se (0.2)

Appendix 3 Procedure for staining vesicular-arbuscular mycorrhiza for rapid assessment of infection

Vesicular-arbuscular mycorrhiza were stained according to the method of Phillips and Hayman (1970). Roots stored in F.A.A. were washed in tap water and placed in a small glass test tube. Roots were covered with 10% KOH solution and heated in a water bath at 90°C for one hour in a fume cupboard. KOH clears the host cytoplasm and nuclei, as well as allowing stain penetration. The KOH solution was poured off. Roots were rinsed with three changes of tap water, and then covered with alkaline H_2O_2 at room temperature for 20 minutes to bleach roots. Alkaline H_2O_2 was made by adding 3 ml of NH_4OH to 30 ml of 10% H_2O_2 and 567 ml of tap water. Roots were rinsed again with tapwater, and covered with 1% HCl. After four minutes the HCl was poured off, but not rinsed.

Roots were stained with analine blue (0.325 g analine blue, 50 ml lactic acid, 100 ml water), and heated to 90°C for 30 minutes. Excess stain was removed using lactic acid solution (50 ml lactic acid, 100 ml water).

Roots were assessed for vesicular-arbuscular mycorrhizal infection by examining them through a stereoscopic microscope. Level of infection was assessed by using a rapid visual non-systematic scanning procedure. Roots were classed from 1 to 5, depending on the percentage of roots colonised by V.A. mycorrhizae.

Class	Roots colonised
1	0-5%
2	6-25%
3	26-50%
4	51-75%
5	76-100%

Results

The results from white clover and browntop roots examined from Chapter Three (low P supply dual labelling trial) are presented.

Species	Unamended subsoil	100 mg P applied per kg subsoil
Browntop	class 1: 6 samples class 2: 1 sample	class 1: 5 samples class 2: 1 sample
White clover	class 1: 4 samples class 2: 1 sample	class 1: 5 samples class 2: no samples

In most root subsamples the level of infection was extremely low, and often there were no observations of V.A. mycorrhizal infection. Therefore, not all the roots were classed.

Appendix 4 Method of counting radioactive phosphorus-32 and -33 in harvested plant material.

The three channels were set as follows:

Channel one: channels 0-500. The counts in channel one were largely due to ^{33}P, with some spillover of ^{32}P. The spillover was calculated from the activity of ^{32}P in channel two.

Channel two: channels 600-1000. The counts in channel two were due to ^{32}P, with insignificant spillover due ^{33}P into this channel.

Channel three: channels 0-1000. Total counts across all the channels were also measured.

The units of measured radioactivity were counts per minute (cpm).
In order to determine the counts attributable to each of the two isotopes in the shoot and root samples the following calculations were carried out:

[1] Readings for blanks with 2.5M HCl (containing no plant digests) were subtracted from all the readings. The readings in each channel for the blanks were as follows:

Channel one	41.7 cpm (se 2.2).
Channel two	17.4 cpm (se 0.5).
Channel three	67.2 cpm (se 2.3).

[2] Channel one readings were adjusted to exclude counts due to the presence of ^{32}P. The spillover ratio of ^{32}P into channel one was calculated from the counts measured in channel two (equation 3.1). This could be done as the counts in channel two were effectively due to ^{32}P only. The amount of spillover varied with pre-ash weight of plant material in the 2.5M HCl digest.

Spillover ratio (CH 1/CH 2) = *SR*

$$SR = \{24.16 + [5.43 \,.\, \text{Pre-ash wt(g)}]\}/100 \quad \text{......} [3.1]$$

(r=0.9804 [5 df])

The amount of actual spillover into channel one was calculated using equations 3.2 and 3.3;

^{32}P in CH 1(cpm) = SR . CH 2(cpm)..[3.2]

^{33}P in CH 1(cpm) = CH 1 reading(cpm) - ^{32}P in CH 1(cpm)..........[3.3]

[3] Finally, the quenching effect of ash on the amplitude of the counts was adjusted for. All readings were adjusted to a level equivalent to "no quenching effect", i.e., at 0.00 g pre-ash weight in the 2.5M HCl digest. A dividing factor, D, was used (equation 3.4).

D = 1 - [0.158 . pre-ash wt(g)]..[3.4]

Appendix 5 Discussion on root/shoot ratio data

The responses of root:shoot ratios for browntop and white clover to P application were atypical in that root:shoot ratios tend to decrease with increasing P supply. For example, Bradshaw *et al.* (1960) found that root:shoot ratios of browntop were 0.28 and 0.16 at P supplies of 2 and 32 ppm P, respectively, when grown in sand. Thus, the root weights were lower than expected, especially at low soil P levels. As roots were collected from the subsoil in the pots using a hydropneumatic elutriation system (Smucker *et al.* 1982), there may have been a loss of root material during the washing process. The highly stable aggregates of Ramiha subsoil were not completely broken down by the jets of water spraying into the subsoil. A substantial amount of fine roots may have remained in the peds and would have been discarded with the waste soil. This is supported by the fact that more roots appeared to have been lost for the more finely rooted browntop plants than for white clover. According to Smucker *et al.* (1982) the degree of recovery of very fine roots (in the range 50-250 μm diameter) is dependent on the technique employed. With the mean root diameter of browntop being in this range (160 μm (Evans 1977)), and white clover roots being thicker (260 μm (Evans 1977)), it is feasible to expect a lower recovery of browntop roots than white clover roots, particularly at low soil P supply, where browntop roots would probably be extremely fine. Increasing levels of P application has been shown to reduce root fineness in other grasses (Christie and Moorby 1975; Bolan *et al.* 1987). The hydropneumatic elutriation system of root harvesting may only be suitable for use with soils that break down into individual soil particles or extremely small peds when wet. This is an area that requires further investigation.