THE EFFECTS OF PHOSPHORUS SUPPLY ON COMPETITION BETWEEN HARD BROME GRASS AND SUBTERRANEAN CLOVER

I. Valentine, B.Sc. (Wales, Bangor)
Department of Agronomy
Waite Agricultural Research Institute

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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

Ian Valentine

SUMMARY

The influence of the nutrient status of the soil on competition between an annual grass and clover was examined in a series of glasshouse experiments. The experiments were designed as a replacement series, in which mixtures and monocultures of Bromus rigidus Roth. and Trifolium subterraneum L. cv. Mount Barker were grown at a constant plant density. Competitive ability of each component was measured by the crowding coefficient calculated from shoot dry weight. The two species concerned commonly grow in association in annual, sown pastures in the winter rainfall area of southern Australia.

In Experiment I the two species were grown over a range of frequencies at a plant density of 0.3 cm^{-2} on an acid siliceous sandy soil taken from under an established pasture that had been fertilized with superphosphate. The addition of phosphorus increased the competitive ability of the grass. The addition of potassium or sulphur produced a frequency dependant competitive response. The frequency dependence was attributed to a response of the grass in mixture to nitrogen fixed by Rhizobium growing in symbiosis with the legume.

The effect of phosphorus supply on the competitive ability of the two species was studied further in Experiments II, III, and

IV. A virgin siliceous sandy soil of low fertility was used in these ~~this and subsequent~~ experiments. The effect of phosphate on competitive ability observed in Experiment I was repeated although the grass became less competitive with time. Variation of plant density (0.08 and 0.29 cm^{-2}) did not alter the effect of phosphorus on competition. Root lengths were measured in this and subsequent experiments, using C^{14} labelling, to identify each component in a mixture.

Experiment III showed that the soil temperature markedly affected growth and competitive ability, favouring the clover at the higher soil temperatures. The effect of phosphorus showed no interaction with that of soil temperature.

In Experiment IV barriers were arranged below ground to allow or to prevent inter-specific mixing of root systems. Canopies were allowed to mix. The grass became more competitive when its roots were mixed with clover roots. Although this indicated that the grass derived some advantage from having its roots mixed with clover roots, there was little other evidence that the plants competed directly for scarce supplies of phosphorus. At the highest phosphate rate the mixtures overyielded at the final harvest, when the clover dominated the grass.

The relative importance of direct competition for scarce

supplies of phosphorus, and of indirect effects of the level of phosphate supply on competition for factors other than phosphorus is discussed in relation to the data from the four experiments. The agronomic implications, including the feasibility of manipulating pasture composition by fertilizer management, are also considered briefly.

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ADDENDUM

At the request of the examiners the following remarks have been added to the introduction, in order to explain further why the experimentation was undertaken, and why glasshouse cultures were employed.

Bray (1954) drew attention to the relationship between competition for nutrients and nutrient mobility, and suggested that competition for immobile nutrients may not occur even when the roots of neighbouring plants intermingle. Bray's ideas have not been tested adequately, and there are few data on the root density and distribution found below plant communities.

Recent theoretical work has postulated that root length is the critical plant attribute involved in the uptake of water (Cowan, 1965) and nutrients (Nye and Tinker, 1969). With improved methods of measuring root length (Newman, 1966) quantitative data on root length indicate the probability of nutrient competition occurring, at least for mobile nutrients.

The relative yields of components of annual grass/clover pastures in southern Australia were known to be influenced in the field by nutrient supply. In analysing such effects, if grass and clover are grown in mixtures and monocultures at relatively high density, interference between neighbouring plants is likely to occur at an early stage. Moreover, if total plant density is kept constant the interference can be measured by using the approach and model proposed by de Wit (1960).

The experiments to be described set out to determine the processes causing a change in competitive ability of grass and clover in response to a change in the level of nutrient supply. Swards of binary mixtures have been used previously to study the interference of canopies, and, in particular, the effects of competition for light (Donald, 1963). In the present work the emphasis is placed on the edaphic environment, and on the collection of root data, in an attempt to describe the nature and extent of root interference. The experiments were conducted in a glasshouse to obtain more uniform conditions than could have been obtained in the field, to facilitate measurements, and to allow closer comparison between experiments.

Firstly the possibility of nutrient competition is considered in relation to the root densities found. Secondly, a hypothesis regarding root competition for phosphorus in sandy soils is tested in an experiment using root barriers. The effect of soil temperature on competitive ability of grass and clover is assessed also, soil temperature being a factor that is likely to have a strong influence on the growth and ion uptake activity of the roots.

COWAN, I.R. (1965).- Transport of water in the soil-plant-atmosphere system. J. appl. Ecol. 2, 221-239.

NYE, P.H. and TINKER, P.B. (1969).- The concept of a root demand coefficient. J. appl. Ecol. 6, 293-300.

1. Introduction

In most natural plant communities the introduction and loss of genotypes occur continually. In contrast the requirements of agriculture call for mixtures and monocultures of known and stable composition. The sown annual pastures of southern Australia are characterised by a marked instability which detracts from their value as feed. A more stable legume component is required to improve the herbage quality (Rae *et al.*, 1963) and as a source of nitrogen for the plant community (Underwood, 1951).

The reasons for this instability of botanical composition are not well understood. The instability is caused partly by the effect of climate on seasonal regeneration (Trumble and Frazer, 1932) and also by management of these pastures (Talbot *et al.*, 1939). Willoughby (1954) and others suggest that the mineral nutrition of the pastures has a critical role in determining the longer term trends in botanical composition. Changes in botanical composition in southern Australian pastures have been attributed to changes in the levels of phosphorus (Rossiter, 1964), potassium (Rossiter, 1947; Lines, 1963; Burford, 1967) and sulphur (Powrie, 1967).

Ecologists have often attributed the succession of species to changes in the edaphic environment. However the technical difficulties of studying the below ground environment have stood in the way of definitive experiments on the role of edaphic factors in plant succession.

The experiments to be described test the significance of changes in the nutrient supply, with particular reference to phosphorus, on the relative yields of components of a mixture. Measurements were confined to the vegetative growth stage of two representative species found in southern Australian pastures. The ecological significance of these effects and the mechanisms involved are considered.

2. Literature Review

2.1 Plant association and competition

The scientific study of interference between plants has followed a historical course similar to that of many other biological subjects. A qualitative descriptive phase has been followed by an experimental phase with progressively greater emphasis on quantifying the effects observed. Analysis has led to mathematical relations being proposed to summarize the results and to allow predictions to be made. The many plant characteristics to which success or failure in a community have been attributed have been discussed by Donald (1963), and more recently for herbage grasses by Rhodes (1970). Here only those experiments directed towards the study of edaphic factors will be considered, although whole plant response has always to be taken into account.

2.1.1 Semantics

Competition has been defined by Donald (1963), following Clements *et al.* (1929), as the phenomena which occur "when each of two or more organisms seeks the measure it requires of any particular factor and when the immediate supply of the particular factor is below the combined demand of the organisms". This definition is profitable only when the particular factor or factors involved are identified. Otherwise the more general term 'interference' may be used (Harper, 1961).

2.1.2 Inferences from morphological observations

Early workers who made a thorough morphological study of root systems, concluded that the root densities found under crops were so high in top-soils that interference must occur (Weaver, 1926; Pavlychenko, 1940). Pavlychenko believed that, because the root was the first organ to support life in the seedling, interference between roots was likely to occur at an early stage. These authors assumed that roots competed mainly for water. However the morphological approach above can lead to false conclusions. For example, Tansley (1939) drew root profiles for successful chalk-grassland species and emphasized the importance of their deep rooting habit in drought avoidance. However it is now known that there is an abundance of water in the chalks concerned but that there is an acute shortage of nutrients.

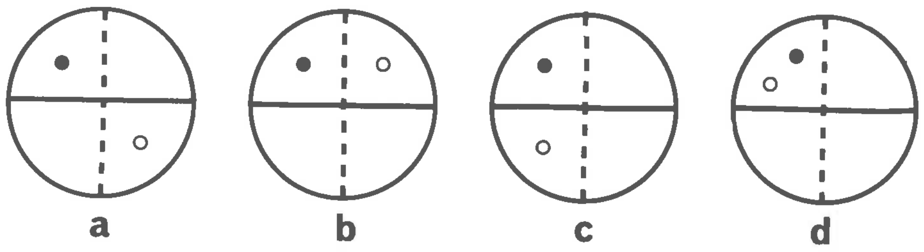
2.1.3 Manipulation of plant associations

Experiments have been designed to ascertain the interactions between edaphic and aerial interference by introducing barriers to separate the above or below ground organs of interfering genotypes. Tansley (1917) reported an experiment where barriers were introduced above and below ground between individuals of a calcicole and a calcifuge to determine the factors that limit the distribution of these species.

More recently Donald (1958) introduced barriers between the shoots and roots of *Lolium perenne* L. and *Phalaris tuberosa* L. in such a way that (a) the species in each pot were separated, (b) the shoots or (c) the roots only were allowed to mix and (d) both the shoots and roots were allowed to mix (see Figure 2.1A). The effect of mixing shoots and roots (d) appeared to be greater than the sum of the effects of mixing shoots or roots only (b and c), and Donald concluded that there were interactions between competitive effects arising below and above ground. Unfortunately the design of Donald's experiment did not permit these conclusions to be drawn, as the soil volume and ground surface area per plant varied between the mixing treatments. The aggregate yields show that reduction in the size of the environment had considerable effects (Table 2.1). The reduction in soil volume per plant in treatments (c) and (d) led to a reduction in the total nitrogen supply per plant, nitrogen being applied uniformly to the whole pot, and this reduction caused a drop in aggregate yield at each rate of nitrogen. The effects of the reduction in ground surface per plant and hence light input per plant in treatments (b) and (d) were found only when the maximum amount of nitrogen was provided per plant.

Figure 2.1. Basic design of pot showing position of shoot barrier (solid line) and root barrier (hatched line) in relation to plant positions. The modes of interference obtained are (a) no mixing, (b) shoots mixed, (c) roots mixed and (d) shoots and roots mixed.

A Donald (1958)



B Snaydon (1971)

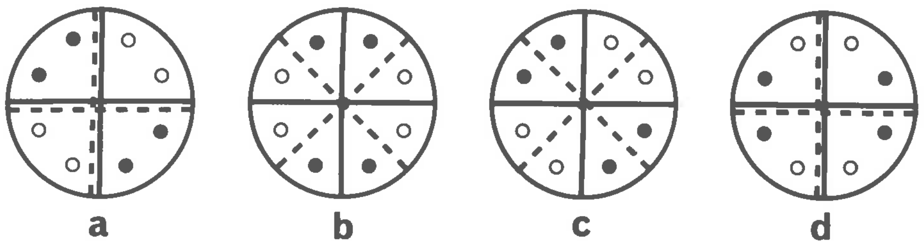


Table 2.1

Mean yield of tops per pot
(dry matter (g))

	Plants Separated (a)	Shoots Mixed (b)	Roots Mixed (c)	Shoots and Roots Mixed (d)
Low Nitrogen	4.5	4.3	2.5	2.9
High Nitrogen	9.4	7.4	5.5	5.0

Source: Donald (1958)

Related pot experiments made by many authors (e.g. Aspinall, 1960; Schreiber, 1967; Lambert, 1968) suffer from a similar source of ambiguity.

Snaydon (1971) overcame this difficulty, when comparing the sites of interference between Trifolium repens L. populations collected from acid and calcareous soils, by adopting a more appropriate geometrical arrangement of the barriers (Figure 2.1B). Snaydon's populations were grown in acid and calcareous soils. The results (Table 2.2) showed that, on acid soils, when the shoots alone were mixed the calcareous population gained at the expense of the acid population. The dominance was reversed when only the roots were mixed, and the effects appeared to counteract each other when both shoots and roots were mixed.

Table 2.2

Total dry weight per plant (g) of acid and calcareous populations of *Trifolium repens* subject to various forms of inter-population mixing

	Acid Soil		Calcareous Soil	
	Acid population	Calcareous population	Acid population	Calcareous population
Populations separate	1.2	1.6	0.8	1.6
Roots mixed	1.3	1.0	0.7	1.8
Shoots mixed	0.9	1.7	0.6	1.2
Roots and Shoots mixed	0.8	1.2	0.7	1.9

Source: Snaydon (1958)

On calcareous soils the calcareous population was dominant in all treatments. The nature of the root attribute which increased the competitive ability of the acid population on acid soils was not determined, but Snaydon suggested that differences in phosphorus uptake could account for this effect.

Rhodes (1968) introduced barriers above and below ground level between rows of H1 ryegrass (*Lolium perenne* L. x *L. multiflorum* Lam.) and *Festuca arundinacea* Schreb. After eight weeks *F. arundinacea* was suppressed by H1 ryegrass when only the roots were mixed or when both roots and shoots were mixed. Rhodes noted that the ryegrass produced a greater number of nodal roots than *F. arundinacea*.

Similar experiments have been conducted in the field by introducing barriers between plant rows. Chamblee (1958) grew mixtures and monocultures of orchard grass (*Dactylis glomerata* L.) and alfalfa (*Medicago sativa* L.) in the field at a uniform plant density with and without soil barriers between the rows. The mixtures without soil barriers yielded more per unit area than the mixtures with the barriers present. However, with or without barriers the mixtures yielded more than either monoculture. Bakhuis and Kleter (1965) conducted an experiment of essentially the same design with mixtures of *Trifolium repens* L. in association with *Lolium perenne* L. or *Dactylis glomerata* L. and obtained similar results to Chamblee. The increased yield per unit area of the mixtures compared to the monocultures was attributed by both authors to the development of a superior canopy for light interception. The additional increase in yield found when the roots of the components were allowed to mix was attributed to an increase in total nitrogen uptake. This phenomenon is commonly observed in grass/legume mixtures and will be discussed later.

In each of the experiments noted above, total plant density was uniform for monocultures and mixtures so comparisons could be made of the effects of botanical composition. However the presence of root barriers had unexpected effects in some experiments. Although Chamblee found no difference between monocultures with and without soil barriers, Bakhuis and Kleter found 50% increases in monoculture yields with barriers present. Bland (1967) found that the introduction of a polyethylene barrier between rows or perennial ryegrass and white clover reduced the total root yields by 20%, the reduction being mainly in the grass component. The effects of barriers were not explained.

2.1.4 Measures of competitive ability and models of plant associations

Several authors have attempted to evaluate the results of experiments on plant associations in terms of a measure of competitive ability. Most published measures are simple and refer to two component mixtures only. They have no predictive value but serve only to describe the response observed. Such measures reflect the increase in the yield per plant of one component (the "aggressor") of a mixture over its yield in monoculture together with the corresponding decrease in yield of the other component. Absolute differences in yield of mixture and monoculture may be treated arithmetically to describe the competitive relationship (for example the p of Sakai, 1955; c of Hanson *et al.*, 1961; γ of McGilchrist, 1965). It has been demonstrated by van den Bergh

(1968) that differences in yield (of opposite sign) are likely to have approximately the same magnitude only if the monoculture yields do not differ appreciably. Models which describe the competitive relationship of components of a mixture as a proportional increase and decrease in mixture relative to performance in monoculture (for example the k of de Wit, 1960; ρ of van den Bergh, 1968; A of McGilchrist and Trenbath, 1971) have wider application.

The proportional model, proposed by de Wit and van den Bergh (1965), compares the relative yields, r, of two genotypes, S_1 and S_2, grown in a mixture at the same total plant density. The relative yields are defined by

$$r_1 = \frac{Y_1}{M_1} \quad \text{and} \quad r_2 = \frac{Y_2}{M_2} \qquad (1)$$

where M_1 and M_2 are yields in monoculture per unit area and Y_1 and Y_2 are yields in mixture. If the responses to mixing are compensatory the expected value for the sum of the relative yields is:

$$r_1 + r_2 = \text{RYT (relative yield total)} \qquad (2)$$

Analysis of many experiments (de Wit, 1960; de Wit and van den Bergh, 1965; de Wit, Tow and Ennick, 1966) show that as a general rule RYT equals 1 and the species show compensatory growth.

De Wit (1960) had earlier proposed a model for a two-genotype mixture by drawing an analogy with the equations used to describe the partial vapour pressures found in binary mixtures of fluids. The two genotypes, S_1 and S_2, are grown in the (fractional) frequencies z_1 and z_2 where $z_1 = Z_1/(Z_1 + Z_2)$ and $z_2 = Z_2/(Z_1 + Z_2)$, Z_1 and Z_2 being the number of seedlings per unit area. Implicit in this model is zero mortality during the growth period. The monocultures together with one or more mixtures comprise a replacement series. The yield per unit area in mixture, Y, is dependent on the yield in the monoculture, M, the seedling frequency, and the crowding coefficients, k_{12} and k_{21}, the latter being defined by the relations:

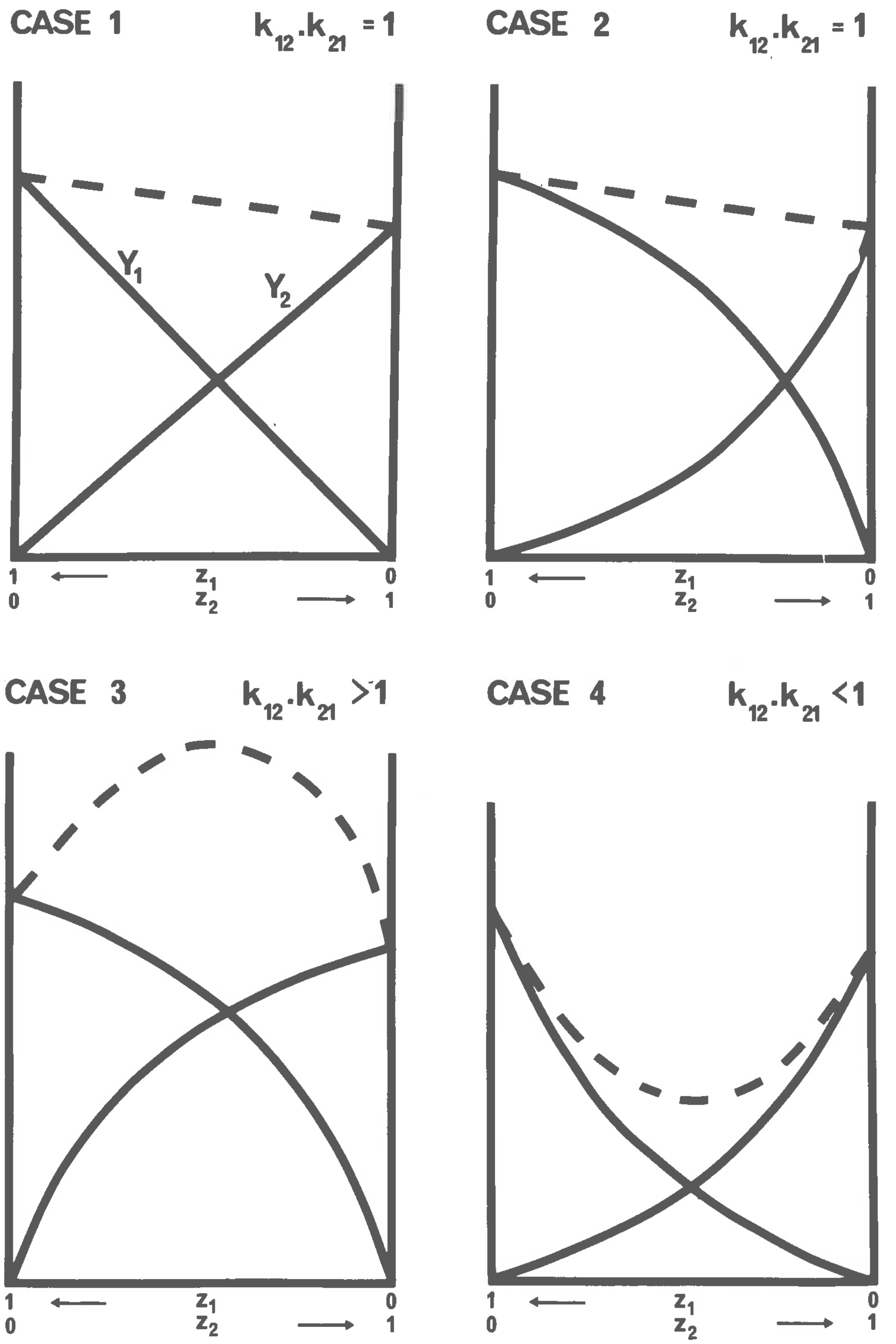
$$Y_1 = M_1 k_{12} z_1/(k_{12} z_1 + z_2) \tag{4}$$

and

$$Y_2 = M_2 k_{21} z_2/(k_{21} z_2 + z_1) \tag{5}$$

The model described by de Wit (1960) is in effect a special case, where $k_{12} . k_{21} = 1$, of the model of de Wit and van den Bergh (1965). Given the values of the crowding coefficients information may be derived on the degree and nature of the exploitation of the environment by the two genotypes. Bakhuis and Kleter (1965) demonstrated the alternative possibilities with replacement diagrams, in which the yield of each genotype per unit area is plotted against the seedling frequency (Figure 2.2). When $k_{12} . k_{21} = 1$ the species may be regarded as sharing the same resources, and the yields of the component genotypes of a mixture may either be proportional to

__Figure 2.2.__ Replacement diagrams showing the theoretical outcome of competition based on the model of de Wit (1960) after Bakhuis and Kleter (1965): yields of components (solid line), total yield (hatched line).



Bakhuis & Kleter (1965)

the seedling frequency (case 1), or one genotype may suppress the other genotype but show compensatory growth (case 2). If both components of a mixture yield more than the expected fraction of the monoculture, then $k_{12}.k_{21} > 1$ and the resources used are not identical for the two components (case 3). Finally, if the mixture does not utilize the environment as fully as expected from the yield of the monocultures, then $k_{12}.k_{21} < 1$ (case 4).

2.2 Mineral nutrition in relation to association and competition

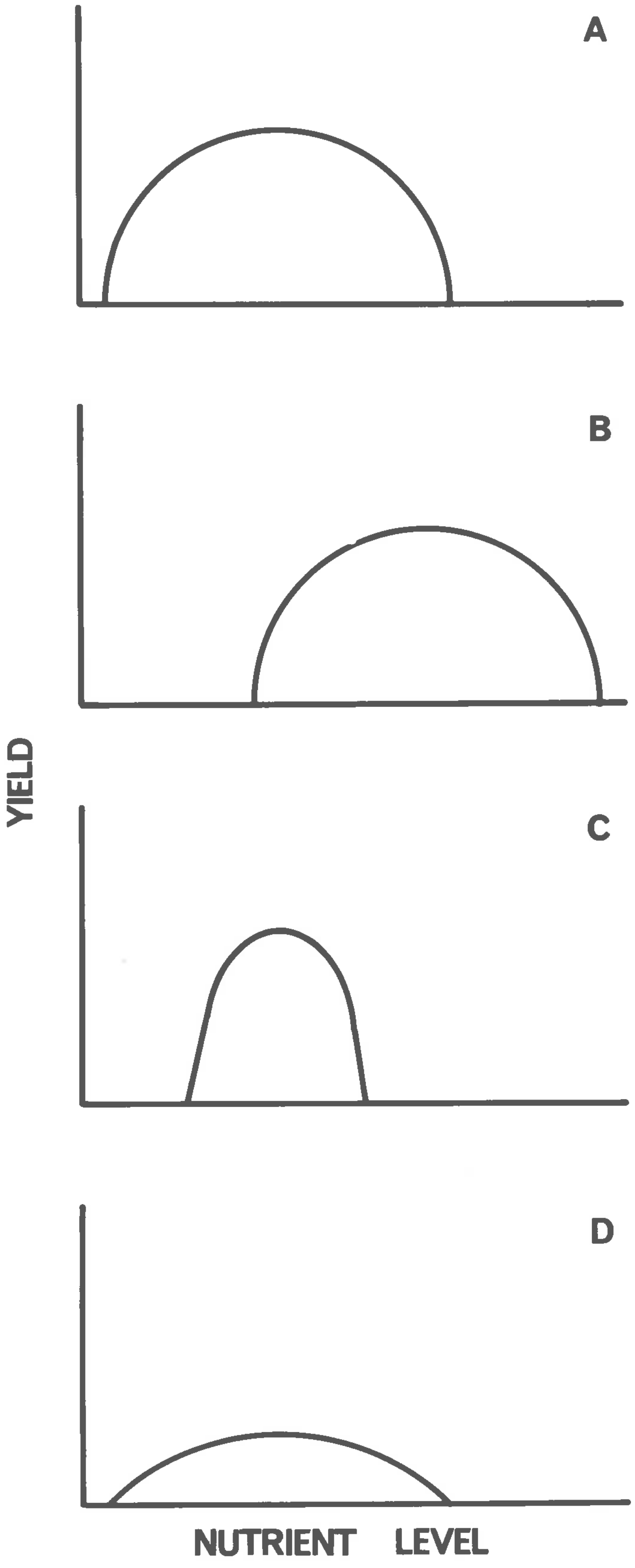
Experiments on plant associations rarely indicate the critical processes involved in competition. However species do differ in their competitive ability, and it is profitable to consider the anatomical, morphological, and physiological differences that occur in the mineral nutrition of species and genotypes, and to consider the possible significance of these differences in competition. Varietal differences in mineral nutrition have been reviewed by Epstein (1956), Vose (1963a) and Langer (1966). Genotypic differences in yield response to a given increment in nutrient supply will be considered firstly.

2.2.1 Genotypic differences in yield response

Antonovics *et al.* (1967) described differences that may occur in the response curves of genotypes and discussed their ecological significance. The authors summarized their observations as shown in Figure 2.3. Over a wide range of nutrient levels the response curve may be expected to show low yield under excessively low or excessively high nutrient levels (case A). A genotype may adapt to higher nutrient levels (case B) or it may be less tolerant to variation (case C). Finally a genotype may respond over a normal range of nutrient supply, but yield may be limited by the potential for growth (case D). This could be brought about, for example, by natural selection for small stature in exposed conditions.

In practice it is often difficult to measure a response by a genotype over the entire nutrient range. A genotype may show a greater absolute response simply by having a greater growth rate. If a differential response in two varieties is lost after log. transformation of the yield data, the relative growth rates have been changed similarly in both varieties by the nutritional treatment. However, in many cases log. transformation of the yield fails to remove genotype x nutrient interactions, suggesting that there are inherent differences in efficiency of uptake or utilization other than those associated with differences in the relative growth rate. For example, Rorison (1968) found differences in efficiency of phosphorus utilization between species that were selected from soils of contrasting fertility, and these differences occurred within sets of species of either low or high growth rate.

Figure 2.3. Theoretical response curves of genotypes showing differences in response (after Antonovics *et al.*, 1967). See text for explanation.


A
B
C
D
YIELD
NUTRIENT LEVEL

2.2.2 Nutrient uptake

The soil and plant-root attributes that may be expected to influence nutrient uptake have been reviewed by Olsen and Kemper (1968) and by Barley (1970). The plant attributes involved are (a) root density, (b) root distribution and (c) physiological uptake ability of the roots.

(a) Root density

Because of the technical difficulties involved, root lengths have rarely been measured. For grasses the root length per unit volume of soil (L_v, cm^{-2}) has been found to vary from 8 (*Avena sativa* L.) to 56 (*Poa pratensis* L.) in the top 15 cm of soil under established swards (Dittmer, 1938). These determinations were made at the flowering stage. Earlier estimates of L_v when mineral uptake is at a maximum might be of more value. Torssell *et al.* (1968) measured the root length under a Townsville stylo (*Stylosanthes humilis* H.B. & K.) stand, and found that the L_v in the top 10 cm was 12 after 100 days and 28 after 120 days from germination.

There is little direct information on the relationship between root length and nutrient uptake. Harris (1968) attributed the outcome of competition in mixtures of *Agropyron spicatum* Scribn. and *Bromus tectorum* L. to differences in root growth. Brouwer (1963) showed a relation between root number and uptake of nitrate and phosphorus in solution by bean plants (*Phaseolus vulgaris* L.)

from which some of the roots had been removed. The direct relationship between root length and mineral uptake could have been due to the reduced turgor of the plants. Gile and Carrero (1917), using young maize plants with roots divided between a complete solution and one lacking a specific nutrient, found that the rate of uptake of N, P, K or Fe per unit weight of root rose to a maximum as the proportion of roots being supplied was reduced.

Such relations are modified when the roots are grown in soil because of the great differences in mobility found between nutrients in the soil. Autoradiographs published by Lewis and Quirk (1967) show wheat roots depleting the phosphorus content of a loamy soil only over short distances from the root surface, a distance corresponding approximately to the length of the root hairs. In contrast nitrate is highly mobile (Wallace, 1954) and plants may quickly reduce the level of inorganic nitrogen in the root zone to a low level. Cornforth (1968) found that when the mean root density was altered by varying the depth of soil in a pot the uptake of the relatively immobile ion, phosphate, was closely related to root density, while the uptake of nitrogen was independent of root density. Andrews and Newman (1970) studied effects on ion uptake found when the root density was altered by root pruning. Wheat plants that were intact and plants that had 60 per cent of the total number of roots cut close to the crown were grown in mixture and monoculture.

Root pruned plants had a similar nitrogen uptake to intact plants when grown in monoculture, but there was a reduction in their nitrogen uptake when grown in the presence of intact plants (Table 2.3). In the mixtures the nitrogen uptake of the intact plants exceeded that of the pruned plants by a factor of 1.6, which was similar to the ratio of the root weights between the treatments. In contrast, phosphorus uptake was reduced by the pruning treatment, but was not affected by mixing pruned and intact plants, suggesting that the volume of soil exploited did not overlap markedly.

Table 2.3

Effect ~~of~~ on uptake of nitrogen and phosphorus of mixing root pruned and unpruned wheat plants (mg/plant)

	Alone	Mixed
Nitrogen		
Unpruned	8.4	13.5
Pruned	10.1	8.4
Phosphorus		
Unpruned	2.8	3.1
Pruned	2.5	2.5

(b) Root distribution

The distribution of roots is partly under genetical control (Subbiah _et al._, 1968; Derera _et al._, 1969). Ozanne _et al._ (1965)

found considerable differences in the vertical distribution of root weight between pasture annuals, and these were correlated with K^{42} uptake from the deeper layers of the soil. The ecological significance of root distribution is shown in mixtures. O'Brien *et al.* (1967) suggested that the greater root density at depth produced by a hybrid of perennial ryegrass (*Lolium perenne* L.) and meadow fescue (*Festcua pratensis* Huds.) led to an advantage over either of its parents when grown in mixture. A similar root layering effect resulted in a mixture of *Avena fatua* L. and *Avena strigosa* Schreb. out-yielding monocultures of either component (Ellern, Harper and Sagar, 1970). *A. strigosa* has a shallow rooting habit habit while *A. fatua* has a deep rooting habit.

The pattern of root distribution is also influenced strongly by the environment. The source of nutrients, the vertical movement of water, and the differences in nutrient fixing ability between soil horizons, gives rise to changes in nutrient concentration with depth and time. The proliferation of roots in fertilizer bands has often been observed. Rooting patterns are also affected by plant density, the orientation of the roots typically having an increasing vertical component as the density rises (Neilson, 1964; Raper and Barber, 1970).

(c) Uptake per unit length of root

The vertical flux of nutrients and water in the soil is too variable and usually too small to provide a satisfactory supply to roots. Consequently, roots largely create fluxes of water and ions, unlike leaves which intercept an existing flux of radiant energy. To generate the gradients necessary for transference, the root must absorb water and ions. Current theories on ion uptake postulate a carrier transport system, in which ions cross a diffusion barrier in association with an organic carrier. The rate of ion uptake is related to the concentration of the ion in the ambient solution, and over longer periods of time, to the transport of ions away from the site of uptake.

The process of ion uptake appears to be under genetic control (Epstein and Jefferies, 1964). Butler *et al.* (1962) found that in crossing *Lolium perenne* with *L. multiflorum* the uptake patterns of nitrate, phosphate, sulphate, sodium, calcium, manganese, aluminium, copper and zinc showed significant heritabilities. Of the ions examined only potassium and iron uptake failed to show significant heritability. Species differ both in the maximum rate of uptake per unit length of root when supply is non-limiting and in the minimum concentration of the solution from which uptake will occur (Rorison, 1968; Asher and Loneragan, 1967).

Differences in cation content, both between and within species, frequently show a general relationship with cation exchange capacity (Asher and Ozanne, 1961; Vose, 1963b). However physical absorption of cations by the root, unlike anion uptake, is non-selective, and Donnan theory does not explain the ratio in which different cations are taken up by the plant. Broeshart (1962) demonstrated that uptake of cation species was unaffected by the ratios in which the cations were adsorbed on the plant root, but was sometimes markedly affected by the ratio of these cations in the ambient solution.

2.2.3 Response to mineral nutrients in plant associations

In an attempt to quantify the effect of competition for nutrients many workers have measured the differential response of components of a mixture when nutrients have been added. Grasses have been shown to compete successfully with legumes for potassium (Blaser and Brady, 1950; Brown and Rouse, 1953) and for sulphur (Walker and Adams, 1958). By rapid proliferation of the root systems, grasses appear to limit the supply of a scarce nutrient available to the associated legume. Addition of the nutrient often gives a relatively greater response by the legume than by the grasses.

Withdrawal of nutrients by weeds in agricultural crops has been assessed in terms of the amount of fertilizer needed to prevent depression in yield of the crop. For example, Jackman and

Mouat (1970) estimated the interference of brown top (Agrostis tenuis Sibth) in terms of the additional amount of superphosphate needed to obtain the same amount of herbage from clover growing with grass as with clover growing alone. Welbank (1961) argued that competition for a nutrient was implied if the response of a component to mixing was less at higher levels of supply of that nutrient than at low levels. Blaser and Brady (1950) found that the addition of potassium increased the yield of ladino clover (T. repens), but not of the grass component, in a herbage mixture. However the response to potassium by the clover was reduced when nitrogen was also added. The addition of nitrogen favoured the grass component and thereby increased the competition for potassium.

It is important to distinguish between a response by a genotype to a nutrient level and change of response to an applied rate due to competition for nutrients in mixture. Comparison must be made with monocultures of each component of the mixture grown over the same range of nutrient supply. Furthermore the monocultures must be grown at the same total plant density as the mixtures. The inter-relationships between the effects of density and interference between species are complex, but it may be premised that density determines the intensity of interference, and the genotype of the nearer neighbours determines the nature of interference. In many early experiments on competition for phosphorus (Trumble and Shapter, 1937) and nitrogen (Walker *et al.*, 1956) the same number of each species was sown in both the monocultures and in the mixtures so that the total plant density differed. With this experimental technique simple mixtures have twice the total plant density of the monocultures and the effects of interference are confounded with the effects of density.

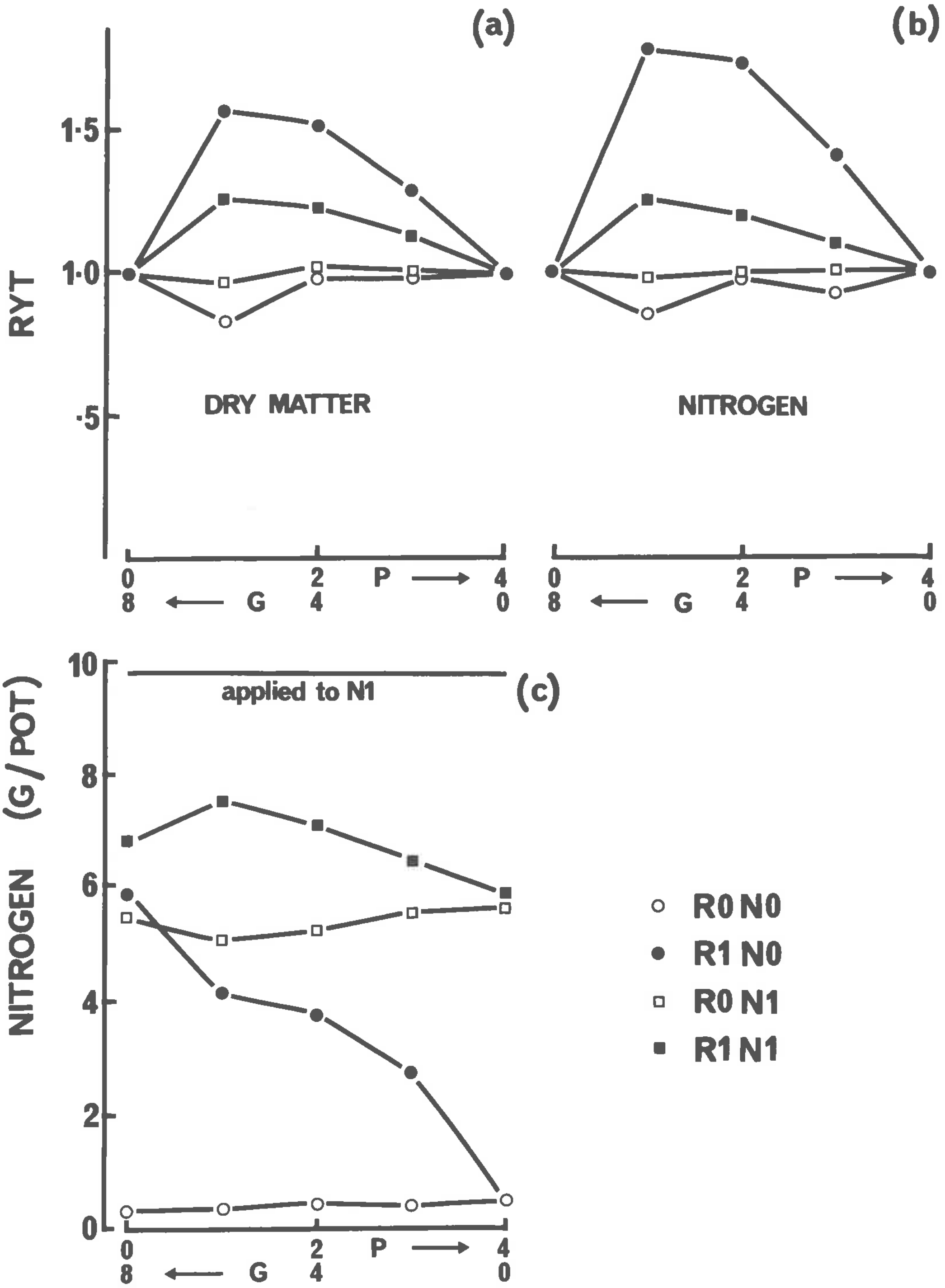
Workers at Wageningen were the first to appreciate the importance of maintaining a uniform total plant density and comparing yield in terms of yield per plant for each treatment. The principles underlying replacement series experiments described by de Wit (1960) have been discussed earlier in the thesis. The method has been used by the Wageningen school to study the effects on plant association of many factors including edaphic factors. For example van den Bergh and Elberse (1962) showed that *Anthoxanthum ordoratum* L. suppressed *Lolium perenne* L. at low fertility levels, but the addition of phosphorus and potassium reversed the suppression. De Wit, Tow and Ennick (1966) elegantly illustrated the role that *Rhizobium* plays in grass/legume mixtures. Glycine (*Glycine javanica* Linn.) and panicum (*Panicum maximum* Jacq.) were sown as monocultures and mixtures. The monocultures and mixtures were grown in a replacement series at the proportions .25, .50 and .75 under two nitrogen levels (N_0 and N_1), and in the presence or absence of Rhizobium (R_0 and R_1). Their results showed that without a source of nitrogen (R_0N_0) yields for both components were low. With the application of nitrogen (R_0N_1), and in the absence of Rhizobium, panicum became dominant, and the yields of the components in the mixture showed compensatory growth with RYT = 1. With the introduction of Rhizobium (R_1N_0) the glycine became dominant but without the compensatory decrease in the panicum yield. Both species responded to the combination of fertilizer nitrogen and Rhizobium (R_1N_1). When Rhizobium was present in the mixtures the response by the legume component was not accompanied by a compensating decrease in yield by the grass component, and RYT > 1. The values of RYT over the range of seedling frequencies calculated from the dry weights are shown in Figure 2.4(a). RYT values can also be calculated from nitrogen yields (Figure 2.4(b)). The increase in total nitrogen yield in the mixtures is apparent. The nitrogen yields (Figure 2.4(c)) show that the amount of nitrogen in the system increased due to fixation when Rhizobium was present.

2.3 Ecological studies of association and competition in southern Australian pastures

2.3.1 Instability in pasture composition

Large areas of southern Australia no longer exist under their original plant cover. The introduction of a European type of agriculture has led to the disappearance of perennial grasses, and these have been replaced by cool season annuals. In particular

<u>Figure 2.4.</u> The average relative yield totals (RYT) of panicum (P) and glycine (G) based on dry matter yields (a) and nitrogen (b) and the total nitrogen yields of seven harvests (c) from de Wit, Tow and Ennick (1966).


(a)
(b)
RYT
1·5
1·0
·5
DRY MATTER
NITROGEN
0
8
2
4
P
4
0
G
10
8
6
4
2
0
NITROGEN (G / POT)
applied to N1
(c)
R0 N0
R1 N0
R0 N1
R1 N1

Themeda australis (R.Br.) Stapf. (kangaroo grass) and *Danthonia* spp. (wallaby grass) have been replaced by introduced Mediterranean species. The increased supply of nitrogen and phosphorus under pastures sown to *Trifolium subterraneum* L. (subterranean clover) and top-dressed with superphosphate fertilizer has led to invasion by a number of introduced annuals including *Hordeum leporin~~i~~um* Link. (barley grass), *Bromus rigidus* Roth. (hard brome), *B. mollis* L. (soft brome), Arctotheca calendula (L.) Levyns ~~*Cryptostemma calendulaceum* (L.) Druce~~ (capeweed), and *Erodium* species.

Once invasion has occurred the pastures become characteristically unstable. Marked fluctuations in botanical composition may occur from year to year. Tiver and Crocker (1951) provided data obtained from pastures at Kybybolite, South Australia. The percentage overlapping cover from two adjacent sites was:

	1945	1946	1947	1948
Field 1				
Sub-clover	38	90	55	45
Capeweed	0	0	25	0
Brome and Barley grass	60	6	20	50
Field 2				
Sub-clover	10	90	50	70
Capeweed	60	5	40	5
Brome and Barley grass	20	5	10	20

Tiver and Crocker attributed these fluctuations to climatic conditions affecting establishment and seed dispersal and to the increasing fertility of the soil.

Similar instability has been described by Meadly (1946) in sown pastures in south-western West Australia, where failure of rains after the initial seasonal break favoured the drought hardy weeds. Meadly noticed that high residual soil phosphate encouraged the invading annuals. More recently Rossiter (1964) assessed the role of soil phosphate in a ten-year study of pastures established on sandy soils of Western Australia. He summarized the long term pattern as follows:

Phosphate Status	Dominant	Subdominant
Low (no applied phosphate)	Erodium (_Erodium_ _botrys_ (Cav) Bertel)	Silver grass (_Vulpia_ _myuros_ (L.) Gmel.)
	Flatweed (_Hypochoeris_ _glabra_ L.)	Subterranean clover
Intermediate	Subterranean clover	Silver grass
	Erodium	Capeweed
High	Brome grass (or Barley grass)	Erodium
	Capeweed	Subterranean clover

Rossiter believed that the changing pattern of dominants and subdominants reflected species differences in the demand for phosphate, their ability to derive supplies from labile soil phosphate, and in the ability of the roots to compete for phosphate.

Willoughby (1954) found in sown pastures of Lolium rigidum Gaud. (Wimmera ryegrass) and subterranean clover that the timing of applications of superphosphate and sulphate of ammonia had considerable effect on the composition of the herbage. The two species exhibited different potential growth responses over a range of temperatures. Wimmera ryegrass had a greater relative growth rate than subterranean clover during the cool winter months, but the growth rate of clover exceeded that of ryegrass during the warmer autumn and spring period. Granted suitable moisture conditions subterranean clover was better adapted to rapid early growth. The subterranean clover competed effectively with the grass for both nitrogen and phosphorus applied early. The ryegrass remained at a disadvantage unless nitrogen and phosphorus were applied later when the temperature had decreased.

There are several examples of major nutrients other than nitrogen and phosphorus influencing botanical composition in southern Australian pastures. Powrie (1967) found that the subterranean clover component of pasture herbage could be increased from 55 per cent to 80 per cent by the application of 30 kg/ha of elemental sulphur. The widespread incidence of sulphur deficiency in pastures in southern Australia has only recently been recognised (Coleman, 1966). Meadly, Rossiter and Willoughby disregarded the effects of sulphur in reaching their conclusions, although they had used fertilizers containing sulphur. Fitzpatrick and Dunne (1956) in Western Australia found that subterranean clover became the dominant species in pastures including brome grass, barley grass and capeweed after applications of potassium. This effect was confirmed by Lines (1963) and Burford (1967) in South Australia.

2.3.2 Plant attributes of some associated southern Australian pasture species

A number of the annual pasture species found in south-western West Australia have been studied as single plants in continuous flow solution culture (Asher and Loneragan, 1967; Asher and Ozanne, 1967). These species showed a range of nutritional responses as well as differences in the rate and depth of rooting. The ecological significance of these differences has not been fully assessed. It may be surmized, however, that an ability to utilize low levels of nutrients, or to maintain a high relative growth rate under nutrient stress, would confer an ecological advantage on a species. Similarly, rapid root growth would lead to early exploitation of the soil, again possibly giving an advantage to one species over another.

It is hazardous to extrapolate from attributes measured on single spaced plants to plant communities. Animal ecologists have found many examples where the response to an environmental factor by one species is modified by the presence of other species (Andrewartha and Birch, 1954), and similar modifications occur in plant communities (Donald, 1958). Nevertheless some useful information may be obtained with spaced plants. For example data on rate of downwards root growth reported by Asher and Ozanne (1966) for spaced plants agree with other observations on the degree of drought susceptibility of seedlings in pastures. Seedlings of subterranean clover and silver grass in which root penetration was found to be slow are known to be drought susceptible (Tiver, 1954), while capeweed, erodium and brome grass in which root penetration was found to be rapid are drought resistant (Tiver, 1954; Rossiter and Pack, 1956).

2.3.3 The agronomic effects of pasture instability

Changes in botanical composition are frequently the consequence of alterations to the environment (for example the addition of nutrients or grazing by livestock). Thus changes in the relative frequency of species are often confounded with changes in the total yield of the pasture. In the agricultural context both the total yield and pasture quality must be considered in relation to the costs of pasture production.

Legumes have been extensively used in the poor soils of the Australian continent as a source of nitrogen and soil organic matter (Underwood, 1951; Donald, 1960). While fertilizer nitrogen has fallen in price in recent years, it still remains expensive relative to the cost of nitrogen derived from legumes topdressed with superphosphate, and to the value of the products sold from the farm (Donald, 1965). It has been shown that the legumes also improve the quality of the herbage by having a higher nitrogen content (Allden, 1959), and sheep show increased live-weight gains when grazing legume-grass mixtures than when grazing grass dominant pastures (Rae *et al.*, 1963). On the other hand subterranean clover dominance may lead to an infertility problem in sheep known as 'clover disease' (Bennetts, Underwood and Shier, 1946).

The introduction of high yielding persistent grasses has so far met with indifferent success in drier areas (Trumble, 1949) and, until a persistent grass with suitable herbage characteristics is available, the aim of pasture management must be to maintain a stable high yielding mixture of the existing grasses and legumes.

3. General Methods

3.1 Origin and treatment of seed

Subsamples from the one lot of each kind of seed were used throughout the experiments.

3.1.1 *Bromus rigidus* Roth (hard brome)

The seed was separated from a mixture of grass seeds collected in 1967 from Kybybolite, in the south-east of South Australia. Eighty two per cent of the cleaned seed germinated after 3 days at 20°C.

3.1.2 *Trifolium subterraneum* L. cv. Mount Barker (subterranean clover)

The seed was obtained from a commercial source and graded for size. Only those seeds were used that passed a 2.5 mm diameter mesh but were retained on a 2.0 mm diameter mesh.

3.2 Method of measuring root length

3.2.1 Separation of roots from soil

Soil sampling methods varied with each experiment and are described in the experimental method sections. Root separation from soil samples was performed as follows:

i) The soil samples were air dried and ground in a 20 cm C & N mill using a coarse screen (diameter of mesh 4 mm). Each ground sample was thoroughly mixed before subsampling for root separation.

Subsamples varied in weight from 60 g to 120 g depending on the rooting density.

ii) Macro-organic matter was separated from the sandy soil by a flotation method similar to that of Barley (1955). Each sample was stirred rapidly in 500 ml water for 30 seconds before decanting the water onto a 250 mμ aperture sieve. Further water was added to the sediment, allowing the jet of water to stir the suspension. The water was again decanted. This was repeated until no further roots could be seen in the sediment; two or three additions of water usually proved adequate.

iii) The root segments, together with other macro-organic matter, were transferred from the sieve to a beaker with a fine jet of water, then onto a filter paper held on a porous plate under suction with a filter pump. A porous plate constructed of sintered nylon(1) was found to be highly permeable and easily cleaned. The flow was confined within a 5 cm x 5 cm perspex retainer placed on the filter paper. The roots and other material were drawn onto the paper as the water was sucked through the paper and plate. The perspex retainer was coated evenly with wax to prevent roots being held at the edge by surface tension. While still wet the filter papers were

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(1) VYON, manufactured by: Porous Plastics Ltd, Dagenham Dock,
Essex, England.

sprayed with a solution of 30% AQUADHERE[2] in water which fixed the roots and other material in position on drying.

3.2.2 Estimation of root length

Root length was determined using the line transect method of Newman (1966). The separate, mounted on the filter paper, was scanned with a x 20 binocular microscope using an 8 x 8 (4 x 4 cm) square grid to locate 64 transects. At each position on the grid the number of intercepts of a hair-line with axes of root segments was recorded. The hair-line, positioned in one eyepiece of the microscope, was rotated after each field was counted. Root segments were distinguished from the remaining macro-organic matter by their lighter colour and by their cylindrical form. Segments of roots produced during the experiment were distinguishable from those roots produced in previous seasons, the latter being darker and more irregular in shape.

Root length per separate, R, was calculated using equation (1) of Newman (1966)

$$R = \frac{\pi NA}{2H} \qquad (6)$$

where N is the number of intercepts counted, A is the area over which

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(2) An emulsion of polyvinyl acetate, supplied by Selleys Chemicals Ltd, 1 Gow Street, Bankstown, New South Wales 2200.

the separate is spread, and H is the total length of the transects. The root length per sample, R, was used to calculate

$$Lv_{\cancel{1}z} \quad = \quad Rb/w \qquad (7)$$

where Lv (cm^{-2}) is the root length per unit volume of soil at depth z (cm), b ($g.cm^{-3}$) is the bulk density of the soil, and w (g) is the dry weight of the soil sample; and

$$L_T \quad = \quad (1/l) \int_0^z Lv \, dz \qquad (8)$$

where L_T (cm^{-1}) is the total root length per plant, where z (cm) is the maximum depth of the root zone, and l (cm^{-2}) is the plant density.

The validity of the results obtained with the transect method was evaluated as follows: 50.0 cm and 100.0 cm lengths of Nylon monofilament, diameter 0.16 mm, were cut into segments, ranging in length from 0.3 to 1.2 cm, and dispersed in a 5 x 5 cm field. The x and y coordinates of the centres of transects were found using random number tables. These were used to locate an x - y stage of the microscope equipped with suitable scales. Intercepts of filament axes on each transect were recorded, and the total length and standard deviation of the length were calculated for increasing numbers of transects. The results are summarized in Table 3.1. Root length in the experimental samples ranged from 50 to 400 cm.

When measuring root length the transects were not positioned randomly but by reference to a grid. Newman (1966) had shown and

Table 3.1

Actual and estimated total length of segments of Nylon filament. [Estimates derived from equation (1) of Newman (1966)]

Number of fields viewed	Total length of filament (cm) Actual	Estimated	Standard deviation
10	50.0	72.4	27.0
20		61.3	7.5
40		51.8	12.1
60		49.9	7.9
80		48.8	6.0
100		50.5	5.6
10	100.0	86.5	69.5
20		106.6	25.5
40		98.5	17.2
60		101.5	13.4
80		102.8	7.4
100		102.5	6.8

Reicosky *et al.* (1970) has confirmed that there is no significant difference in the results obtained by the two methods of positioning the transect. By using a regular grid considerable saving in time for each determination was made.

3.2.3 Identifying the roots of each botanical component in the mixture

Root components in a mixture have previously been identified by labelling with radioisotopes and preparing autoradiographs (Neilson, 1964; Litav and Harper, 1967). A similar procedure was used in these experiments:

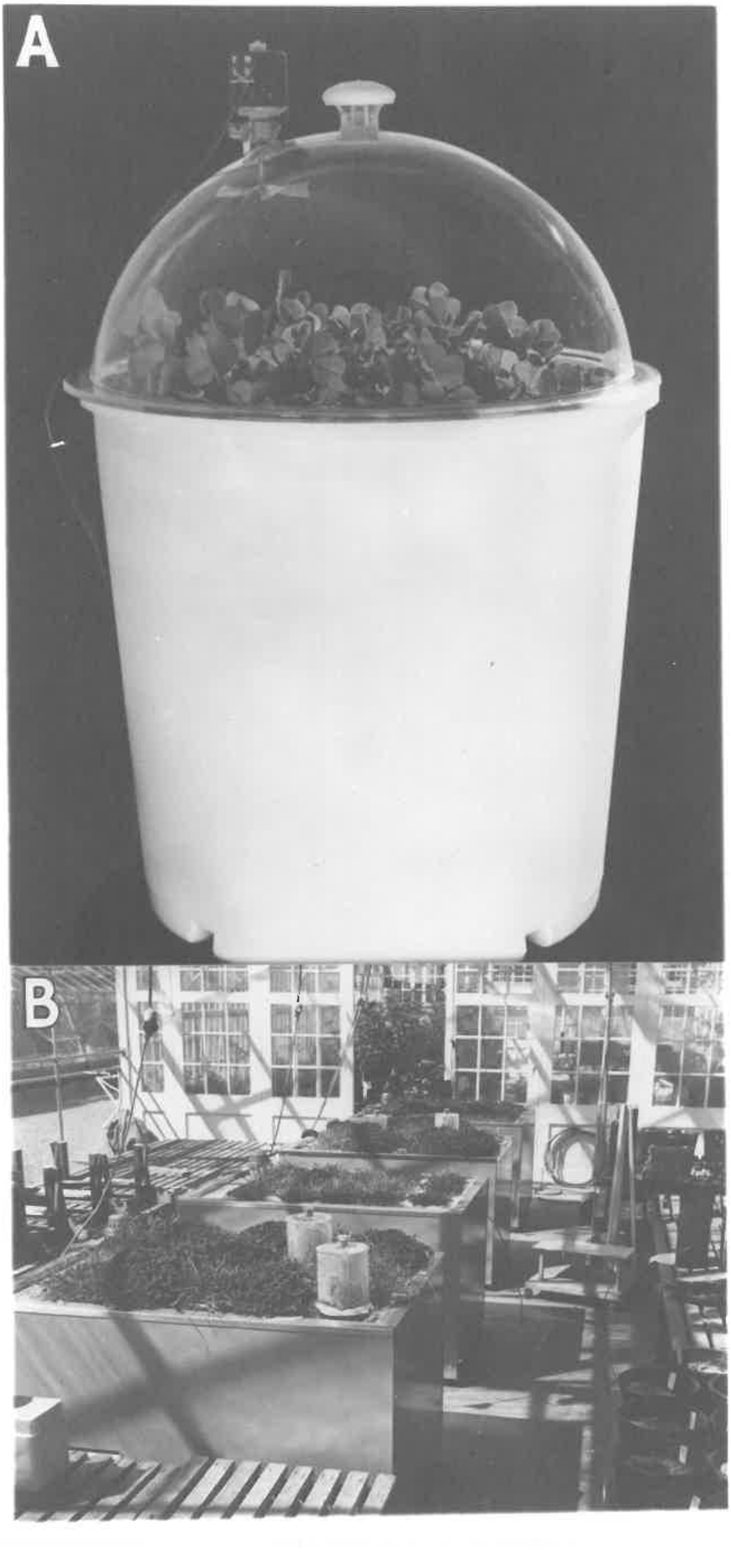
i) At each harvest one component of a mixture was cut at ground level and the tops of both the sample plants and border plants of that species were removed. The remaining plants were enclosed in a clear perspex compartment equipped with a fan to circulate the air (Figure 3.1). The compartment was sealed to the soil.

ii) Radioactive $C^{14}O_2$ was released in each compartment by the action of excess lactic acid on an aqueous solution of radioactive sodium carbonate. The radioactive sodium carbonate had a specific activity of 1.000 mc/millimole and was diluted to a radioactivity of 80 µm/ml of aqueous solution. Dose rate was calculated to give approximately 0.5 µc/plant in experiments II and III, and increased to 1.0 µc/plant in experiment IV because of the larger compartment volume. Precise doses are given in the experimental method sections.

The gas chambers (Figure 3.1) were covered to exclude light until the $C^{14}O_2$ had been released and circulated. The plants were then allowed to photosynthesise for one hour in daylight before removing the perspex cover of the chamber. The plants were harvested after a further 24 hours. Previous tests had shown that activity had reached the extremities of the root system by this time.

iii) Autoradiographs were made by exposing the root separate to KODIREX x-ray film for one week. The developed films were placed over the corresponding root separates and intercepts of the

__Figure 3.1.__ Perspex compartments with air-circulating fans used to expose plants to $C^{14}O_2$. Type A was used in Experiment II; type B was used in Experiment III. The large perspex container used in Experiment IV is not shown but is described in the text.


A
B

transects counted as in 3.2.2 (Figure 3.2). A two-channel tally counter was used to record the number of intercepts made by radioactive and by non-radioactive root segments. The labelled component was alternated in each replicate in experiments III and IV. The agreement between replicates was good. The sum of the lengths of the two components accounted for at least 90 per cent of the total root length.

3.3 Chemical analyses

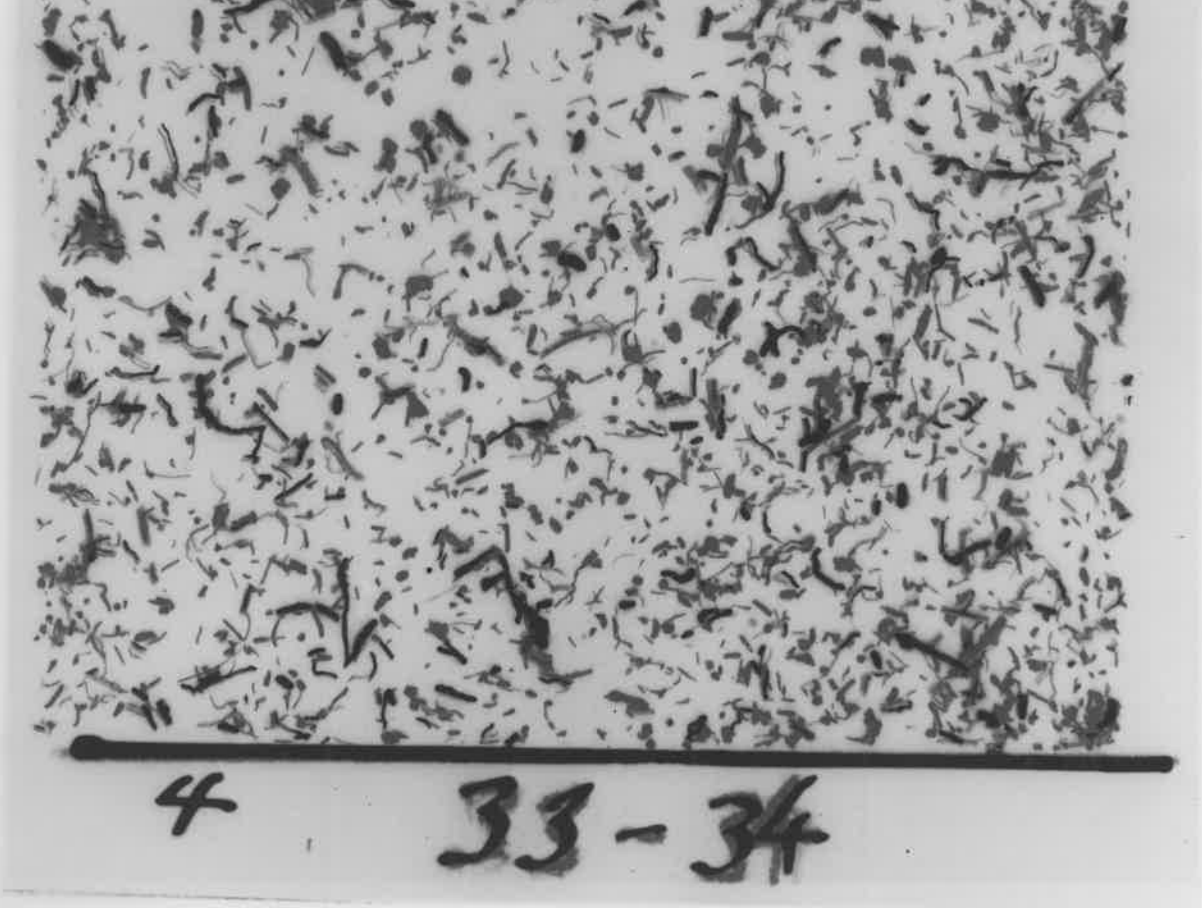
3.3.1 Digestion procedure

Plant material, oven dried at 90°C, was ground in a laboratory hammer mill to pass a 1 mm screen. The ground material was mixed, and a 0.5 g subsample was digested in a nitric-perchloride acid mixture as described by Johnson and Ulrich (1959). The extracts were made up to volume with distilled water (50 ml or 100 ml were appropriate). Blank digests were included in each run.

3.3.2 Determination of plant phosphorus

The extracts were analysed for phosphorus using the phosphovanado-molybdate complex method described by Hanson (1950). The developed colour was read on a UNICAM S.P.600 spectrophotometer at a wavelength of 390 μm using a 1 cm cuvette. The readings were calibrated with standard phosphate solution over the 1 ppm to 5 ppm range.

Figure 3.2. Photograph of macro-organic matter separated from soil and fixed on filter paper. The transparent overlay is an autoradiograph of the labelled root segments (x 3).


4
33 - 34

3.3.3 Determination of plant potassium

After suitable dilution the extracts were atomised in an air/acetylene flame on an atomic absorption spectrophotometer (Techtron AA4). Lithium chloride was added to both the samples and the standards to give a final concentration of 200 ppm Li in order to suppress interference from sodium.

3.3.4 Determination of plant sulphur

Plant sulphur levels were determined by the method described by Little and Reeve (1967). Sulphate in the digests was precipitated as lead sulphate in the presence of ethanol, and the residual lead in solution was measured using the atomic absorption spectrophotometer.

3.3.5 Soil phosphate soluble in 1N H_2SO_4

Air dried soil samples were ground in a 'Seibtechnik' mill, mixed and a 10 g subsample was extracted with 100 ml 1N H_2SO_4 overnight at room temperature (Powrie and Jennings, in press). The phosphate in the supernatant fluid was determined by the molybdate blue method of Watanabe and Olsen (1962). The colour reaction was read on a UNICAM S.P.600 at a wavelength of 830 μm. Samples with less than 0.5 ppm P present were measured in a 4 cm cuvette, otherwise a 1 cm cuvette was used.

3.4 Evaluating parameters of the competition models

3.4.1 4 parameter model

As noted in section 2.1.4 de Wit (1960) has proposed a two-species competition model, in which k_{12} and k_{21} are crowding coefficients defined by equations (4) and (5). In the following experiments examination of the data revealed that the yields had standard errors approximately proportional to their means. Following Thomas (1970) the appropriate equations are then:

$$Y_1 = \frac{m_1 k_{12} z_1}{k_{12} z_1 + z_2} (1 + \varepsilon_1) \qquad (9)$$

and

$$Y_2 = \frac{m_2 k_{21} z_2}{k_{21} z_2 + z_1} (1 + \varepsilon_2) \qquad (10)$$

Where ε_1 and ε_2 are normally distributed variates with zero mean and variances δ_1^2 and δ_2^2, and the other symbols are as defined earlier (section 2.1.4) except that m_1 and m_2 are estimates of M_1 and M_2, the monoculture yields. The unknown parameters in the model, m_1, m_2, k_{12} and k_{21} can be estimated by the least squares method (see Thomas (1970) equations 8, 9 and 10), as can δ_1 and δ_2. The estimates of m, k and δ, derived independently for each species in the model, provide information on the plant interaction (section 2.1.4).

3.4.2 3 parameter model

Subject to the condition $k_{12} = 1/k_{21}$, it follows that:

$$Y_1 = \frac{m_1 k_{12} z_1}{k_{12} z_1 + z_2} (1 + \varepsilon_1) \qquad (9)$$

and

$$Y_2 = \frac{m_2 z_2}{k_{12} z_1 + z_2} (1 + \varepsilon_2) \qquad (11)$$

A test for the hypothesis $k_{12} = 1/k_{21}$ has been described by Thomas. The assumption that the variance of the two sets of data is not significantly different must be tested first. An F ratio of the residual mean squares of the two species is calculated. If this statistic is less than the tabulated value at the selected significa~~nt~~ce level, the hypothesis is tenable.

Again the unknowns m_1, m_2 and k_{12} are found by least squares methods (Thomas, 1970; equations 13, 14 and 15). A second F ratio test is then made comparing the residual mean squares obtained using the 4 parameter model with those obtained with the 3 parameter model. If the F ratio is less than the tabulated value at the selected significance level, then the extra parameter has not improved the fit of the model to the data and the hypothesis that $k_{12} = 1/k_{21}$ is tenable.

4. Experiment I

4.1 Introduction

Experiment I was designed to measure changes in interference between brome grass and subterranean clover brought about by the addition of the nutrients P, K and S. Interference was measured by the crowding coefficients, k_{12} and k_{21}, derived from dry weights of the tops of these two species when grown in monocultures and mixtures. The two species were sown as a replacement series as described by de Wit (1960).

4.2 Methods and Materials

4.2.1 Design and treatments

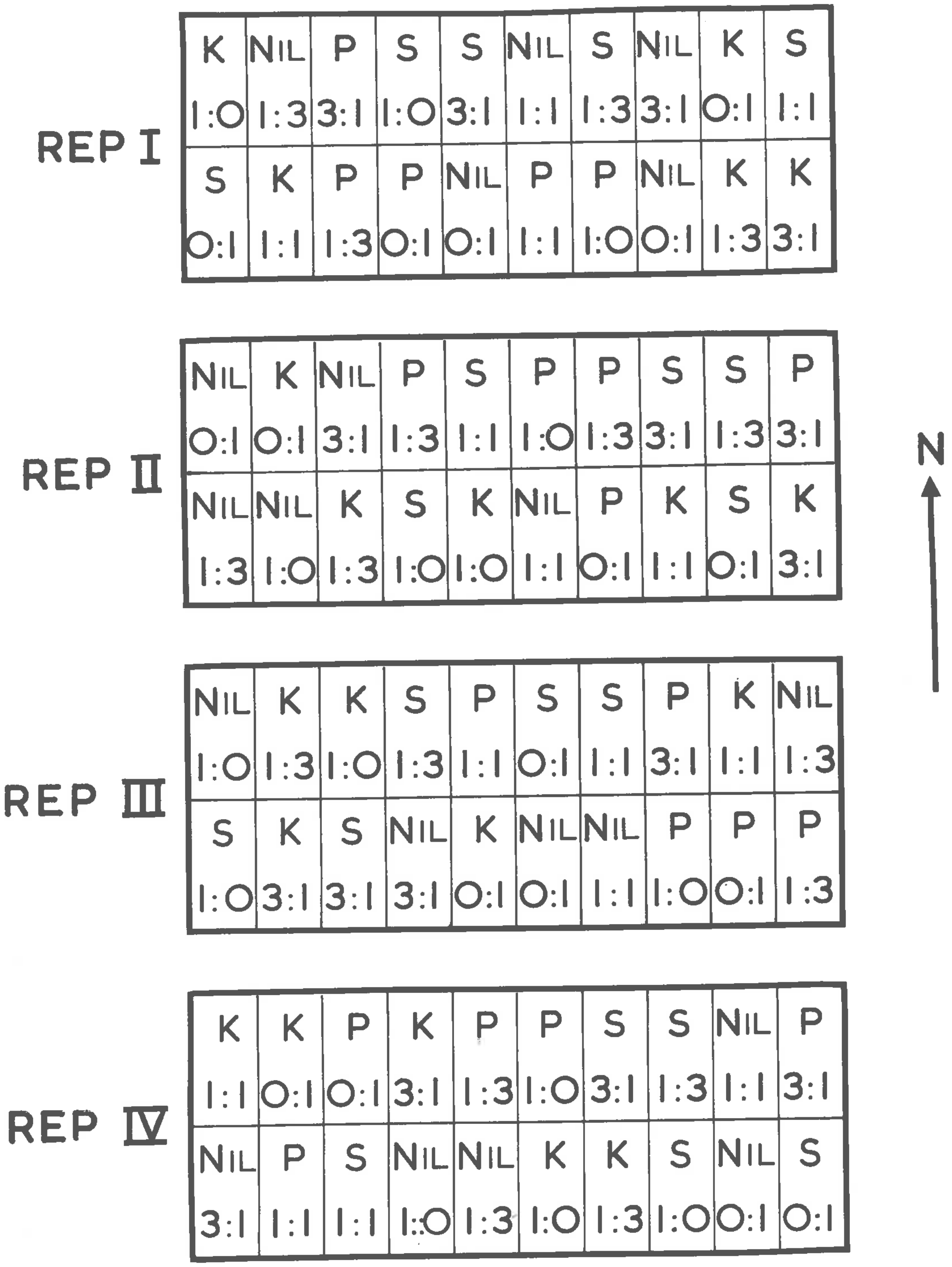
i) Plan. The two species were grown in monoculture and in mixture at three plant frequencies. The treatment combinations were:

Nutrient treatments		Seedling frequency		Replicates		Total No. of pots
4	x	5	x	4	=	80

Within each replicate the pots were fully randomized. Each replicate was sited on a separate bench. The experiment was conducted in an open-sided glasshouse. The layout is shown in Figure 4.1.

Brome grass and subterranean clover were established as a replacement series at a density of 0.3 plants/cm^2. The mixtures

<u>Figure 4.1.</u> The layout of the pots in Experiment I showing the treatments (Nil, P, K and S) and the seedling frequency shown by the seedling ratio (grass : clover).



REP I

K	NIL	P	S	S	NIL	S	NIL	K	S
1:0	1:3	3:1	1:0	3:1	1:1	1:3	3:1	0:1	1:1
S	K	P	P	NIL	P	P	NIL	K	K
0:1	1:1	1:3	0:1	0:1	1:1	1:0	0:1	1:3	3:1

REP II

NIL	K	NIL	P	S	P	P	S	S	P
0:1	0:1	3:1	1:3	1:1	1:0	1:3	3:1	1:3	3:1
NIL	NIL	K	S	K	NIL	P	K	S	K
1:3	1:0	1:3	1:0	1:0	1:1	0:1	1:1	0:1	3:1

REP III

NIL	K	K	S	P	S	S	P	K	NIL
1:0	1:3	1:0	1:3	1:1	0:1	1:1	3:1	1:1	1:3
S	K	S	NIL	K	NIL	NIL	P	P	P
1:0	3:1	3:1	3:1	0:1	0:1	1:1	1:0	0:1	1:3

REP IV

K	K	P	K	P	P	S	S	NIL	P
1:1	0:1	0:1	3:1	1:3	1:0	3:1	1:3	1:1	3:1
NIL	P	S	NIL	NIL	K	K	S	NIL	S
3:1	1:1	1:1	1:0	1:3	1:0	1:3	1:0	0:1	0:1

were composed in the proportions by number of grass to clover 0.75, 0.50 and 0.25. The plant arrangement in the mixtures is shown in Figure 4.2. The row direction within the pots was not standardized.

ii) Soil preparation and potting. A bulk sample of the top 10 cm layer of an acid sandy podsol, Northcote (1960) classification Uc 2.32, was collected from a pasture at Mount Compass, South Australia. The site (map ref. 163630 Barker S1 54-13) had been under a subterranean clover pasture for 22 years, during which time it had received approximately 2,000 kg/ha of superphosphate. Field responses to further applications of phosphorus and sulphur (Powrie, 1967), and to potassium (Burford, 1967) had been recorded in the season immediately preceding sampling.

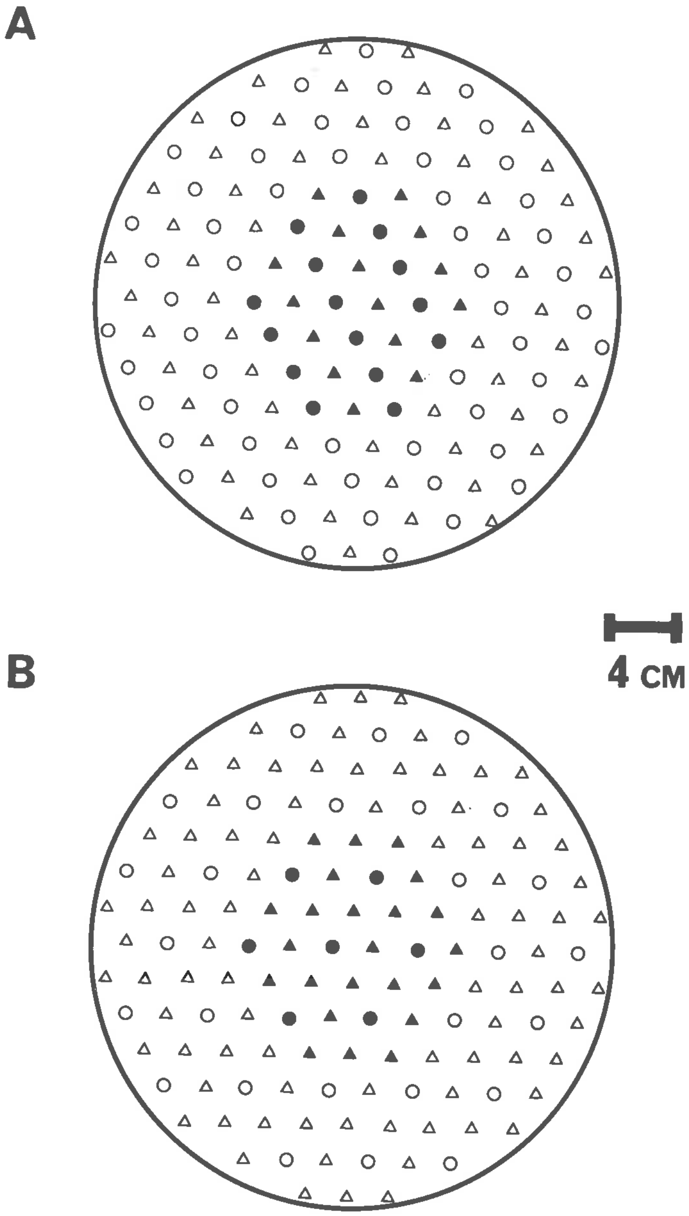
The soil was air dried, mixed, and passed through a 2 mm mesh to remove the larger pieces of organic material. The soil had the following properties:

pH	4.8 to 5.0	1 : 2.5 soil/water paste measured with a glass electrode
P	15 ppm	extracted with 1N H_2SO_4 (a)
K	80 ppm	extracted with 0.25N NaCl (b)
Bulk density	1.30 to 1.35	$g.cm^{-2}$

(a) Section 3.3.5

(b) Burford (1967)

<u>Figure 4.2.</u> Sowing pattern of brome grass (o) and subterranean clover (Δ) to give the seedling frequencies of grass 0.50 (A) and 0.25 (B). Solid symbols indicate harvested plants. The two species were transposed in (B) to give the 0.75 seedling frequency of grass. The monocultures were planted at each point in the grid, and harvested at all the points indicated by solid symbols.


A
B
4 cm

Thirteen kilograms of the soil was placed in each pot. The pots were 25 cm in diameter and 22 cm deep. They were free draining, the soil being placed on top of a layer of coarse gravel retained by a Nylon mesh. A volume of 2.0 litre of distilled water was added to each pot, this being 80 per cent of the amount retained after drainage of a liberally watered pot.

iii) Fertilizer treatments. The four fertilizer treatments were:

Treatment	Salt	Element mg/pot	 kg/ha
Nil	-	-	-
P	$Ca(H_2PO_4)_2.H_2O$	196	40
K	KCl	196	40
S	$CaSO_4.2H_2O$	196	40

A basal dressing of micronutrients was added to all pots as follows:

Treatment	Salt	Element mg/pot	 kg/ha
Cu	$CuSO_4.5H_2O$	3.5	0.71
Zn	$ZnSO_4.7H_2O$	3.5	0.71
Mo	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.5	0.10

The appropriate macronutrient, together with the basal dressing of micronutrients, were ground to a fine powder and thoroughly mixed into the top 10 cm of moist soil in each pot.

4.2.2 Establishment and management

i) Sowing. Seeds of brome grass and subterranean clover were soaked overnight. The clover seed was inoculated with an effective strain of Rhizobium before sowing. The sowing pattern was indicated by a template which made 5 mm indentations in the soil surface. Two seeds of the required species were sown in each position. After sowing the pots were covered for four days to promote germination and then thinned to the required density.

ii) Management. The pots were sown on 29th April, 1968, and harvested on 15th and 16th July, a growing period of 71 and 72 days. The weekly minimum and maximum shade temperatures over this period are given in Appendix Figure A1.

The soil was maintained at the desired water content by weighing the pots and adding distilled water. Initially this was done once a week but later this was done twice a week. A slight yellowing of the grass leaves was observed forty days after sowing, when 1.4 g/pot of NH_4NO_3 (100 kg/ha of N) dissolved in 100 ml distilled water was added to each pot. The shoots were also sprayed with a dilute solution of a systemic insecticide 'EKATIN'(1) at this time to control an aphid infestation.

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(1) Manufactured by Amalgamated Chemicals Victoria Pty, Melbourne.

4.2.3 Harvest procedure

At harvest the plants were cut at ground level from a sampling area centred in the pot. The plants were identified from the planting pattern in Figure 4.2. This gave a total of 30 plants for each harvest, and 7 plants of the minority component in the 0.25 frequency mixtures.

Leaf area, A, was measured on the fresh material with an electronic planimeter(1) (Wilkinson and Silsbury, 1966). The samples were then oven dried at 90°C and weighed. The dried plant material was ground in a hammer mill to pass a 1 mm screen. After mixing, a 0.5 g subsample was digested and analysed for total phosphorus, potassium and sulphur (see section 3.3).

4.3 Results

Negligible mortality occurred during the experimental period and apart from the slight yellowing of the grass, which responded to nitrogen, the plants appeared healthy.

An analysis of variance was performed on untransformed data, the levels of significance attained by the treatment effects are listed in Table 4.1.

4.3.1 Yield of tops

The yield of brome grass in monoculture did not respond to

---

(1) Manufactured by Paton Industries, Stepney, South Australia.

Table 4.1

Experiment I - Analysis of variance table showing significance of F ratio

Treatment	Dry weight of tops per plant	Leaf area/pot	% Nutrient in tops P	K	S
<u>Brome</u>					
Nutrient	***	(NS)	***	***	***
Frequency	***	**	(NS)	(NS)	(NS)
Nutrient x Frequency	*	(NS)	(NS)	(NS)	(NS)
<u>Clover</u>					
Nutrient	***	(NS)	***	***	***
Frequency	***	*	(NS)	**	*
Nutrient x Frequency	*	(NS)	(NS)	(NS)	(NS)

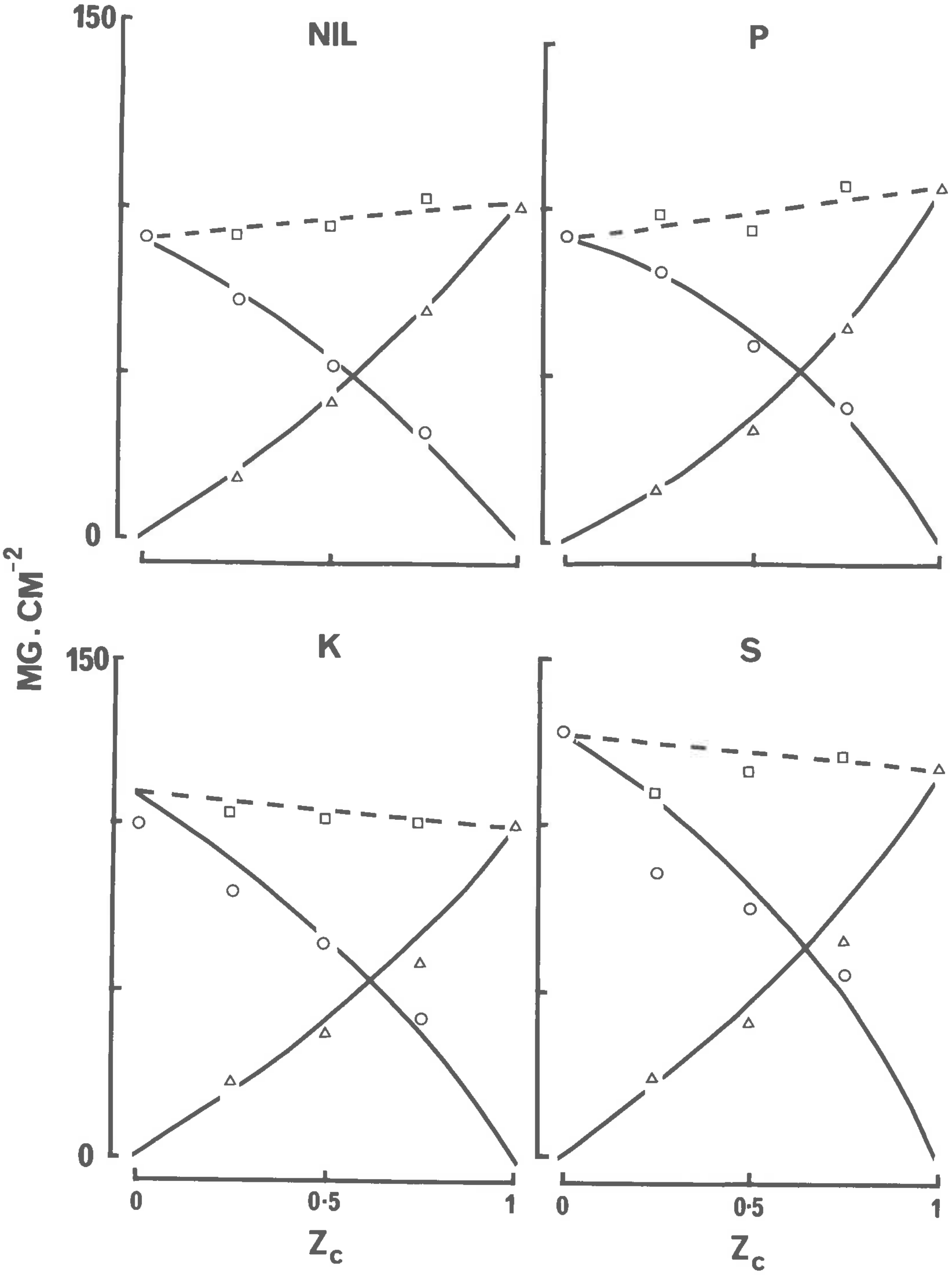
Level of significance

NS	Not significant
5%	*
1%	**
0.1%	***

applications of phosphorus or potassium but showed a highly significant increase ($P \leqslant 0.001$) over the nil treatment when sulphur was applied. The results for clover in monoculture were similar but the response to S less marked ($P \leqslant 0.05$). The yields per plant are given in Appendix Table A1, and yields per unit area are plotted as a replacement diagram in Figure 4.3.

The yield per plant of each species was affected markedly by the plant frequency. In all nutrient treatments the yield per plant

Figure 4.3. Replacement diagram showing the dry weight per unit area ($mg.cm^{-2}$) of brome grass (o), clover (Δ) and the total yield (□). Fitted lines are plotted from Equations (4 & 5) using the 4 parameter model from Table 4.2.


150
0
NIL
P
K
S
MG . CM⁻²
0
0·5
1
Zc

of clover was reduced when grown in association with grass, and the yield per plant of grass showed the reverse effect, being greater in the mixture than in the monocultures. This effect was also observed in the leaf area measurements (Appendix Table A2).

4.3.2 Mineral content of tops

Chemical analysis of the shoots indicated that the phosphorus concentration of neither species was affected by plant frequency (Table 4.1, Figure 4.4 and Appendix Table A3). In contrast the concentration of potassium (Table 4.1, Figure 4.4 and Appendix Table A4) and sulphur (Table 4.1, Figure 4.4 and Appendix Table A5) in the clover shoot was reduced in the presence of the grass, although the clover had little influence on the concentration of these nutrients in the grass.

4.3.3 Estimation of crowding coefficients

Both species showed significant changes in shoot dry weight per plant when grown in mixture. In order to summarize the effects of interference, the data were used to calculate the crowding coefficients, k, of the two species using equations 9 and 10. Single subscripts are used to identify the crowding coefficients for grass when mixed with clover, k_b, and for clover when mixed with grass, k_c. The calculated values for the 4 and 3 parameter models are given in Table 4.2.

<u>Figure 4.4.</u> The concentration of phosphorus, potassium and sulphur in the shoot of brome grass (o) and subterranean clover (Δ) at each of four plant frequencies (per cent dry weight).

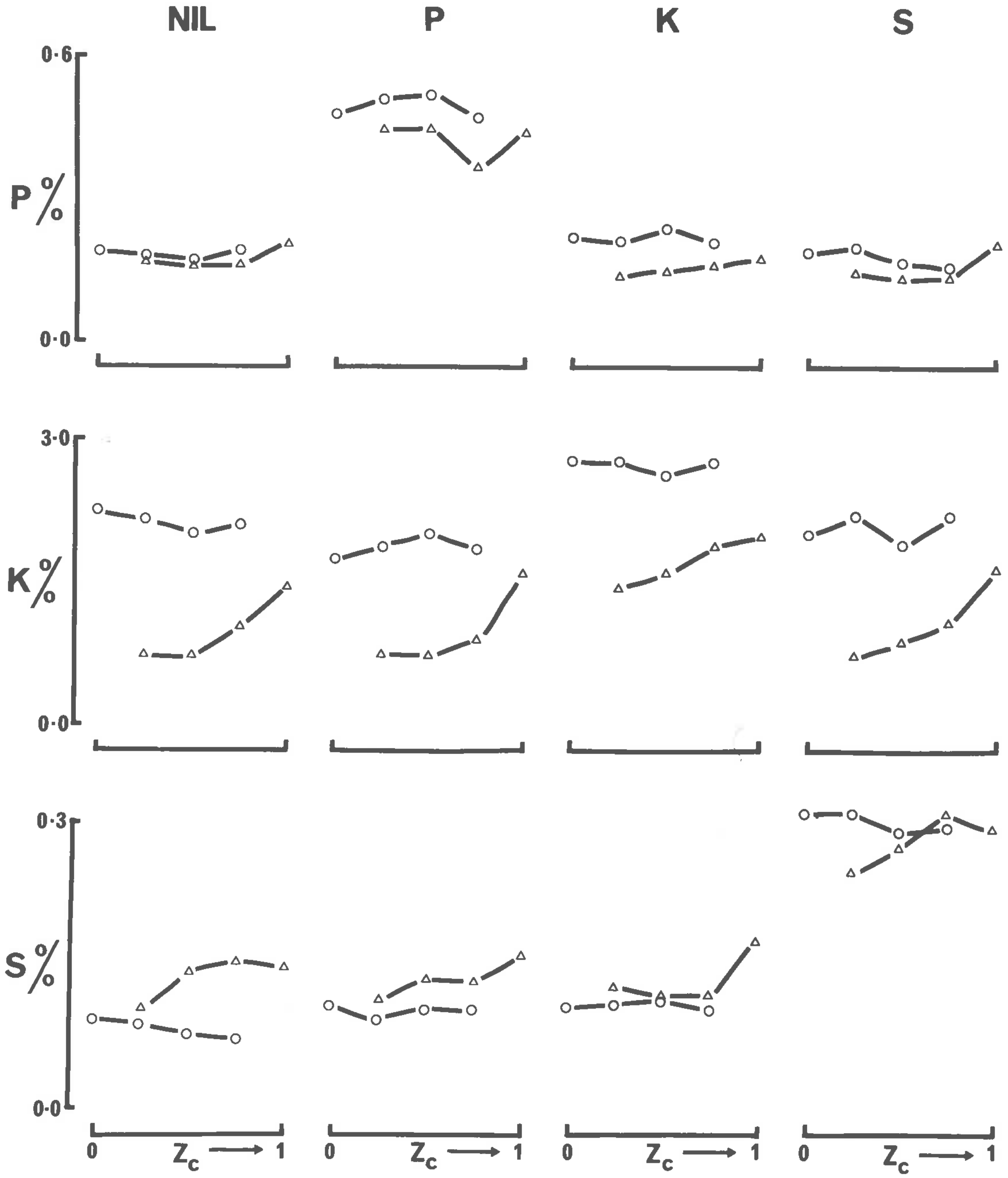

NIL
P
K
S
0·6
P %
0·0
3·0
K %
0·0
0·3
S %
0·0
0
Z_c
1

Table 4.2

Calculation of the crowding coefficient, k, for the
4 and 3 parameter models after de Wit (1960)
and Thomas (1970)

Treatment	No. of parameters in model	Crowding coefficients k_b	k_c	$k_b x k_c$	Between species F ratio 14,14 df	Between models F ratio 1,28 df
Nil	4	1.69	.71	1.20	2.06 (NS)	1.57 (NS)
	3	1.51	.66	1.00		
P	4	2.39	.51	1.21	1.56 (NS)	2.28 (NS)
	3	2.07	.48	1.00		
K	4	2.21	.87	1.92	1.60 (NS)	16.3 ***
	3	1.44	.70	1.00		
S	4	2.56	.79	2.03	1.64 (NS)	12.4 **
	3	1.58	.63	1.00		

The variances of the two species were not significantly different (between species F ratio, Table 4.2), so the further test of the condition $k_b.k_c = 1$ could be made using the procedure devised by Thomas (1970) and outlined in section 3.4). The test showed that the condition $k_b = 1/k_c$ was statistically invalid in two instances, as shown by the between models F ratio (Table 4.2). From Table 4.2, it can be seen that the condition $k_b.k_c = 1$ held for the Nil and P treatments but the condition $k_b.k_c > 1$ held for the K and S treatments. This implies that mixing the two species had enlarged the environment when K or S was added and overyielding had occurred.

However, inspection of Figure 4.3 shows that the model does not give a good fit to the data for the K or S treatments, nor is there any evidence of overyielding in the mixtures. De Wit's model assumes that k is independent of z. When k is calculated for each frequency (Table 4.3) it can be seen that the values of k for the K and S treatments increase with a decrease in z_b. The crowding coefficient appears to be a function of z ~~or~~ so that z is inadequately expressed in the model.

It would be better to have a model applicable to all seedling frequencies. Noting that, when $z_c = 0$, $k_b = 1$ and when $z_c = 1$, $k_b > 1$ the effect might alternatively be described by including z_c as an exponent of the crowding coefficient so that equations 9 and 10 become:

$$Y_1 = M_1 k_{12}^{z_2}.z_1/(k_{12}^{z_2}.z_1 + z_2) \qquad (12)$$

and

$$Y_2 = M_2 k_{21}^{z_2}.z_1/(k_{21}^{z_2}.z_2 + z_1) \qquad (13)$$

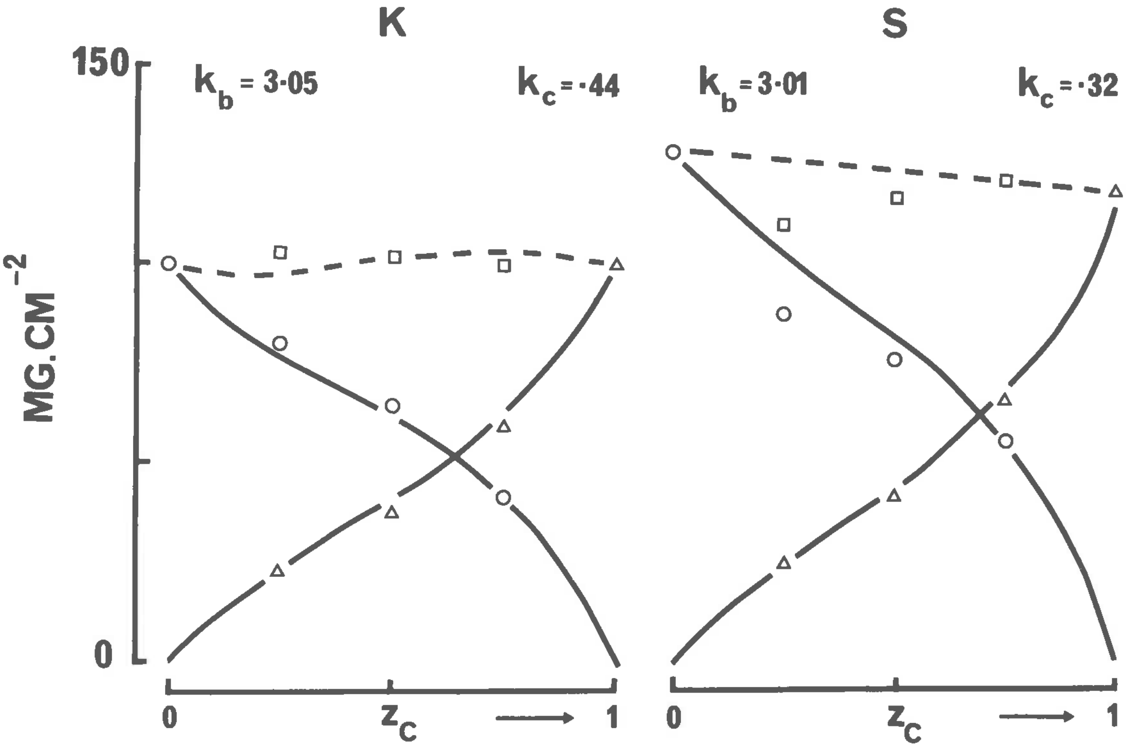
Substituting k_b and k_c for k_{12} and k_{21} in equations (12) and (13), and plotting the calculated yield per unit area as a replacement diagram, a good fit to the data appears to be obtained (Figure 4.5). It has not been possible however, to devise a method to test the fit

Table 4.3

Calculation of the crowding coefficient, k, for the 4 and 3 parameter models for each mixture proportion (z_b), after de Wit (1960) and Thomas (1970)

Treatment	No. of parameters in model	Crowding coefficients			Between species F ratio 6,6 df	Between models F ratio 1,12 df
		k_b	k_c	$k_b x k_c$		
Nil z_b = .75	4	1.38	.72	.99	1.07 (NS)	.001 (NS)
	3	1.39	.72	1.00		
= .5	4	1.42	.70	.99	16.1 **	
	3	1.43	.70	1.00		
= .25	4	1.70	.75	1.27	6.79 *	
	3	1.56	.64	1.00		
P z_b = .75	4	2.49	.53	1.31	2.83 (NS)	.23 (NS)
	3	1.91	.52	1.00		
= .5	4.	1.77	.46	.81	1.46 (NS)	1.29 (NS)
	3	2.13	.47	1.00		
= .25	4	2.46	.52	1.27	1.39 (NS)	1.91 (NS)
	3	2.15	.46	1.00		
K z_b = .75	4	1.37	.88	1.21	1.16 (NS)	.32 (NS)
	3	1.15	.87	1.00		
= .5	4	1.82	.59	1.07	2.08 (NS)	.14 (NS)
	3	1.73	.58	1.00		
= .25	4	2.15	.48	1.03	3.10 (NS)	.02 (NS)
	3	2.12	.47	1.00		
S z_b = .75	4	.67	.80	.54	6.23 *	
	3	1.16	.86	1.00		
= .5	4	1.42	.56	.79	7.70 *	
	3	1.71	.59	1.00		
= .25	4	2.32	.41	.96	1.05 (NS)	.08 (NS)
	3	2.38	.42	1.00		

Figure 4.5. Replacement diagram showing the dry weight per unit area (mg cm^{-2}) of brome grass (o) and clover (Δ) and total yield (□). Fitted lines calculated from Equations (12 & 13) using k values shown.


K
S
150
0
MG.CM^{-2}
$k_b = 3{\cdot}05$
$k_c = {\cdot}44$
$k_b = 3{\cdot}01$
$k_c = {\cdot}32$
0
z_c
1
0
z_c
1

of the data to this model statistically.

4.4 Discussion

The absence of response in total yield to applications of phosphate was unexpected. Powrie and Jennings (in press) reported responses in herbage yield by pastures grown on similar soils containing up to 30 ppm acid extractable phosphorus in the top 10 cm. As noted in section 4.2.1(ii) the concentration of acid extractable P in the sample of soil used for this experiment was 15 ppm. By using a greater than usual depth of top soil in this experiment, a sufficient total amount of extractable phosphorus may have been present in each pot to sustain growth. The responses to sulphur and potassium were nevertheless similar to those reported previously (Powrie, 1967; Burford, 1967).

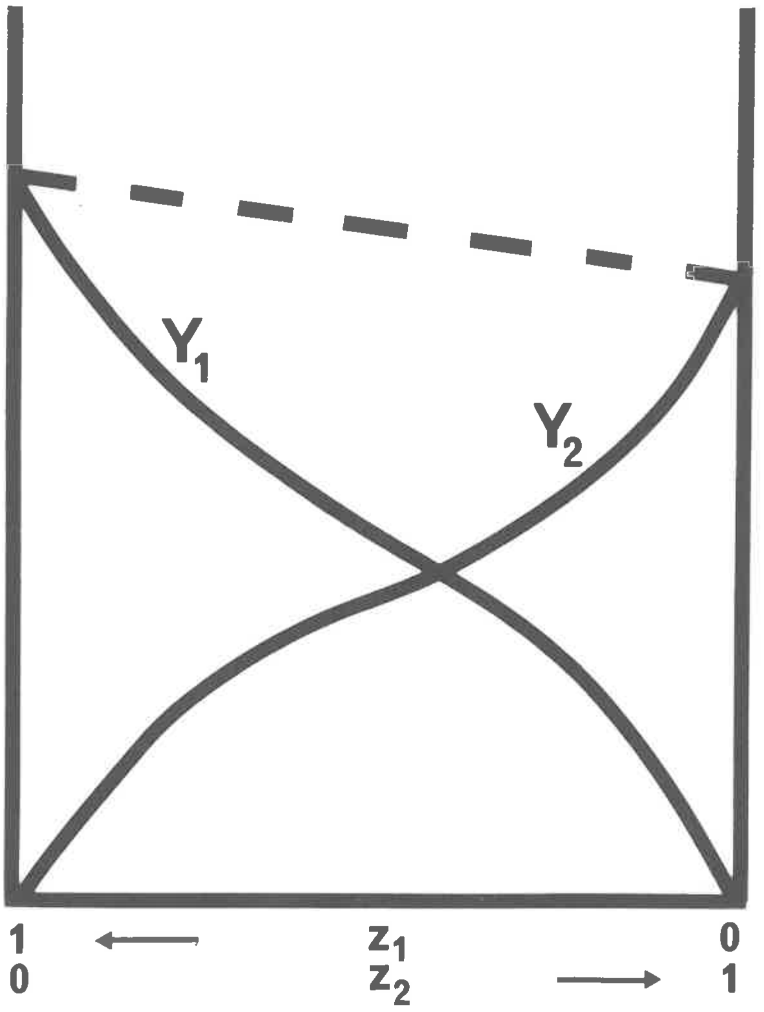
The application of phosphorus increased the relative yield of the grass in mixture, having made the grass more competitive with the clover. However the clover maintained a fairly uniform phosphorus concentration in its shoots at all values of z, both in the Nil and in the P treatments (Figure 4.4). This suggests that the change in competitive ability with the application of phosphorus may not have been due to competition for scarce supplies of phosphorus. Differences in total phosphorus per plant may result from differences in dry weight, arising from dissimilar response curves or competition for another essential resource, rather than from a change in the share of the total phosphorus supply available to the plants.

Frequency dependant responses to nutrients by components of a mixture have been reported elsewhere (de Wit, Tow and Ennick, 1966; Seaton and Antonovics, 1967). The possible mechanisms have been discussed in section 2.2.4. The simplest examples occur when the two components use different resources (case 3 in Figure 2.2). When total production becomes limited by some factor common to both components during the growing season, although the minority component may retain its earlier advantage, case 3, in which RYT > 1, is modified to conform with the condition RYT = 1 as shown in Figure 4.6 (Antonovics and Ford, 1972). The apparent frequency dependant response found when K or S was applied may have arisen from an early response to nitrogen by the grass in the mixtures of high legume content. Subsequent competition for light may have limited total yield and kept RYT = 1.

The concentration of potassium and sulphur in the clover was much reduced when grown in a mixture with grass. Earlier experiments have shown that certain other legumes are poor competitors with grasses for potassium (Blaser and Brady, 1950) and for sulphur (Walker and Adams, 1958).

Figure 4.6. Replacement diagram showing theoretical outcome of competition with a frequency dependent relationship favouring the minority component. Showing yields of components Solid line) and total yield (hatched line).

$k_{12} . k_{21} = 1$



After Antonovics & Ford 1972

5. Experiment II

5.1 Introduction

In Experiment I it was found that, even although the species were unresponsive to applied phosphate on the soil used when grown in monoculture, the level of phosphorus supply altered the crowding coefficients of the two species. The results were in agreement with those obtained previously by Rossiter (1964), who concluded that brome grass dominated subterranean clover at high levels of phosphorus supply, and that the reverse happened at intermediate levels of supply.

As phosphate fertilizers are widely used on the poor soils of southern Australia, Experiment II was designed to study the effects of phosphorus nutrition on competition between brome grass and subterranean clover in more detail. Observations were made at two growth periods and at two densities. Root length was measured as a plant attribute likely to be related to phosphate uptake, and to help interpret the nature of the competition. The soil used for Experiment II was a siliceous sand similar in its general properties to the soil used in Experiment I, but no fertilizer had been applied previously and its phosphate content was extremely low.

5.2 Materials and Methods

5.2.1 Design and preparation

i) Plan. Monocultures and 1:1 mixtures of grass and clover were grown at two densities and at two rates of applied phosphate. Sufficient pots were sown to provide for two harvests during the season. To reduce the total number of pots to a manageable number the high density treatment was replicated four times but the low density treatment was replicated only three times. The following combinations were used:

	P rates		Mixtures		Harvests		Replicates		No. of pots
Low density	2	x	3	x	2	x	4	=	48
High density	2	x	3	x	2	x	3	=	36
									84

Each replicate was sited on a separate bench in an open-sided glasshouse. Within each replicate the high and low density treatments were separated in two groups to reduce border effects. Otherwise the treatments were fully randomised within each replicate. The pots used for the second harvest remained in their positions after the first harvest was taken. The layout is shown in Figure 5.1.

ii) Soil preparation and potting. A bulk sample of the top 10 cm layer of a siliceous sandy soil, Northcote (1960)

Figure 5.1. Layout of treatments in Experiment II showing the phosphate treatments (P1 & P2), for brome grass (B), clover (C) and the mixture (M) for each harvest (1 & 2).

	HIGH DENSITY						LOW DENSITY					
REP I	P2 C2	P1 B1	P2 M2	P1 M1	P2 C1	P1 C1	P1 B1	P1 C1	P2 M1	P1 M1	P2 B1	P1 M1
	P1 M2	P2 B1	P1 B2	P1 C2	P2 B2	P2 M1	P1 B2	P2 B2	P2 M2	P1 C2	P2 C1	P2 C2
REP II	P1 M2	P2 M1	P1 B1	P2 M2	P2 B1	P2 B2	P2 B1	P1 M1	P1 M2	P1 C1	P2 M2	P2 M1
	P1 C1	P1 M1	P2 C2	P1 B2	P1 C2	P2 C1	P1 B2	P2 B2	P1 B1	P1 C2	P2 C2	P2 C1
REP III	P2 M1	P1 C1	P1 B2	P1 C2	P1 M2	P1 B1	P1 M2	P1 B1	P2 C2	P2 B1	P2 M1	P2 B2
	P2 B2	P2 C2	P2 B1	P1 M1	P2 M2	P2 C1	P1 C2	P1 M1	P2 C1	P1 C1	P2 M2	P1 B2
REP IV	P2 B1	P2 M1	P2 C2	P2 C1	P1 B2	P1 M1						
	P1 C1	P2 M2	P2 B2	P1 M2	P1 C2	P1 B1						

N

classification Dy 5.83, was collected from a recently cleared site near Keith, South Australia (map ref. 324562 Pinnaroo S1 54-14). The site had no history of fertilizer application. Similar soil in this area had shown marked responses to phosphorus and sulphur (Powrie and Jennings, in press).

The soil was air dried, mixed, and passed through a 2 mm mesh to remove the larger organic particles. The soil had a pH of 6.4, measured with a glass electrode on a 1:25 soil/water paste. The amount of phosphorus extracted with 1N H_2SO_4 was 3 to 4 ppm (section 3.3.5) and after ashing was 12 ppm (correlated to and a little less than total phosphorus (Piper, 1942)). The particle size analysis of the soil was as follows:

Percentage by weight retained on mesh of aperture shown

(μm)	% weight
> 1000	0.0
1000 - 500	4.0
500 - 250	35.1
250 - 125	47.7
125 - 63	11.5
< 63	1.6

As the soil was generally poor in mineral nutrients a basal dressing was added. This has the following composition:

Macronutrient	Element mg/pot	Element kg/ha
K	450	92
S	320	65
Ca	245	50
N	178	36
Mg	100	20
Micronutrient		
Mn	16	3.3
Zn	11	2.2
Cu	6	1.2
Fe	4	0.8
Bo	3	0.7
Mo	2	0.5

The nutrients were supplied as $Ca(NO_3)_2.4H_2O$ (1.5 g/pot); K_2SO_4 and $MgSO_4.7H_2O$ (1.0 g/pot); $MnSO_4.H_2O$ and $ZnSO_4.7H_2O$ (0.05 g/pot); $CuSO_4.5H_2O$; ferric citrate; $Na_2B_4O_7.10H_2O$ (0.025 g/pot) and $(NH_4)_2Mo_7O_{24}.4H_2O$ (0.005 g/pot). Sufficient salts for two pots were dissolved in two litres of water and mixed with 26 kg of soil in a cement mixer. This gave a moist, homogeneous soil that could be handled without separation of organic matter. Fourteen kg lots of the prepared soil were tamped into white plastic pots 25 cm in diameter and 22 cm deep (as used in Experiment I).

iii) Phosphorus treatment. Phosphorus was applied to the surface of the pots after sowing as a solution of monocalcium phosphate ($Ca(H_2PO_4)_2.H_2O$). The rates of P were:

	Element mg/pot	kg/ha
P1	63	13
P2	196	40

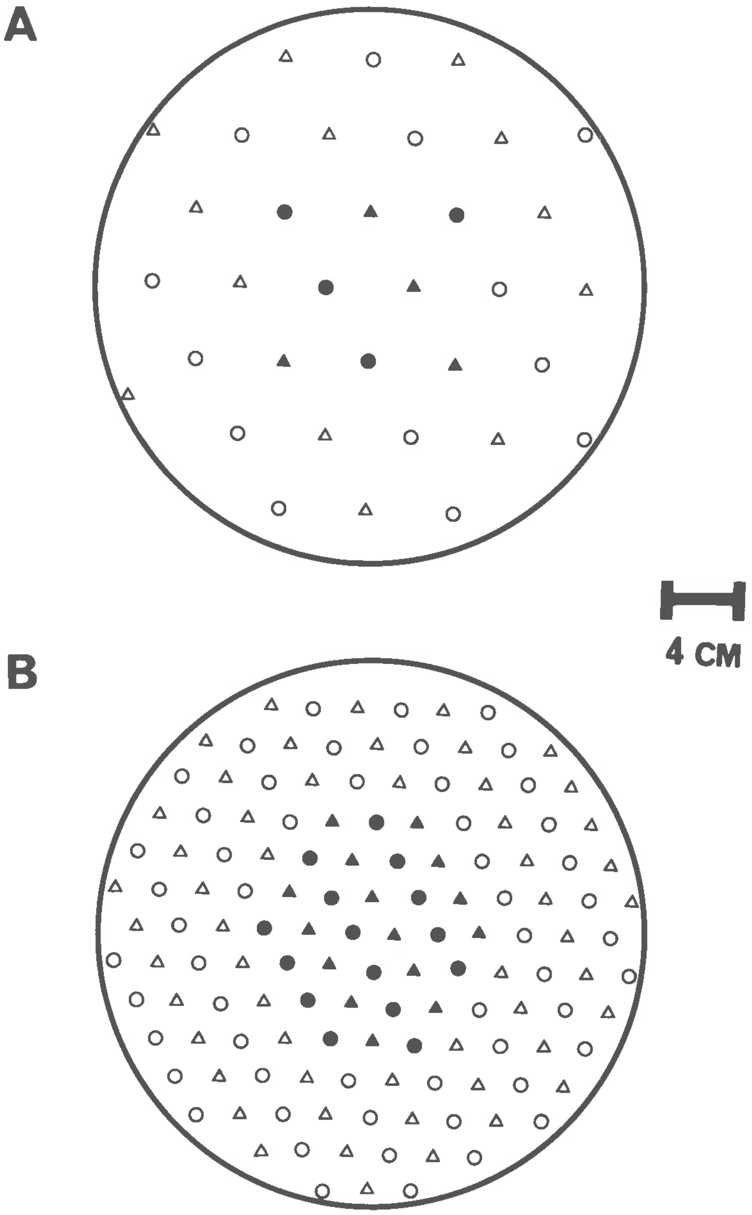
The salt was dissolved in 100 ml distilled water, and the solution was watered evenly onto the pot. The pots were then watered with a further 1.0 litre of distilled water to bring the gravimetric water content to 16 per cent.

iv) Seedling frequency. Brome grass and subterranean clover were sown in monocultures and 1:1 mixture at two total plant densities. The plants were arranged in a filled hexagon pattern, with the mixtures disposed as in Figure 5.2. Plants were 2 cm apart in the high density treatment and 4 cm apart in the low density treatment, giving 0.29 and 0.08 plants/cm^2 respectively.

5.2.2 Management

The pots were covered for the initial five days to keep the top soil moist and promote germination, after which the seedlings were thinned to the required density. The pots were weighed regularly, and any water lost was replaced by overhead watering with distilled water. This was done at weekly intervals initially, but later, as the plants grew and used more water, twice per week. In addition to the regular weighings, small uniform amounts of water were given to the pots daily during the last few weeks of the

Figure 5.2. Sowing pattern of brome grass (o) and subterranean clover (Δ) in mixture in Experiment II. The solid symbols represent the harvested plants at the low density (A) and high density (B). In the monocultures seedlings were planted at each point in the grid, and harvested at all points indicated with solid symbols.


A
4 CM
B

experiment. A further amount of nitrogen was added to the pots remaining after the first harvest at the rate of 0.5 g/pot of nitrogen (100 kg/ha of NH_4NO_3-N).

The experiment was sown on 9th and 10th September, 1968. The first harvest was made on 24th and 25th October, at 45 days, and the second harvest was made on the 21st and 22nd November, at 73 days. The minimum and maximum weekly shade temperatures over the growing period are given in Appendix Figure 1.

5.2.3 Harvest procedure

At harvest plants were cut at ground level, the plants for harvest being identified from the planting pattern (Figure 5.2). This gave 30 plants per pot in the high density treatment and 8 plants per pot in the low density treatment.

Leaf area was measured on the fresh material with an electronic planimeter (see section 4.2.3). The leaf area of the whole sample was determined at the first harvest. A sub-sample of approximately one-third of the total fresh weight was taken at the second harvest. After measurement of the leaf area the tops were oven dried at 90°C and weighed, and the total leaf area was calculated from the dry weight of the subsample and the total dry weight. The dried plant material was ground, and a 0.5 g subsample was taken and analysed for phosphorus content.

One component of the mixture was labelled, as described in section 3.2.3, by releasing 40 μc of radioactive $C^{14}O_2$ in a compartment of 4 litre volume (Figure 3.2A). By randomly allocating the component to be labelled within each replicate, each species was labelled in two replicates for each treatment at the high density and in two and in one replicate at the low density. Core samples were taken from the centre of each pot with a 7.5 cm diameter corer. Root length measurements were made on a bulk sample composed, in the monocultures, from all replicates for each of three layers sampled at depths of 0 to 2, 6 to 8 and 12 to 14 cm. In the mixtures samples were bulked for each labelled component.

5.3 Results

An analysis of variance was conducted on the untransformed phosphorus content and leaf area data, and on the logarithmically transformed dry weight data. The levels of significance established for the treatments and interactions are listed in Table 5.1.

5.3.1 Monocultures

i) Dry weight of shoots

The dry weight yields (Table 5.2) of shoots of both species showed a significant positive response ($P \leqslant 0.001$) to additional phosphate. Increased density significantly ($P \leqslant 0.001$) reduced

Table 5.1

Analysis of variance table. Dry weight and leaf area (A) data expressed per plant. Per cent P refers to content of shoot (per cent dry matter)

Treatment	df	Brome Log dry weight	Brome A	Brome % P	Clover Log dry weight	Clover A	Clover % P
Harvest (H)	1	***	***	***	***	***	***
Phosphate (P)	1	***	**	***	***	***	***
Frequency (z)	1	***	***	***	***	***	**
Density (D)	1	***	***	***	***	*	***
H x P	1	***	(NS)	***	(NS)	**	***
H x z	1	(NS)	*	***	(NS)	*	(NS)
H x D	1	***	***	***	***	**	(NS)
P x z	1	(NS)	(NS)	*	*	(NS)	(NS)
P x D	1	***	**	***	***	(NS)	*
z x D	1	*	(NS)	(NS)	(NS)	(NS)	**
H x P x z	1	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)
H x P x D	1	**	(NS)	***	(NS)	(NS)	(NS)
H x z x D	1	*	(NS)	**	(NS)	(NS)	(NS)
P x z x D	1	(NS)	(NS)	(NS)	(NS)	(NS)	*
H x P x z x D	1	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)
Residual	40						
Total	56						

Table 5.2

Dry weight of shoot per plant (mg). Log dry weight in brackets

Treatment		Harvest 1 Mono	Harvest 1 Mix	Harvest 2 Mono	Harvest 2 Mix
			Brome		
Low density	P1	72 (1.85)	58 (1.76)	499 (2.69)	551 (2.74)
	P2	132 (2.12)	153 (2.18)	633 (2.80)	815 (2.91)
High density	P1	44 (1.64)	54 (1.73)	170 (2.23)	208 (2.32)
	P2	50 (1.69)	67 (1.83)	191 (2.28)	226 (2.35)
			Clover		
Low density	P1	43 (1.63)	34 (1.53)	468 (2.67)	418 (2.62)
	P2	127 (2.10)	84 (1.92)	1086 (3.04)	837 (2.92)
High density	P1	37 (1.56)	31 (1.49)	188 (2.27)	171 (2.22)
	P2	73 (1.86)	50 (1.70)	344 (2.54)	286 (2.45)
		L.S.D. 1%	Within density	Low D	(0.12)
				High D	(0.10)
			Within P rate		(0.11)

yield per plant at both phosphate rates. A phosphate x density interaction ($P \leqslant 0.001$) arose from the relatively greater response to phosphate at the lower density by both species. By the second harvest the two densities had produced similar yields of tops per unit area at the P2 rate but not at P1.

ii) Leaf area

The leaf area per plant, A, (Table 5.3) of both species showed a significant response ($P \leqslant 0.001$) to additional phosphorus. The effect of phosphate on clover was especially marked. However the two species differed in their response to increased density. Brome grass showed a marked reduction in leaf area per plant while clover showed little change with density. Consequently, the grass maintained a relatively constant leaf area per unit ground surface (F) at the two densities, but clover F was low at low density and high at high density (Table 5.4).

iii) Root length

As replicates were bulked no estimate of error can be given. The length of root per plant, L, in each of the three layers measured are given in Table 5.5.

The grass almost invariably had a greater value of L than clover. Both species showed a reduction in L with an increase in density but were not affected by the phosphate rate.

Table 5.3

Leaf area per plant (cm^2)

Species	Treatment		Harvest 1		Harvest 2	
			Mono	Mix	Mono	Mix
Brome	Low density	P1	28	20	54	82
		P2	49	58	84	146
	High density	P1	13	16	19	30
		P2	14	25	24	37
			L.S.D. 1%	Low D	19	
				High D	14	
Clover	Low density	P1	8	4	30	24
		P2	30	12	69	54
	High density	P1	7	4	25	22
		P2	18	12	50	43
			L.S.D. 1%	Low D	15	
				High D	10	

Table 5.4

Leaf area per unit ground surface (F)(1)
at 73 days

Treatment		Monocultures		Mixtures		
		Brome	Clover	Brome	Clover	Total
Low density	P1	4.5	2.5	3.4	1.0	4.4
	P2	7.0	5.8	6.1	2.3	8.4
High density	P1	5.5	7.2	4.3	3.2	7.5
	P2	6.9	14.4	5.3	6.2	11.5

(1) $F = Al$, where A is the leaf area per plant and l is the plant density.

Table 5.5

Root length per plant in each layer (cm)

Layer	P1 0-2	 6-8	 12-14	P2 0-2	 6-8	 12-14
Harvest 1 (45 days)						
Low density						
Brome monoculture	282	60	72	282	89	53
mixture	208	31	20	220	15	46
Clover monoculture	183	34	46	163	34	36
mixture	232	35	11	217	8	9
High density						
Brome monoculture	132	44	23	130	68	51
mixture	119	15	14	109	22	29
Clover monoculture	114	33	17	85	14	10
mixture	97	25	13	80	25	27
Harvest 2 (73 days)						
Low density						
Brome monoculture	505	96	51	462	279	81
mixture	475	156	80	460	269	90
Clover monoculture	338	54	19	302	106	40
mixture	447	62	15	590	115	21
High density						
Brome monoculture	207	89	65	206	115	57
mixture	179	102	96	193	119	71
Clover monoculture	143	55	43	114	71	63
mixture	218	101	57	232	88	47

iv) Phosphorus content

Both species had very low levels of phosphorus in the shoots in the P1 treatment (Table 5.6). The addition of further phosphorus gave a significant response ($P \leqslant 0.001$) in phosphorus content at each harvest.

5.3.2 Mixtures

i) Dry weight of shoots

The dry weight per plant of both species was significantly ($P \leqslant 0.001$) altered when grown in mixture compared to monoculture. As in Experiment I the yield of the grass increased while that of clover decreased in mixtures. However the degree of interference varied with treatment. The competitive ability of brome grass, expressed as the crowding coefficient, k (Table 5.7), increased with the addition of phosphorus. The effect was similar to that observed in Experiment I, but the effect had diminished by the second harvest.

The hypothesis that $k_b = 1/k_c$ was tenable in all cases (Appendix Table A6), indicating that the species used a common environment, and that a response by one species caused a compensatory change in growth by the other.

ii) Leaf area

Both species showed a significant response ($P \leqslant 0.001$) to

Table 5.6

Concentration of P in shoots (%)

Species	Treatment		Harvest 1 Mono	Harvest 1 Mix	Harvest 2 Mono	Harvest 2 Mix
Brome	Low density	P1	.195	.246	.072	.060
		P2	.286	.381	.156	.142
	High density	P1	.123	.164	.057	.056
		P2	.305	.354	.140	.162
			L.S.D. 1%		Low D	.034
					High D	.030
Clover	Low density	P1	.140	.162	.083	.090
		P2	.224	.211	.106	.054
	High density	P1	.126	.104	.076	.056
		P2	.208	.195	.107	.081
			L.S.D. 1%		Low D	.027
					High D	.023

Table 5.7

Crowding coefficients calculated from shoot dry weight after de Wit (1960) and Thomas (1970) using 3 parameter model ($k_c = 1/k_b$)(1)

Treatment		Harvest 1 k_b	Harvest 2 k_b
Low density	P1	0.92	1.23
	P2	1.95	1.61
High density	P1	1.39	1.26
	P2	1.90	1.41

(1) Comprehensive data given in Appendix Table A6.

mixing. As with dry weight A of the grass increased while that of clover decreased in mixture.

iii) Root length

In mixture the grass generally showed a decrease in L. Clover, however, showed either little absolute change or an increase in L.

iv) Phosphorus content

At the first harvest brome grass had significantly ($P \leqslant 0.01$) higher concentrations of phosphorus in the shoot when in mixture than when in monoculture (Table 5.6). The difference in P concentration in the grass between mixtures and monocultures had disappeared by the second harvest. At this stage the clover usually had a lower concentration of phosphorus in the shoot in mixture than in monoculture.

5.4 Discussion

As in Experiment I brome grass became more competitive at P2. The amount of phosphorus present in the tops, derived from the dry weights and concentrations is shown in Table 5.8. The grass shoots contained more phosphorus per plant in mixture than in monoculture at P2. Conversely, the phosphorus uptake to the tops of clover showed a reduction in mixture at P2. At P1, when the grass was less competitive, mixing had relatively little effect on the phosphorus content of the shoot but where the differences

Table 5.8

Total phosphorus content of shoot per plant (μg)

Species	Treatment		Harvest 1		Harvest 2	
			Mono	Mix	Mono	Mix
Brome	Low density	P1	140	143	359	331
		P2	378	583	987	1157
	High density	P1	54	89	97	116
		P2	153	237	267	366
Clover	Low density	P1	60	55	388	376
		P2	284	177	1151	452
	High density	P1	47	32	143	96
		P2	152	104	368	232

existed they were generally in the same direction as at P2.

The relation between phosphorus uptake and competitive ability may be due to direct competition for scarce supplies of phosphate by the components of a mixture, to differences in growth response to phosphate affecting competition for some other factor, or to some combination of the two.

Competition for scarce phosphorus, as defined by Clements et al. (1929), has never been demonstrated unequivocally in an experiment. Cornforth (1968) and Andrews and Newman (1970) suggested that root density was the major factor influencing phosphorus uptake and that, due to the relative immobility of phosphate in soil, interference between roots was unlikely to occur. However, even in a soil in which phosphate is highly immobile, Lewis and Quirk (1967) demonstrated a clearly defined zone of phosphate depletion that extended around individual roots for a distance at least equal to the length of the roots' hairs. The L_v below a sward may reach a value where there is significant overlap of such depletion zones.

From probability theory Roach (1968) found that γ, the proportion of a plane covered by randomly distributed circular laminae of radius r, having n centres per unit plane is given by:

$$\gamma = 1 - \exp(-\pi r^2 n) \tag{14}$$

If the distribution of the root axes in the soil is assumed to be uniformly random, the number of axes intersecting unit plane n, is given by $n = L_v/2$ (Kendall and Moran, 1963). Neglecting the elliplicity of the intercepts made by the roots on unit plane, and assuming that the radius of the cylinder passing through the tips of the root hairs is 0.1 cm, the proportion of overlapping area (β) may be found from:

$$\beta = \pi r^2 n - \gamma$$

In the 0-2 cm layer in the mixture the total L_v was approximately 20 at the lower density and 30 at the higher density by the second harvest (Appendix Table A7). This corresponds to β values of 0.045 and 0.096. If there were greater movement of phosphate through the sandy soil used in this experiment so that the depletion zone were wider, for example, $r = 0.2$ cm, the β values for L_v values of 20 and 30 would be 0.54 and 1.04.

Dittmer (1938) found an L_v of 56 in the top 15 cm soil under a sward of the perennial grass Poa pratensis. The L_v values in this experiment do not appear to be unusually high for grasses judging from Dittmer's result, however few measurements of L_v under grassy swards have been made.

The apparent recovery of applied phosphorus, C (per cent) is given by:

$$C = 100(M_h - M_0)/H \qquad (16)$$

where H is the amount of P added as fertilizer, M is the amount of P in the plant, assuming root P is 0.25 of shoot P, when no fertilizer was applied (M_O) or when fertilizer was applied (M_h). In the absence of a nil data for the P_O treatment, Experiment IV has been used for M_O. By the second harvest the values of C varied between 17 and 33 per cent, indicating a moderate degree of depletion of the available soil phosphorus.

Brouwer (1963) suggested that an increase in root/shoot ratio was indicative of a limiting edaphic factor. Loneragan and Asher (1967) showed that the root/shoot weight ratio for brome grass and subterranean clover did in fact increase at low phosphate levels. In the experiment both species showed a general increase in root length/shoot weight ratio at lower phosphate levels.

The calculated percentage overlap of zones of depletion around roots, the degree of depletion of applied phosphorus, and the change in root/shoot ratio suggest that the two species may well have competed directly for phosphorus. On the other hand it is important to note that density had little effect on the competitive ability of the two species (Table 5.7). If the two species had been competing for a limited supply of phosphorus, then a reduction in density should have been comparable in its effect to an increase in phosphorus supply per plant. Secondly, differences in competitive ability were present at 45 days, indicating that changes in the growth of components in the mixture started at an early stage when less root interference would have occurred. Differences in P uptake in mixtures may alternatively have been caused by differences in growth response to the intensity of soil phosphate supply. A component of a mixture growing rapidly may, for example, compete successfully for light, thereby creating a larger sink for phosphorus.

It is probable that both direct competition for phosphorus and an indirect influence of phosphate supply on competitive ability affected yield of the components in mixture. Separation of the root systems when canopies are grown together might be expected to indicate the relative importance of these two effects.

A change in the crowding coefficients occurred with time (Table 5.7). The clover had become more competitive by the latter harvest. This effect could conceivably have been due to physiological changes brought about by differences in the ontogeny of the two species. However the experiment was conducted largely during the vegetative phase of growth, stem elongation having begun in the grass only just before the final harvest. During the latter half of the experiment the mean weekly ambient air temperature rose by 5°C (Appendix Figure 1). The change in crowding coefficients could have been due to different growth responses to temperature by the grass and clover components. Differences of this kind between annual grasses and subterranean clover have been suggested previously by Willoughby (1954), and the effects of temperature will be examined further in the next experiment.

6. Experiment III

6.1 Introduction

Experiment II showed that the crowding coefficient of brome grass and subterranean clover altered during the season. This change was accompanied by an increase in ambient air temperature (Appendix Figure A1). Willoughby (1954) considered that the seasonal temperature prevailing during the time when there was sufficient moisture for growth affected the botanical composition of Wimmera ryegrass/subterranean clover swards. Brouwer (1964) showed that root temperature had a marked influence on plant growth rate. This change in growth rate has been attributed to the effect of root temperature on the supply of water to the leaves (Kuiper, 1964), to its effect on the rates of nutrient uptake (Brouwer and van Vliet, 1960), and, more specifically, to effects of temperature on the production of kinins in the root (Atkin and Barton, 1970).

The temperature of the roots in Experiment II would have been close to the mean ambient air temperature in the open-sided glasshouse.

Experiment III was designed to determine whether soil temperature affected the crowding coefficients of the two species by growing them in mixtures and monocultures over a range of soil temperatures. The temperatures chosen were within the range of soil temperatures found during the growing season in the pasture regions of southern Australia (Appendix Figure A2).

6.2 Materials and Methods

6.2.1 Design and treatments

i) Plan

Mixtures and monocultures of brome grass and subterranean clover were sown at a constant total plant density at two rates of applied phosphate and at four soil temperatures. Due to lack of sufficient controlled temperature tanks, it was not possible to replicate the temperature treatments. A split pot design was used in which the phosphate and mixture treatments were arranged as a completely randomized block within each temperature tank. The array of treatments and replicates comprised 72 pots, arranged as shown below:

Mixtures		Phosphate rates		Replicates		Sub-total
3	x	2	x	3	=	18
		Temperatures		Sub-total		Total
		4	x	18	=	72

The layout of the experiment is given in Figure 6.1 and a general view of the experiment is shown in Figure 6.2. The experiment was conducted in an open-sided glasshouse. The mean weekly maximum and minimum air temperatures from the Waite Institute meteorological

Figure 6.1. The layout of pots in the temperature tanks. The phosphate rates (P1 and P2) and botanical composition (Brome - B, Clover - C, and Mixture - M) were arranged randomly within each replicate.

BATH 1 (18°C)

REP I

REP II

REP III

P2 M	P1 C	P1 M	P2 C	P1 M
P2 B	P1 B	P2 C	P2 M	P1 B
P2 B	P1 M	P1 B	P1 C	
P2 C	P2 M	P1 C	P1 B	

BATH 2 (10°C)

P1 C	P1 M	P2 C	P1 B	P1 C
P1 B	P2 B	P2 M	P1 M	P2 M
P1 C	P2 B	P2 M	P2 C	
P1 M	P2 C	P1 B	P2 B	

N ↑

BATH 3 (22°C)

P2 C	P1 C	P1 M	P2 C	P2 M
P2 M	P2 B	P1 B	P1 M	P1 B
P1 B	P1 M	P2 B	P2 B	
P2 M	P1 C	P2 C	P1 C	

BATH 4 (14°C)

P2 B	P1 B	P1 M	P2 C	P2 B
P2 M	P1 C	P2 C	P1 C	P1 B
P2 B	P1 M	P2 C	P1 M	
P2 M	P1 C	P1 B	P2 M	

Figure 6.2. A general view of the temperature tanks facing south. Note the uneven pattern of shade from the roof members.

station over the duration of the experiment are given in Appendix Figure A1. Air temperatures were recorded in the glasshouse over part of the experimental period. The minimum air temperatures recorded in the glasshouse were similar but the maximum air temperatures were approximately 10 per cent greater than those recorded at the meteorological station.

ii) Soil preparation and potting

The soil, the basal fertilizer dressing, and the handling procedure were the same as described in Experiment II (Section 5.2.1(ii)). Metal pots, 18 cm in diameter and 35 cm deep, were coated with a bituminous paint and lined with a polyethylene tube sealed at the lower end. A 12.5 kg lot of the moist prepared soil was tamped into each pot to a dry bulk density of 1.3 g/cm^3.

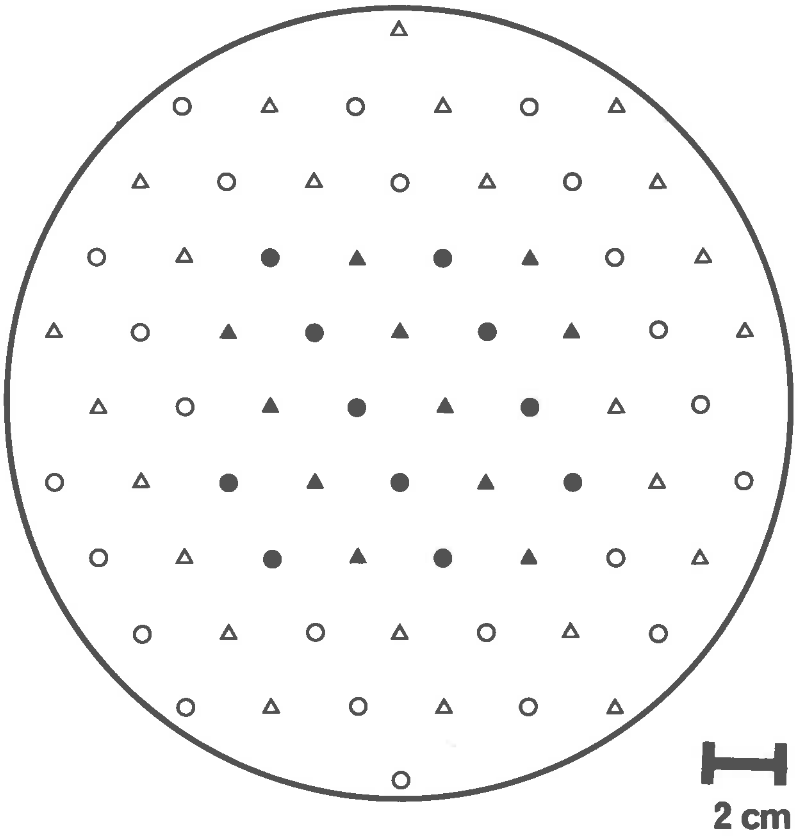
iii) Botanical composition

Brome grass and subterranean clover were sown in monoculture and in 1:1 mixture at a constant total plant density of 0.3 plants cm^{-2}. The plants were sown in a filled hexagon pattern (Figure 6.3). This corresponded to the density and pattern used in Experiment I and in the high density treatment in Experiment II.

iv) Phosphate treatment

Monocalcium phosphate ($Ca(H_2PO_4)_2.H_2O$) was applied in solution to the surface of the pots after sowing. The rates of

Figure 6.3. The sowing pattern of brome grass (o) and subterranean clover (Δ) in mixture in Experiment III. The solid symbols indicate harvested plants and open symbols indicate border plants. In the monocultures seeds were planted at each point in the grid, and harvested at all the points indicated with solid symbols.


2 cm

addition of phosphorus were:

	$Ca(H_2PO_4)_2.H_2O$ mg/pot	P mg/pot	P kg/ha
P1	135	33	13
P2	415	102	40

v) Temperature treatment

Except during the germination period when all tanks were held at 18°C, the tanks were maintained at temperatures of 10, 14, 18 or 22°C. Four stainless steel tanks equipped with reverse cycle refrigeration units and stirrers were used to control the temperature of water surrounding the pots. Twenty pots were arranged in each tank on an open-mesh base so that the water level was 2 cm from the top of the pot and level with the surface of the soil within the pot. The water was circulated below and around the pots. The water surface was insulated from the air with a 2 cm thick layer of expanded polystyrene beads. This also reduced evaporation from the tanks. The water temperature in the tanks was monitored continuously and ranged within ± 1°C of the set temperature, except for the 22°C level at which the water cooled to 20°C on several occasions during cold nights.

Marked temperature gradients were measured in the pots with decreasing soil depth. Fluctuations in the temperature of the soil surface were reduced by a 1 cm layer of coarse gravel applied carefully 15 days after sowing. Thereafter the soil temperature at a depth of 1 cm varied by not more than ± 1°C from the set water temperature.

Dry bulb temperature 2 cm above the top of the insulation and centrally above each tank was close to the air temperature in the glasshouse. However the wet bulb temperature over the tanks at this height showed a smaller depression than the glasshouse air. The corresponding increase in relative humidity above that of the glasshouse air was as high as 12 per cent over the warmest tank. No difference in wet bulb reading from glasshouse air was detected above the coolest tank; conditions over the other tanks being intermediate.

6.2.2 Management

The pots were sown as in Experiments I and II (section 4.2.2(i)) and covered for five days. During this period the four tanks were held at 18°C. After this the plants were thinned to the desired number. The temperature controls on the tanks were then set at either 10, 14, 18 or 22°C, these temperatures being attained in the water baths within eight hours. Fifteen days after sowing the pots were mulched with 300 g per pot of a coarse gravel to insulate the surface of the pot.

The pots were maintained at a gravimetric water content of 16 per cent by weighing the pots and adding distilled water. The pots were weighed once a week initially, and later twice weekly. In addition a uniform volume of distilled water was added to the pots daily during warmer weather. A second application of nitrogen at the rate of 140 kg/ha as $Ca(NO_3)_2.4H_2O$ (3.0 g per pot) was watered in on the 29th day.

The experiment was sown on 4th August and harvested on 9th and 10th October, 1969 after a growing period of 67 to 68 days.

6.2.3 Harvest procedure

At harvest twenty two plants were cut at ground level, the plants having been identified from the pattern shown in Figure 6.3. Fresh weight, leaf area, and dry weight measurements were made on the shoot samples.

One component of the mixtures was labelled, as described in section 3.2.3, by releasing 40 μc of radioactive $C^{14}O_2$ in a 4.5 litre compartment (Figure 3.2(b)). Root length estimations were made on the 0 to 2, 2 to 4, 4 to 6, 6 to 8 and 12 to 14 cm layers, using cores cut from the centre of each pot with a 7.5 cm diameter corer.

The dried shoot material was ground, subsampled and analysed for phosphorus (section 3.3.2). The soil remaining from the root length determinations was bulked by replicates and analysed for soil phosphorus soluble in 1N H_2SO_4 (section 3.3.5).

6.3 Results

An analysis of variance was conducted on the untransformed data. The levels of significance found are listed in Table 6.1.

6.3.1 Monocultures

i) Dry weight

Unexpectedly, subterranean clover gave no response to an increase in phosphate supply, and brome grass showed only a small response ($P \leq 0.05$) to additional phosphate (Tables 6.1 and 6.2). Increasing the soil temperature from 10 to 22°C doubled the dry weight of the tops of both species.

ii) Fresh weight and leaf area

Both species showed large increases in fresh weight and leaf area with increasing soil temperature (Tables 6.3 and 6.4). Additional phosphate produced a small but significant increase in these attributes in clover. The grass showed a temperature x phosphate interaction where the addition of phosphate gave a decrease in fresh weight and leaf area at 10°C but showed an increase at 22°C.

iii) Root length

The root length per plant in each of five soil layers is

Table 6.1

Analysis of variance table⁽¹⁾. Dry weight of shoot, leaf area (A), and shoot-P expressed per plant

Treatment	df	Dry weight	Fresh weight	A	Shoot-P %	Shoot-P Total
Brome						
Temperature (T)	3	***	***	**	(NS)	**
Reps within temp.	8					
Phosphate (P)	1	**	*	***	***	***
Frequency (z)	1	***	***	***	***	***
T x P	~~1~~ 3	(NS)	*	*	(NS)	(NS)
T x z	3	(NS)	(NS)	(NS)	(NS)	**
P x z	~~3~~ 1	**	***	(NS)	(NS)	**
T x P x z	3	(NS)	(NS)	(NS)	(NS)	(NS)
Residual	24					
Total	47					
Clover						
Temperature (T)	3	***	***	***	***	***
Reps within temp.	8					
Phosphate (P)	1	(NS)	***	***	***	***
Frequency (z)	1	***	***	***	(NS)	*
T x P	~~1~~ 3	(NS)	(NS)	(NS)	(NS)	(NS)
T x z	3	**	***	*	(NS)	***
P x z	~~3~~ 1	(NS)	(NS)	(NS)	(NS)	(NS)
T x P x z	3	(NS)	*	(NS)	(NS)	(NS)
Residual	24					
Total	47					

(1) Mean squares are given in Appendix Table A8.

Table 6.2

Dry weight of shoots per plant (mg)

Treatment		Brome Mono	Brome Mix	Clover Mono	Clover Mix
10°C	P1	123	168	110	89
	P2	109	169	120	82
14°C	P1	141	171	176	141
	P2	147	206	154	127
18°C	P1	181	207	220	194
	P2	209	225	216	191
22°C	P1	246	221	248	259
	P2	253	241	299	294

	Brome	Clover
L.S.D. 1%(1)	12	15

---

(1) L.S.D.'s corresponding to the subtreatments in this and subsequent tables are given as a guide even though second order interactions occur only occasionally (see Table 6.1).

Table 6.3

Fresh weight of shoots per plant (mg)

Treatment		Brome		Clover	
		Mono	Mix	Mono	Mix
10°C	P1	650	968	629	444
	P2	528	1023	808	442
14°C	P1	683	1158	1102	765
	P2	757	1315	1004	648
18°C	P1	892	1158	1269	1050
	P2	856	1258	1412	1346
22°C	P1	1088	1192	1490	1408
	P2	1182	1281	1827	1453

	Brome	Clover
L.S.D. 1%	63	71

Table 6.4

Leaf area per plant (cm^2)

Treatment		Brome		Clover	
		Mono	Mix	Mono	Mix
10°C	P1	20.6	27.5	15.6	11.3
	P2	15.6	30.6	18.8	11.3
14°C	P1	21.2	33.7	28.8	22.6
	P2	21.8	40.6	28.8	19.4
18°C	P1	25.0	36.8	36.3	30.0
	P2	26.3	38.7	38.7	38.1
22°C	P1	33.1	35.0	45.6	41.9
	P2	36.9	46.3	50.6	43.8

	Brome	Clover
L.S.D. 1%	6.3	7.1

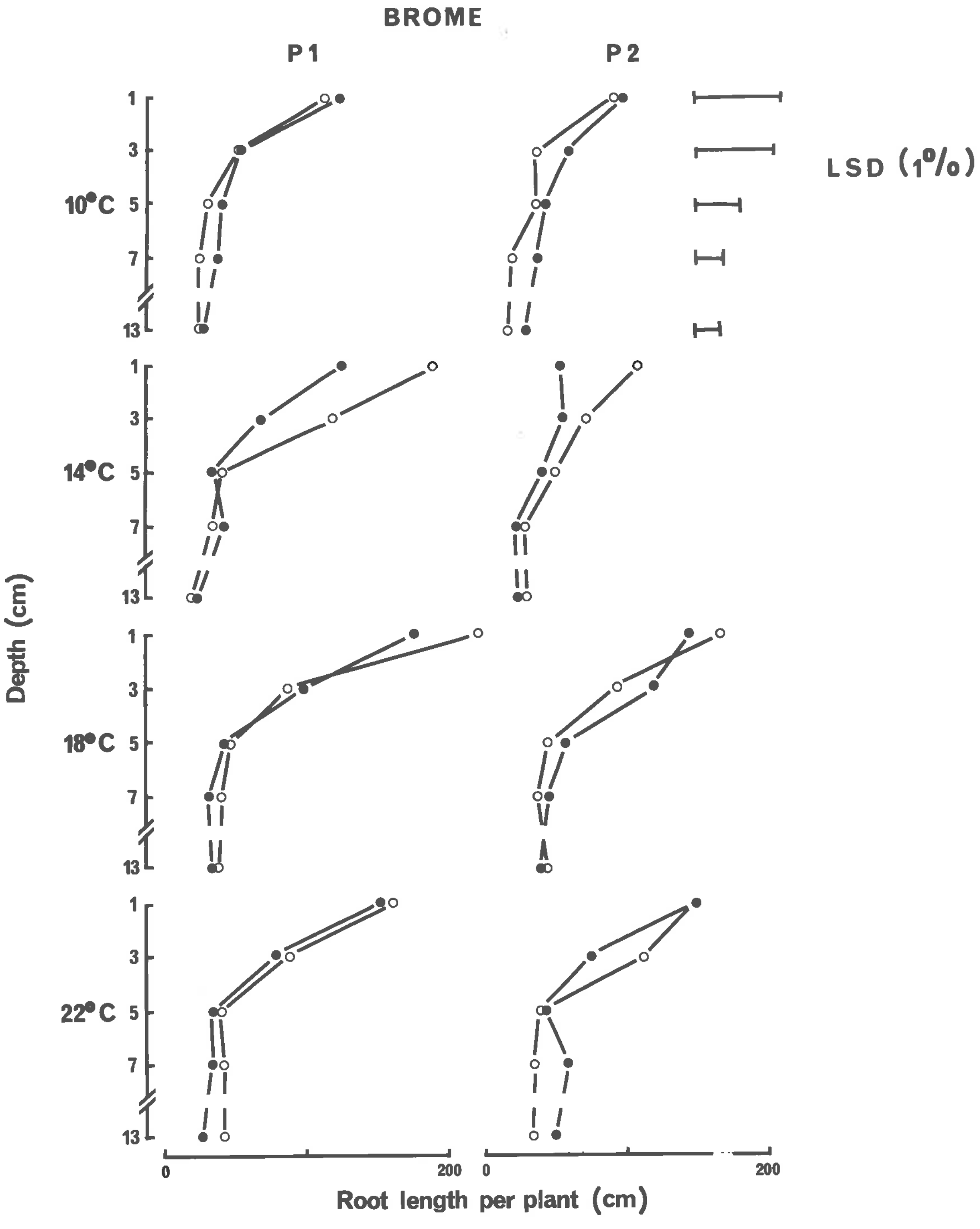
shown in Figure 6.4. The results of an analysis of variance on L_{0-8} and L_{12-14} are given in Table 6.5 and the mean values in Table 6.6.

The two species differed markedly in their response to the treatments imposed. The grass showed a response in L to soil temperature, L being greater at the intermediate temperatures than at either extreme. However, the grass showed no response to phosphate. In contrast for clover L_{0-8} did not differ significantly between temperatures and L_{12-14} was greater at the higher temperatures. At 0-8 cm L for clover decreased significantly ($P \leqslant 0.01$) at the higher level of phosphate supply.

iv) Phosphorus content

Additional phosphate had a marked effect on the concentration of phosphorus in the shoots (Table 6.7). The high levels of phosphorus in the P2 treatment suggest luxury uptake, judging from the results of Ozanne, Keay and Biddiscombe (1969), and the levels of phosphorus in the shoots appeared adequate for growth at both P1 and P2. The concentration of phosphorus in the shoots tended to decrease with increasing soil temperature at P1, while at P2 the level of phosphorus remained high at all temperatures. The total phosphorus uptake to the shoots (Table 6.8) doubled in the P2 treatment from 10 to 22°C, however, because of the decreased concentration, the increase in phosphorus uptake at P1 over this

Figure 6.4. The root length per plant for brome grass (o) and subterranean clover (Δ) (overleaf) as a function of depth at two rates of applied phosphate. The open symbols represent the monocultures and the solid symbols represent the mixtures. Values for the 2 cm layers are plotted at the depth corresponding to the mid-point of each layer.


BROME
P 1
P 2
LSD (1%)
10°C
14°C
18°C
22°C
Depth (cm)
1
3
5
7
13
0
200
Root length per plant (cm)

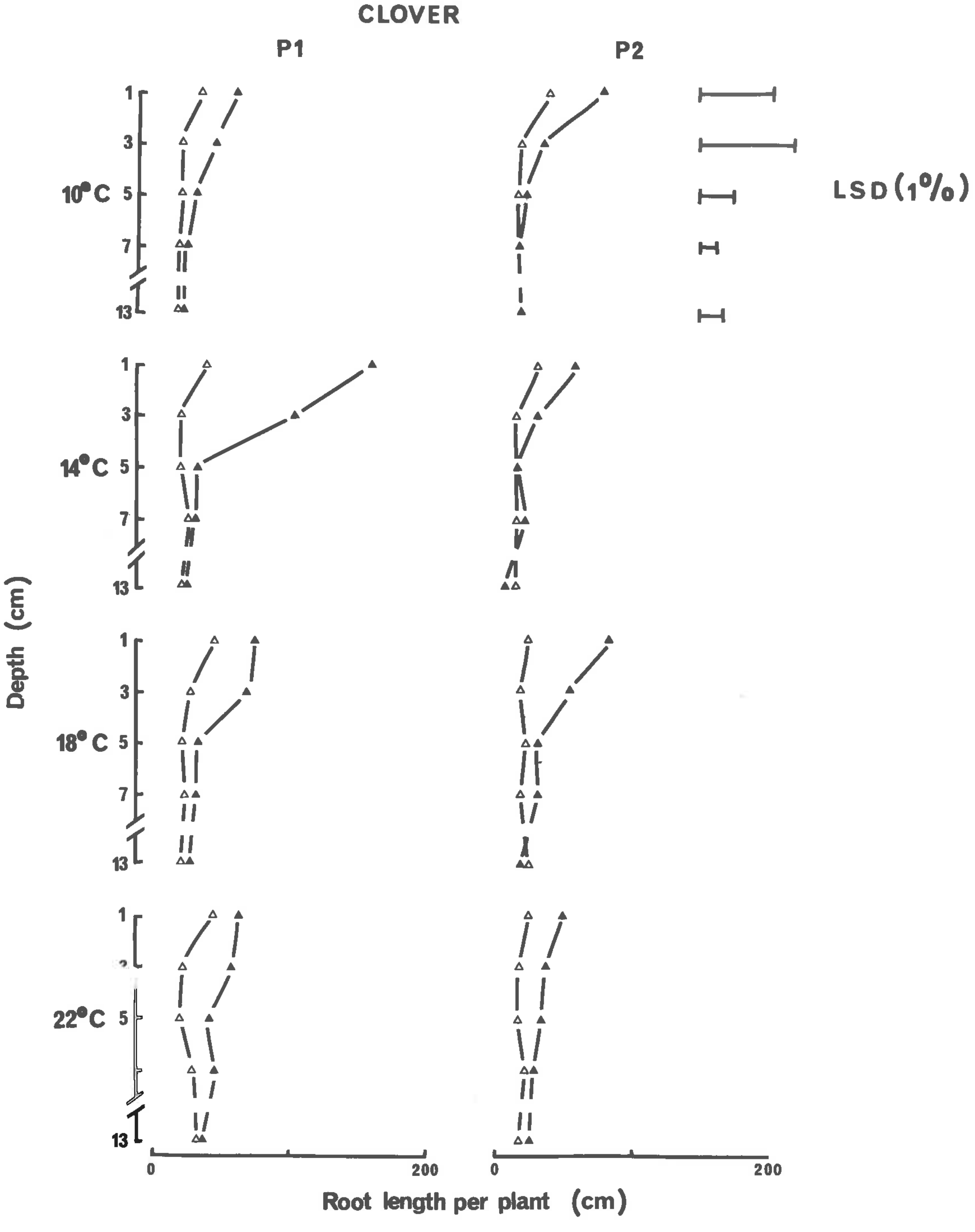

CLOVER
P1
P2
LSD (1%)
10°C
14°C
18°C
22°C
1
3
5
7
13
Depth (cm)
0
200
0
200
Root length per plant (cm)

Table 6.5

Analysis of variance table for L_{0-8} and L_{12-14} (cm per plant)(1)

Treatment	df	Variate Brome L_{0-8}	 L_{12-14}	 Clover L_{0-8}	 L_{12-14}
Temperature (T)	3	**	***	(NS)	**
Reps within temp.	8				
Phosphate (P)	1	(NS)	(NS)	**	(NS)
Frequency (z)	1	(NS)	(NS)	*	(NS)
T x P	1	(NS)	(NS)	(NS)	(NS)
T x z	3	(NS)	(NS)	(NS)	(NS)
P x z	3	(NS)	(NS)	(NS)	(NS)
T x P x z	3	(NS)	*	(NS)	(NS)
Residual	24				
Total	47				

(1) Mean squares are given in Appendix Table A9.

Table 6.6

Root length per plant in the 0-8 and 12-14 cm layers (cm)

		L_{0-8}		L_{12-14}	
		Mono	Mix	Mono	Mix
			Brome		
10°C	P1	226	257	20	23
	P2	182	239	30	25
14°C	P1	373	267	38	33
	P2	261	175	45	40
18°C	P1	393	353	43	28
	P2	341	368	33	50
22°C	P1	328	302	32	28
	P2	330	325	31	37
			Clover		
10°C	P1	95	158	18	21
	P2	90	151	19	19
14°C	P1	110	329	18	21
	P2	78	132	16	6
18°C	P1	120	208	22	27
	P2	83	201	25	22
22°C	P1	113	208	32	36
	P2	84	152	19	25

L.S.D. 1%	L_{0-8}	L_{12-14}
Brome	43	7
Clover	40	7

Table 6.7

Concentration of phosphorus in the shoot (%)

Treatment		Brome		Clover	
		Mono	Mix	Mono	Mix
10°C	P1	.171	.183	.197	.192
	P2	.231	.245	.406	.447
14°C	P1	.152	.176	.170	.168
	P2	.213	.234	.419	.409
18°C	P1	.136	.188	.140	.137
	P2	.187	.275	.388	.407
22°C	P1	.125	.145	.117	.101
	P2	.227	.235	.339	.383

	Brome	Clover
L.S.D. 1%	.028	.033

Table 6.8

Total phosphorus in shoot per plant (μg)

Treatment		Brome		Clover	
		Mono	Mix	Mono	Mix
10°C	P1	210	307	215	170
	P2	254	416	486	367
14°C	P1	215	301	298	233
	P2	313	484	642	524
18°C	P1	245	390	307	265
	P2	391	617	838	771
22°C	P1	309	320	290	263
	P2	574	566	997	1124

	Brome	Clover
L.S.D. 1%	59	71

temperature range was relatively small.

6.3.2 Mixtures

i) Dry weight

At the two lower soil temperatures the dry weight of the grass shoots increased and that of the clover decreased when grown in mixture; but the effect of mixing diminished at the higher soil temperatures, and at 22°C it tended to be reversed (Table 6.2). The replacement diagram (Figure 6.5), constructed using the crowding coefficients given in Table 6.9, shows that the dominance of the grass was reduced with increasing soil temperature. The temperature effect was superimposed on the effect of soil phosphate level on the crowding coefficients observed previously in Experiments I and II. The hypothesis that $k_b = 1/k_c$ was tenable, except possibly in the 14°C P1 treatment where the two species had significantly different variances (Appendix Table A10), and the 3 and 4 parameter models could not be compared.

ii) Fresh weight and leaf area

Fresh weight (Table 6.3) and leaf area (Table 6.4) were also significantly altered ($P \leqslant 0.001$) for each species when grown in mixture at the lower temperatures. At the highest temperature (22°C) at the P1 rate the effect of mixing had disappeared but persisted at the P2 rate.

Figure 6.5. Replacement diagram showing the shoot dry weight per unit area (mg.cm^{-2}) of brome grass (o), subterranean clover (Δ) and the total yield (□). Fitted lines are plotted with Equations (4 & 5) using the crowding coefficients given in Table 6.6.

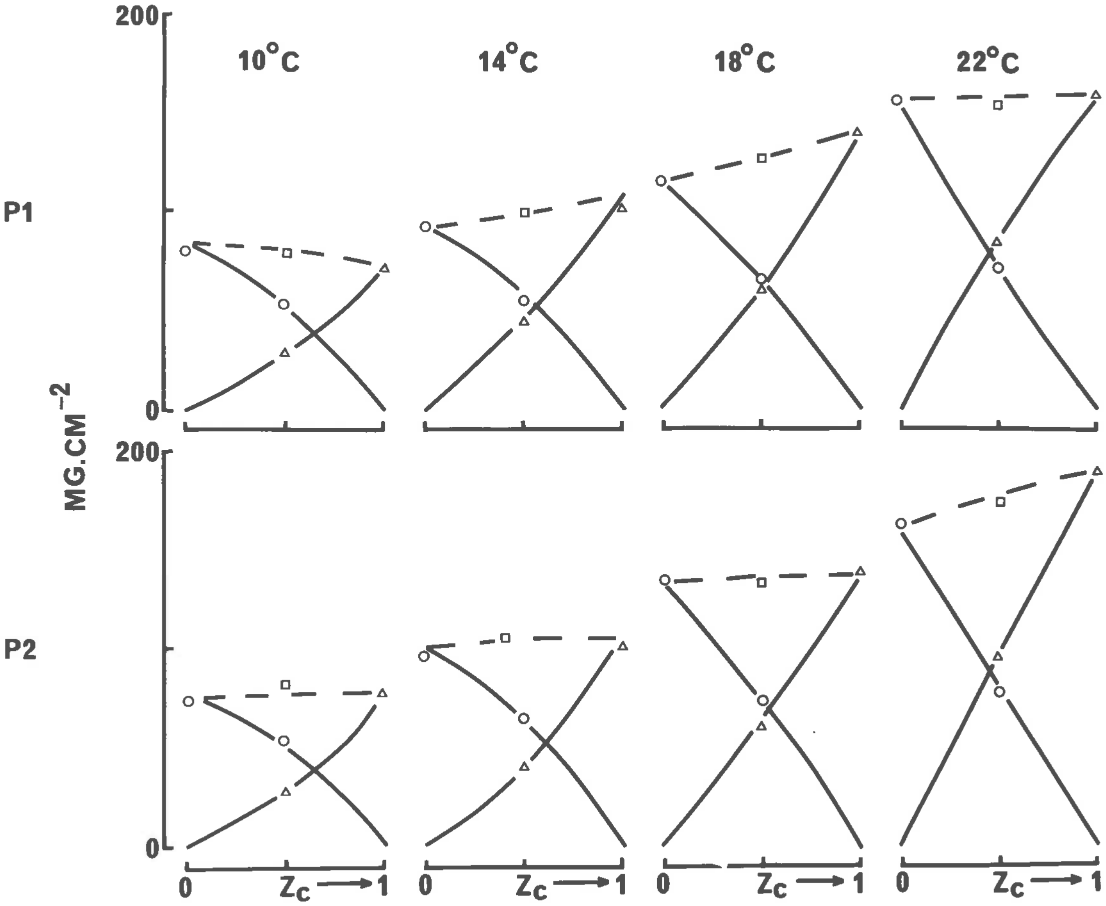

200
0
200
0
MG.CM^{-2}
P1
P2
10°C
14°C
18°C
22°C
0
Z_c ⟶ 1
0
Z_c ⟶ 1
0
Z_c ⟶ 1
0
Z_c ⟶ 1

Table 6.9

Crowding coefficients, k, for shoot-dry weight after de Wit (1960) and Thomas (1970)

Soil temperature (°C)	P1		P2	
	k_b	k_c	k_b	k_c
10	1.55	$1/k_b$	2.06	$1/k_b$
14	1.53	.74	1.60	$1/k_b$
18	1.27	$1/k_b$	1.23	$1/k_b$
22	.85	$1/k_b$	.96	$1/k_b$

Values from Appendix Table A10. 3 parameter model used when $k_c = 1/k_b$; otherwise 4 parameter model used.

iii) Root length

The root length of grass showed no significant response to being grown in mixture. In contrast clover showed marked increase ($P \leqslant 0.05$) in L_{0-8} when grown in mixture. As in monoculture, L_{0-8} for clover was higher at P1 than at P2, and L_{12-14} was increased at the higher temperatures.

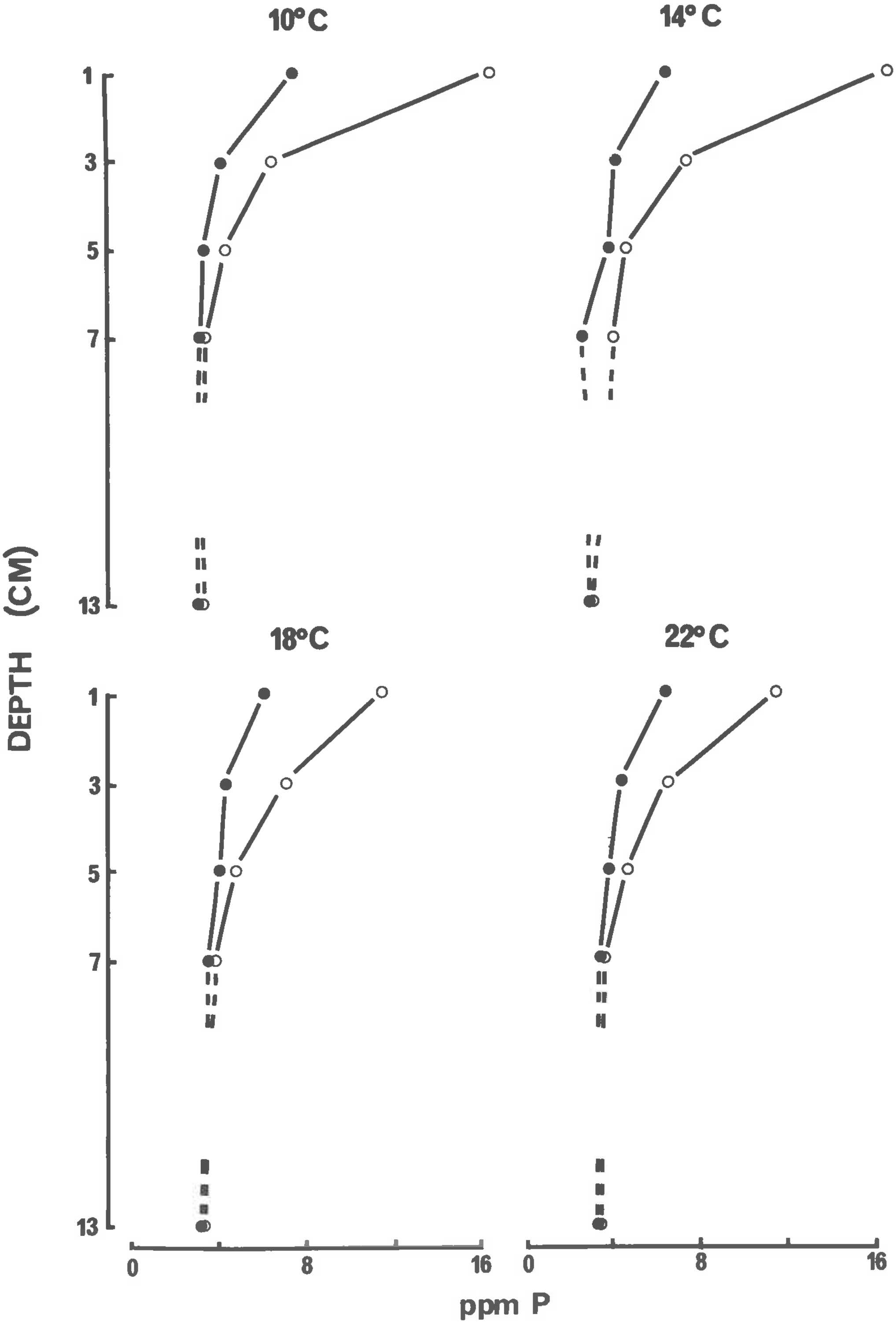
iv) Phosphorus content

Brome grass in mixture with clover showed an increase ($P \leqslant 0.001$) in phosphorus concentration compared to the monoculture. In contrast the phosphate level of the clover was little affected by being grown in mixture (Table 6.7). At the three lower soil temperatures total phosphorus uptake to the shoot was increased in the grass and decreased in the clover when grown in mixture compared with monoculture, but the effect of mixing was either reduced or reversed at the highest soil temperature (Table 6.8).

6.3.3 Soil phosphate

The amount of phosphate extractable from the soil with 1N H_2SO_4 at harvest varied little between frequencies, but differed between temperatures and P rates (Appendix Table A11). Figure 6.6 shows the extractable phosphorus content as a function of depth at each soil temperature for each rate of P. Less phosphorus remained in the upper layers of the soil at higher temperatures at

Figure 6.6. Amounts of phosphate extractable from the soil with 1N H_2SO_4 (ppm air dried soil) as a function of depth for each temperature treatment and phosphate rate (P1 = ● and P2 = ○). Means of amounts for the various seedling frequencies determined on soil samples bulked from replicates (comprehensive data in Appendix A8). Values for the 2 cm layers are plotted at the depth corresponding to the mid-point of each layer.


10°C
14° C
18°C
22° C
DEPTH (CM)
1
3
5
7
13
0
8
16
ppm P

each rate of P.

6.4 Discussion

The low degree of response to the phosphorus application was unexpected. The yield level and phosphorus content of the shoot at the P1 rate in Experiment III were considerably higher than in Experiment II. The additional supply of phosphorus probably came from the greater depth of soil in Experiment III - 35 cm compared with 22 cm in Experiment II. The additional depth of soil represents an increase of 13 mg acid extractable P per pot, and this may be compared with the 33 mg P per pot added as the P1 treatment.

Large effects of soil temperature on plant growth have often been observed. The subject has been reviewed by Richards, Hagan and McCalla (1952). It has been difficult to identify the mechanisms involved. Brouwer (1964) concluded that a reduction in growth rate of *Phaseolus vulgaris* L. with lower root temperature was due to a restriction in the water supply to the leaves. Brouwer argued that the effect was too rapid to have been caused by changes in the pattern of nutrient uptake or growth hormone distribution. Kramer (1942) attributed the decrease in water uptake at lower root temperatures to an increase in viscosity of water and of the protoplasm. Kuiper (1964) showed that the increased water viscosity restricted water inflow with reduced root temperatures until a critical temperature was reached. Kuiper suggested that below this critical temperature the lipid membranes in the root became relatively impermeable and severely limited water flow into the plant. This pattern was repeated when a constant suction was artificially applied to the base of an excised root system, indicating that stomata in no way controlled the sudden fall in water uptake observed when root temperatures were reduced to a critical value.

There is growing evidence that physiologically active quantities of gibberellins (Skene, 1967) and kinetins (Kende, 1965) are produced in the roots and translocated to the shoots. Thus the shoot may be influenced indirectly over extended periods by the level of growth substances produced by the root, a theory first postulated by Went (1943). Low soil temperature has been shown to lower the production of these growth substances in vines (Woodham and Alexander, 1966) and in grasses (Atkin and Barton, 1970).

Soil temperature affects the temperature of the shoot as well as the root, particularly in the region near the ground surface. During the vegetative stage the apical meristem of brome grass was just below the soil surface. The apical meristem of the subterranean clover was raised by the hypocotyl~~e~~ to a level just above the gravel mulch. Experiments on *Zea mays* by

Kleinendorst and Brouwer (1970) indicate that, while plants may maintain turgor at a low root temperature, by increasing the osmotic suction within the plant, the temperature at the apical meristem will in general control the rate of enlargement of the leaves. Watts (1971) showed that the temperature of the transpiration stream passing the leaf meristem influenced the cell extension rate in that leaf, even when the rest of the lamina was maintained at a non-limiting temperature.

The observed effect of soil temperature on the crowding coefficients may have considerable ecological significance. Table 6.9 shows that clover improves its competitive ability with an increase in soil temperature over the range encountered during the growing season (Appendix Figure A2). These results are consistent with field observations made by Willoughby (1954), who concluded that clover tended to become the dominant pasture species during the autumn and spring growth periods but that ryegrass became dominant during growth in colder winter months. The results from this experiment show that, either by its influence on the roots or on the apical meristem, soil temperature can bring about changes in dominance at a uniform air temperature.

Changes in root length per plant, L, do not appear to have accounted for changes in competitive ability with temperature.

For the grass L was inversely related to the crowding coefficients (compare Tables 6.6 and 6.9). Clover L_{0-8} showed no response to temperature but did respond at a greater depth, indicated by L_{12-14}.

Williams (1972) found that the root/shoot weight ratio of several lines of subterranean clover, grown as spaced plants, remained constant over the solution culture temperature range of 10 to 20°C. On the other hand, Davidson *et al.* (1970) found a decrease in the root/shoot ratio in *T. subterraneum* cv. Tallarook grown at a plant density of 0.02 cm^{-2} over the temperature range 12 to 22°C. Brouwer (1963) argues that the root/shoot ratio will be reduced when conditions are unfavourable for the shoot and the carbohydrate supply is inadequate to maintain the whole plant at maximum growth rate for the conditions prevailing. The decrease in root/shoot weight observed by Davidson *et al.* and the decrease in root length/shoot weight of the clover observed in this experiment may indicate increased competition for light occurring at higher temperatures.

Although smaller in magnitude, additional phosphate caused changes in crowding coefficients in the same direction to those found in Experiments I and II. The root length per plant of clover relative to that of the grass was greater at P1 than at P2, and this could perhaps have contributed to greater competitive ability of clover at P1. As pointed out in Experiment II the separation of root systems when canopies are grown together might be expected to indicate the relative importance of competition for phosphate. The next experiment attempts this by introducing barriers below ground to give inter- and intra-specific mixing of the root systems.

7. Experiment IV

7.1 Introduction

In the discussion of the previous experiments two mechanisms have been proposed for the effect of added phosphorus on the competitive ability of the two species. The species may have competed directly for a limited supply of this essential nutrient with the more competitive species attaining the larger share. Alternatively the two species may have responded differently in their growth to changes in the level (intensity) of phosphorus supply, and the more successful species may have obtained more of the phosphorus simply by establishing the greater sink. Although it then firstly affects the growth rate of the plant, the added phosphorus may indirectly affect the ability of the plant to compete for some other scarce resource, for example, light.

Experiment IV was designed to test these alternatives by comparing the growth of the grass and clover where the roots of the two species were either separated or mixed. If the two species were to compete directly for phosphorus to a greater or lesser degree when phosphorus is in scarce supply, separation of the root systems would be expected to reduce any difference between the relative yields of the two species. If, at the other extreme, the response of the two species to mixing were influenced only indirectly by varying the phosphate supply, separation of the root systems would not be expected to affect the relative yield of the components at any level of phosphorus supply.

7.2 Materials and Method

7.2.1 Design and treatments

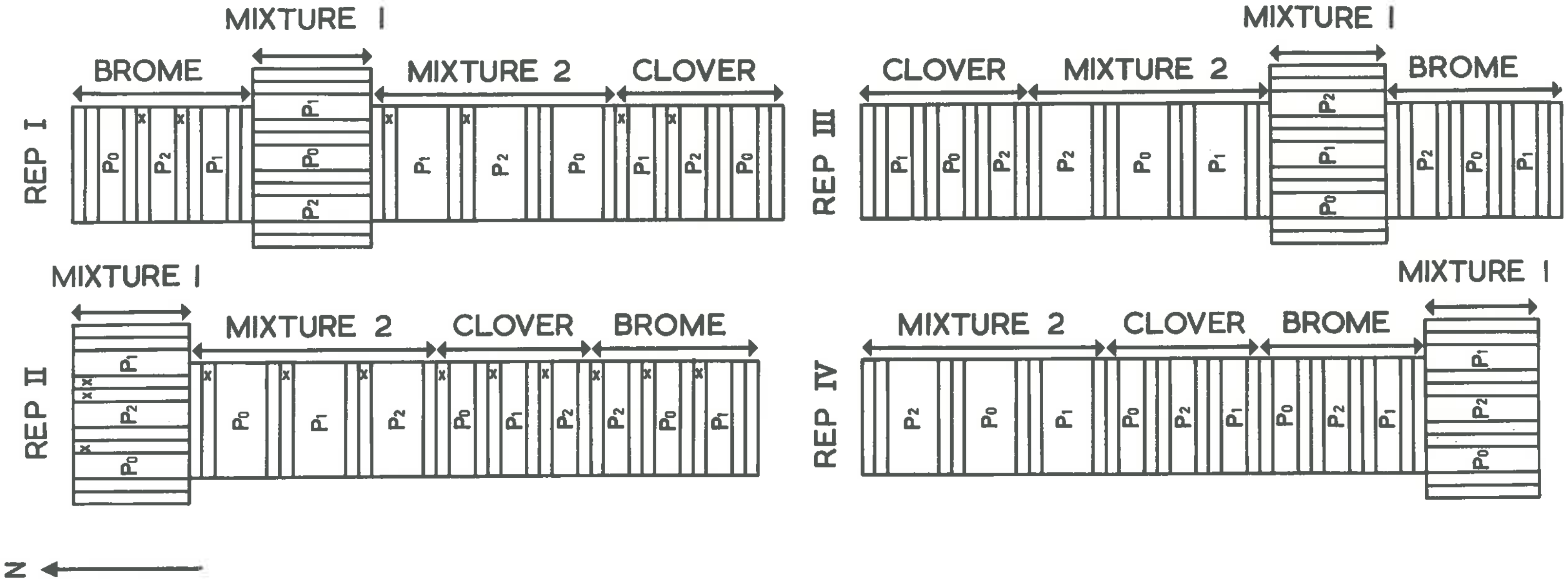
i) Plan

Monocultures and mixtures of brome grass and subterranean clover were grown at three phosphate levels. Barriers were arranged to prevent interspecific root mixing in one treatment. Canopies were allowed to mix in all treatments. Sufficient material for four harvests was included in each treatment, and the treatments were replicated four times. The following array shows the combinations and the total number of containers:

Harvests		Frequency and type of mixture (composition)		Phosphate rates		Replicates		Total
4	x	4	x	3	x	4	=	192

Each composition was located randomly as a main treatment within a replicate block, and the three phosphate rates were allocated randomly as sub-treatments using a split plot design. The four replicates were positioned on separate benches in an open-sided glasshouse (Figure 7.1).

Figure 7.1. The layout of containers in Experiment IV showing the rates of applied phosphate and the composition treatments. The composition treatments are given in detail in Figure 7.2. The location of the thermal conductivity probes (X) in the border containers in Replicates I and II are indicated.


REP I
BROME
MIXTURE I
MIXTURE 2
CLOVER
P0 P2 P1
P1 P0 P2
P1 P2 P0
P1 P2 P0
REP III
CLOVER
MIXTURE 2
MIXTURE I
BROME
P1 P0 P2
P2 P0 P1
P2 P1 P0
P2 P0 P1
REP II
MIXTURE I
MIXTURE 2
CLOVER
BROME
P1 P2 P0
P0 P1 P2
P0 P1 P2
P2 P0 P1
REP IV
MIXTURE 2
CLOVER
BROME
MIXTURE I
P2 P0 P1
P0 P2 P1
P0 P2 P1
P1 P2 P0
N

ii) Design and arrangement of containers and barriers

The plants were grown in slabs of soil separated by metal panels. Inter- and intra-specific root mixing were obtained by altering the alignment of the panels with respect to the rows of the two species (see Figures 7.2 and 7.3). A border slab treated with the same rate of phosphate was placed on either side of each treatment. The slabs containing the treatments had internal dimensions of 2.5 x 40 x 43 cm deep, and those used as borders were twice as wide, that is 5 x 40 x 43 cm. The containers were made from 0.5 mm galvanised iron sheet attached to timber spacers. The slabs were clamped together with screw jacks, using concrete paving stones to support and insulate each end of a set of slabs. After the slabs had been clamped together in order, the metal panels were lined with a continuous strip of polyethylene sheet. The sides of the clamped slabs were insulated from radiation with SISALATION 410(1).

iii) Soil preparation

The soil used to fill the slabs came from the same bulk sample as used for Experiment II and was mixed with the same basal fertilizer as in Experiment II. The soil was moistened, weighed amounts were added to each slab (3 x 2 kg lots of moist soil for

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(1) Manufactured by: Regis-ACI Pty Ltd, Kilkenny, South Australia.

Figure 7.2. The arrangement of the soil barriers and sowing pattern of brome grass (o) and subterranean clover (Δ) to give the composition treatments in Experiment IV. The solid symbols indicate harvested plants and open symbols indicate border plants.

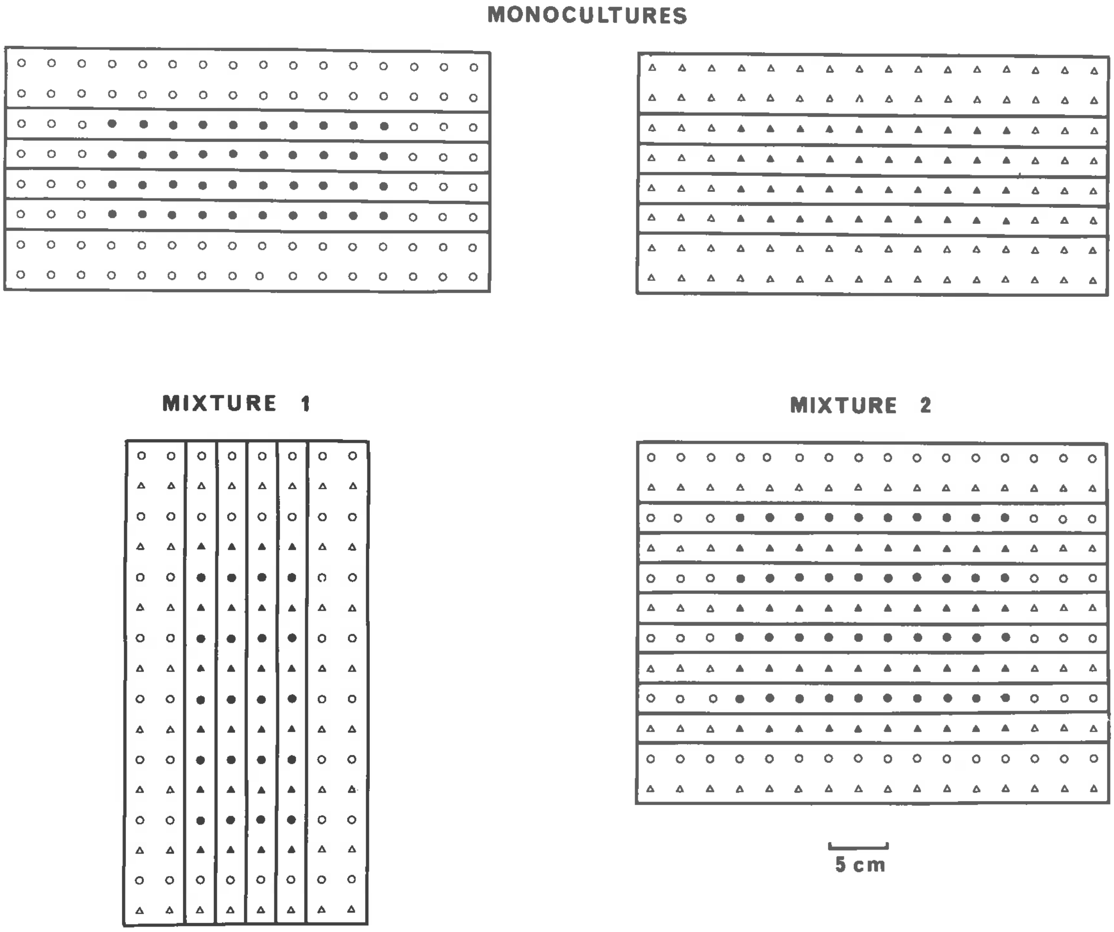
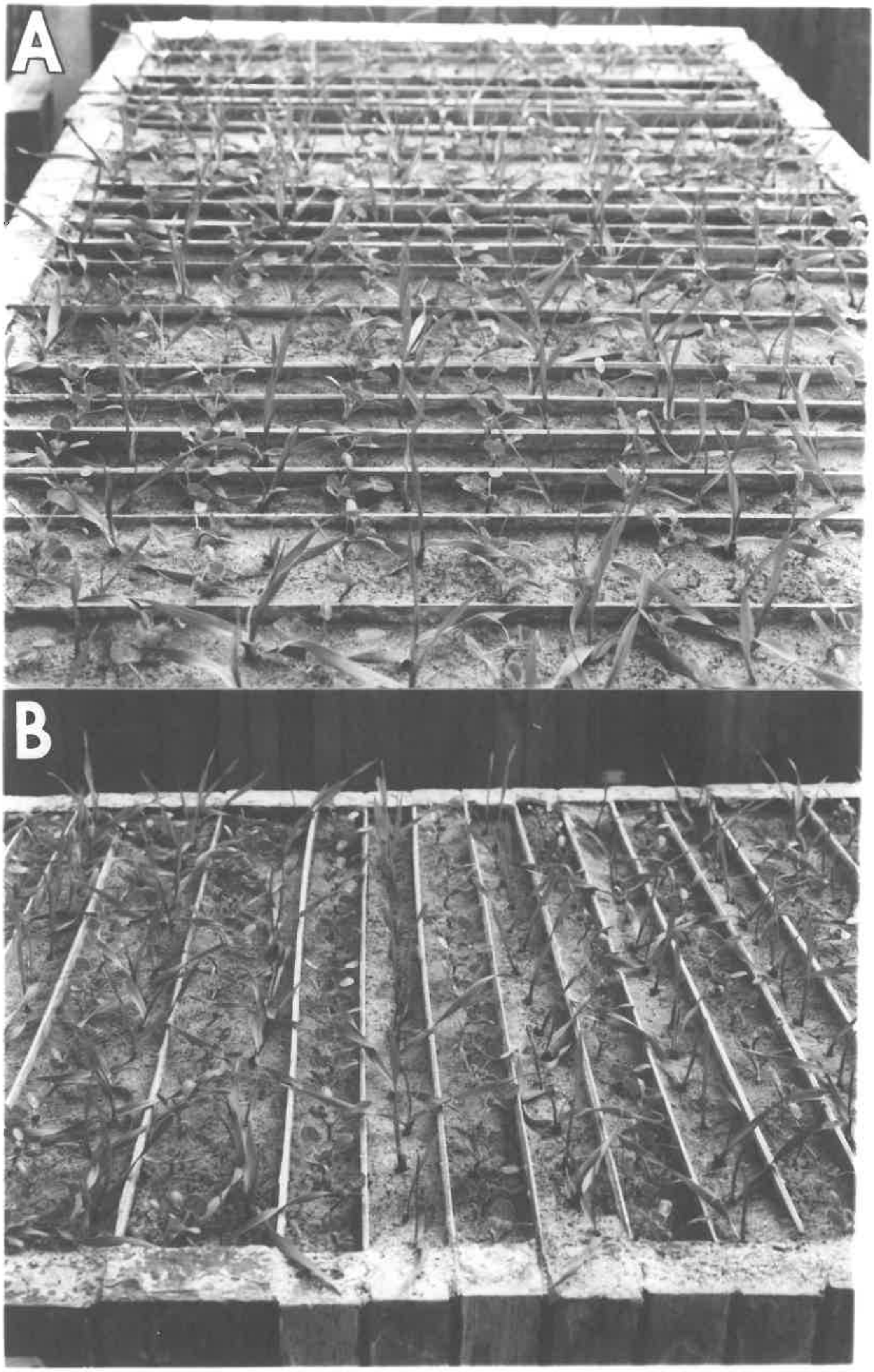

MONOCULTURES
MIXTURE 1
MIXTURE 2
5 cm

Figure 7.3. The mixture compositions showing root and shoot mixing (A) and shoot only (B).


A
B

the 2.5 cm wide slabs and 3 x 4 kg lots for the border slabs) and tamped, care being taken not to distort the panels. This filled the slabs to a level 2 cm below the tops of the panels. The final bulk density was 1.35 $g.cm^{-3}$ of dry soil.

iv) Phosphate treatment

Phosphate was applied to the top of the soil as a solution of monocalcium phosphate in 50 ml of deionised water, and was watered in with a further 400 ml of deionised water. The wider border rows received twice these amounts. The addition raised the soil water content of the soil to 16% by weight. The phosphate treatments were:

Rate	$Ca(H_2PO_4)_2.H_2O$ (mg/pot)	P (kg/ha)
P0	0	0
P1	53	13
P2	159	39

v) Measurement of soil water and watering procedure

The volumetric water content of the soil, w, was measured with thermal conductivity probes. De Vries (1953) showed theoretically that the temperature of a constant infinite line source of heat in a homogeneous conducting medium increased logarithmically with time. The thermal conductivity of a medium can be derived from the rate of temperature rise, and for a moist granular medium the latter is related to the moisture content of the medium. De Vries (1953) obtained consistent results in sandy soils using a thermal conductivity probe, and the method compared favourably with other nondestructive methods of measuring soil water (Cope and Trickett, 1965). The method is particularly useful in sandy soils where w generally decreases considerably with little decrease in pore water pressure, so that instruments of the tensiometer type are insensitive to changes in w.

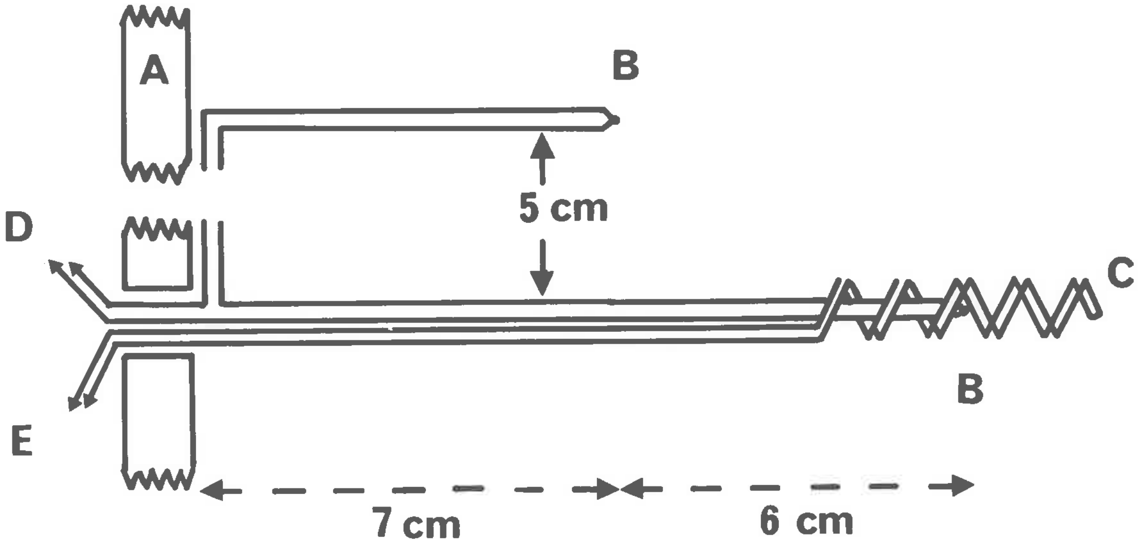
The probes were similar to those constructed by de Jager and Charles-Edwards (1969). The heat source was a 4 cm long coil of NICROM wire; the 20 cm of wire used in each coil had a resistance of approximately 1 Ohm. A copper/constantan thermocouple was positioned within the bore of each coil, and the assembly was embedded in ARALDITE. A second thermocouple used in the circuit as the cold junction was embedded separately in ARALDITE and placed 5 cm above the heating coil (see Figure 7.4). The coil was heated by a constant A.C. current of 450 mA, and reproducible results were obtained by recording temperature for three minutes with the heater on.

The probes were calibrated by placing them in 0.5 kg lots of the experimental soil at known values of w and at 1.35 $g.cm^{-3}$ bulk density, and recording the e.m.f. produced on a logarithmic recording volt meter. The calibration curve-mean for 18

Figure 7.4. Thermal conductivity probe.

Key:

A - Timber spacer
B - Copper-constantan thermocouple
C - Heating coil
D - To millivolt recorder
E - To constant current supply


A
B
5 cm
D
C
B
E
7 cm
6 cm

probes is shown in Appendix Figure A3. Eighteen probes with similar calibration curves to that shown in Figure A3 were selected from a set of twenty four, and placed singly in the containers bordering all treatments in Replicate I and those bordering P2 and P3 treatments in Replicate II (see Figure 7.1). The probes were located centrally between the panels, 10 cm below the soil surface and 12.5 cm from the timber side wall of the slabs on the side shown in Figure 7.1.

Measurements of w were made once a week beginning at the first harvest. The water use was then calculated, assuming a uniform moisture content within the container. The water use was assumed to be the same for all borders and treatment-slabs at given rates of P, and equal to the mean amounts determined in Replicates I and II. The actual amounts used in Replicates I and II did not differ by more than 14%. Deionised water was applied to the top of the soil to replace that used, once a week initially increasing to three times a week as the plants grew.

vi) Sowing

The seeds of brome grass and clover were soaked as in Experiment I. The clover seeds were inoculated with *Rhizobium*. Pairs of seed were placed 2.5 cm apart in rows in 2 mm deep indentations and covered with soil. The containers were covered with black polyethylene sheet for five days during germination. After this period the plants were thinned to the required plant density, a few missing plants being replaced with transplants from the border rows. A 1 cm mulch of coarse sand was then spread over the soil.

7.2.2 Management, Labelling and Harvesting

A second application of nitrogen was given 50 days after planting at the rate of 100 kg/ha of N as $Ca(NO_3)_2$ dissolved in 50 ml of water.

Harvests were made on the following dates:

	Date	Time from sowing (day)
Seeds soaked	2nd August, 1970	0
1st Harvest	12th September, 1970	42
2nd Harvest	2nd October, 1970	62
3rd Harvest	16th October, 1970	76
Final Harvest	26th October, 1970	86

At each harvest the 10 central plants from the 16 present in a slab were cut at ground level, and samples were made by combining alternate plants. This gave two subsamples of 5 plants of each species for the container where the roots were separate, and one sample of 5 plants of each species where the roots were mixed (see Figure 7.2). The slabs were chosen systematically for each harvest to minimize disturbance to the canopy. Shoots were generally severed with the slabs in place. Clamps were then loosened and the harvested slabs were removed. Finally the remaining slabs were closed up and again clamped. At the last harvest only, one component in the mixtures with mixed roots (mixture 1, Figure 7.2) was labelled as described in section 3.2.3. On this occasion, after the shoots of the non-labelled component had been harvested, slabs containing the component to be labelled were removed, temporarily clamped together, and covered with a PERSPEX chamber of size 54 x 60 x 120 cm. An amount of 0.2 millimoles of $C^{14}O_2$, having a total activity of 200 μc, was released in the chamber and circulated with a fan for one hour. The labelled plants were harvested one day after labelling.

Leaf area measurements were made with the electronic planimeter on the fresh material. The samples were then dried at 60°C and weighed. Phosphate determinations were made on the ground material, taking an 0.5 g subsample when the sample exceeded this weight, and otherwise the entire sample.

The harvested slabs from the 1st, 2nd and 4th harvests were held at 2°C until soil samples could be taken. The metal panels were then removed, exposing the soil. Root systems from the monocultures in one replicate from harvests one and two were photographed after being washed free of soil by carefully spraying the exposed soil slabs on a fine mesh with a jet of water. The remaining slabs from Harvest 2 and the slabs from Harvest 4 were sampled by cutting the soil into 2 cm wide horizontal layers. The central 15 cm length of the 0-2, 2-4, 4-6, 6-8 and 12-14 cm layers was retained for root length determinations by the method described in Section 3.2.

7.3 Results

The results of analysis of variance of the logarithmically transformed dry weight and leaf area data, and of the shoot-phosphorus concentration data are given in Table 7.1.

7.3.1 Monocultures

i) Shoot dry weight

The phosphate supply at P0 was acutely low, and the yield of shoots of both species was increased significantly ($P \leqslant 0.001$) by the addition of phosphorus (Table 7.2), the clover being somewhat more responsive than the grass after the first harvest. The weight of the shoots of each species increased continuously during the experiment at each rate of P, although at P0 the shoots of either species increased in weight by 20 to 40 mg, that is only two or three times the initial dry weight of the seed in 86 days (Figure 7.5).

Table 7.1

Analysis of variance table

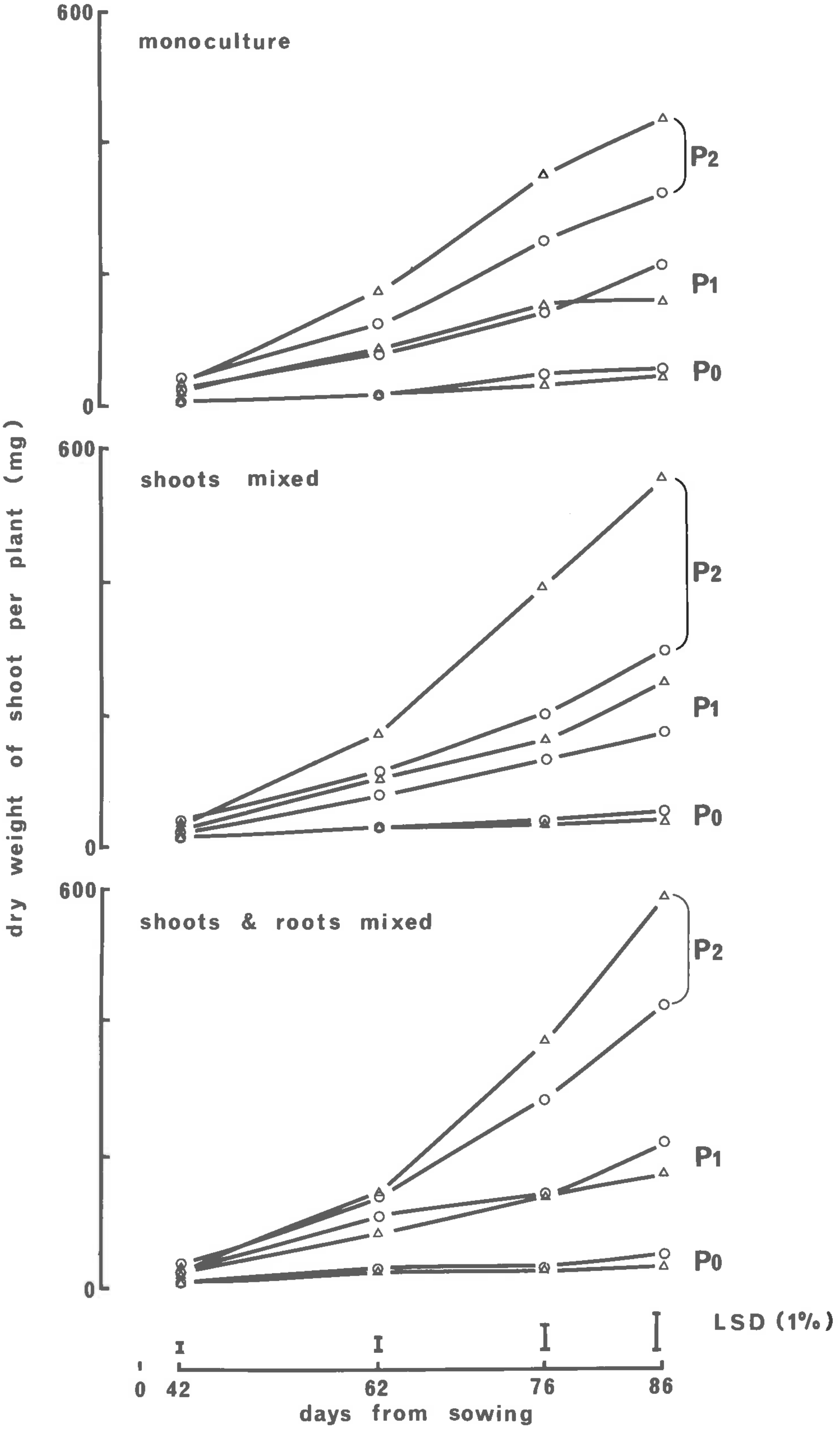
		Variate					
		Brome			Clover		
Treatment	df	Log dry wt shoot	Log A	% P in shoot	Log dry wt shoot	Log A	% P in shoot
Reps	3						
Composition (C)	2	*	***	(NS)	***	**	**
Error 1	6						
Phosphate (P)	2	***	***	***	***	***	***
P x C	4	***	***	(NS)	(NS)	(NS)	(NS)
Error 2	18						
Harvest (H)	3	***	***	***	***	***	***
H x P	6	***	***	***	***	***	***
H x C	6	(NS)	***	**	(NS)	*	*
H x P x C	12	(NS)	**	(NS)	(NS)	(NS)	(NS)
Error 3	81						
Total	143						

Table 7.2

Dry weight of shoots per plant (mg)
Logarithms in brackets

	Harvest			
	1	2	3	4
Brome				
P0				
Monoculture	15 (1.18)	27 (1.44)	43 (1.60)	54 (1.73)
Shoot mixed	15 (1.17)	31 (1.47)	44 (1.63)	59 (1.77)
Root & shoot mixed	12 (1.06)	27 (1.43)	34 (1.52)	53 (1.70)
P1				
Monoculture	27 (1.42)	79 (1.90)	138 (2.14)	214 (2.33)
Shoot mixed	23 (1.35)	77 (1.88)	133 (2.12)	172 (2.23)
Root & shoot mixed	27 (1.42)	110 (2.04)	147 (2.17)	221 (2.34)
P2				
Monoculture	38 (1.57)	123 (2,09)	248 (2.39)	320 (2.51)
Shoot mixed	40 (1.60)	115 (2.06)	202 (2.30)	292 (2.46)
Root & shoot mixed	38 (1.58)	141 (2.15)	285 (2.45)	429 (2.63)
Clover				
P0				
Monoculture	17 (1.23)	28 (1.45)	33 (1.51)	44 (1.64)
Shoot mixed	16 (1.21)	29 (1.46)	35 (1.54)	45 (1.65)
Root & shoot mixed	15 (1.18)	24 (1.38)	30 (1.48)	35 (1.53)
P1				
Monoculture	27 (1.43)	83 (1.92)	148 (2.16)	187 (2.27)
Shoot mixed	30 (1.47)	103 (2.01)	163 (2.21)	244 (2.38)
Root & shoot mixed	28 (1.45)	85 (1.93)	141 (2.15)	178 (2.25)
P2				
Monoculture	33 (1.52)	173 (2.24)	347 (2.54)	432 (2.63)
Shoot mixed	35 (1.54)	169 (2.23)	393 (2.50)	552 (2.74)
Root & shoot mixed	27 (1.43)	145 (2.16)	357 (2.55)	588 (2.77)
LSD 5%	(0.10)	(0.09)	(0.09)	(0.10)
1%	(0.14)	(0.12)	(0.12)	(0.14)

<u>Figure 7.5</u>. The dry weight of shoot per plant (mg) of brome grass (o) and subterranean clover (Δ) as a function of time at three rates of applied phosphorus for each mixture composition.


monoculture
shoots mixed
shoots & roots mixed
dry weight of shoot per plant (mg)
600
0
P2
P1
P0
LSD (1%)
0
42
62
76
86
days from sowing

ii) Leaf area

The leaf area per plant, A, responded strongly to applications of phosphorus (Table 7.3). Unlike dry weight, the area A of the grass reached maximum values at the second harvest where phosphate had been applied. The leaf area per unit ground surface, F, of the monocultures of both species did not exceed 1 at P0, indicating that the canopy remained open. The canopies developed rapidly at P1 and P2, where they had covered the ground completely by the second harvest.

iii) Root length

The results of an analysis of variance on the sum of the root length per plant in the 0 to 8 cm layer and the 12 to 14 cm layer are given in Table 7.4 and the data are given in Table 7.5. In both species L_{0-8} increased significantly with the addition of phosphate. No response was detected at the 12 to 14 cm depth except by the clover at the final harvest, but it should be noted that the variability was high.

Intact root systems of P0 and P2 monocultures from harvest 1 and 2 are shown in Figure 7.6. The root systems shown are those grown in a 2.5 cm thick slab of soil. For comparison, L_v values measured in the 0-2 cm layer on the other replicates at harvest 2 and at P0 and P2 were 4.7 and 9.3 cm for the clover and 5.4 and 12.2 cm for the grass. The total root length per plant

Table 7.3

Leaf area per plant (cm^2)

Logarithms in brackets

	Harvest			
	1	2	3	4
Brome				
PO				
Monoculture	1.7 (0.21)	3.3 (0.50)	4.3 (0.63)	4.0 (0.59)
Shoot mixed	2.2 (0.28)	3.8 (0.51)	4.1 (0.59)	4.8 (0.68)
Root & shoot mixed	2.3 (0.33)	2.0 (0.24)	4.2 (0.62)	5.0 (0.69)
P1				
Monoculture	5.6 (0.69)	18.7 (1.23)	16.7 (1.15)	14.2 (1.14)
Shoot mixed	6.1 (0.68)	18.4 (1.10)	16.7 (1.16)	14.2 (1.14)
Root & shoot mixed	5.5 (0.72)	17.2 (1.18)	21.7 (1.32)	17.9 (1.20)
P2				
Monoculture	9.3 (0.94)	34.0 (1.52)	32.8 (1.51)	22.6 (1.33)
Shoot mixed	11.9 (1.03)	30.4 (1.45)	32.2 (1.50)	19.3 (1.24)
Root & shoot mixed	7.5 (0.71)	28.7 (1.45)	60.4 (1.78)	46.9 (1.66)
Clover				
PO				
Monoculture	1.2 (0.07)	1.7 (0.17)	2.6 (0.41)	3.1 (0.48)
Shoot mixed	1.3 (0.11)	2.3 (0.34)	3.2 (0.48)	4.2 (0.62)
Root & shoot mixed	1.1 (0.04)	1.9 (0.26)	2.9 (0.46)	3.4 (0.53)
P1				
Monoculture	3.2 (0.47)	7.6 (0.83)	8.4 (0.89)	11.3 (1.04)
Shoot mixed	4.0 (0.56)	13.4 (1.01)	17.0 (1.18)	20.5 (1.28)
Root & shoot mixed	3.3 (0.48)	10.6 (0.93)	18.9 (1.22)	27.2 (1.29)
P2				
Monoculture	4.4 (0.64)	25.4 (1.37)	37.0 (1.56)	36.9 (1.54)
Shoot mixed	4.4 (0.74)	19.5 (1.21)	44.8 (1.64)	51.9 (1.70)
Root & shoot mixed	3.0 (0.46)	18.6 (1.24)	46.5 (1.66)	69.5 (1.83)
LSD 5%	(0.34)	(0.43)	(0.27)	(0.34)
1%	(0.43)	(0.58)	(0.37)	(0.45)

Table 7.4

Analysis of variance of L_{0-8} and L_{12-14}

for Harvest 2 and 4

Treatment	df	Brome		Clover	
		L_{0-8}	L_{12-14}	L_{0-8}	L_{12-14}
<u>Harvest 2</u>					
Composition (C)	1	(NS)	(NS)	(NS)	(NS)
Phosphate (P)	2	**	(NS)	***	(NS)
C x P	2	(NS)	(NS)	(NS)	*
<u>Harvest 4</u>					
Composition (C)	2	*	(NS)	(NS)	**
Phosphate (P)	2	***	(NS)	***	**
C x P	4	(NS)	(NS)	*	(NS)

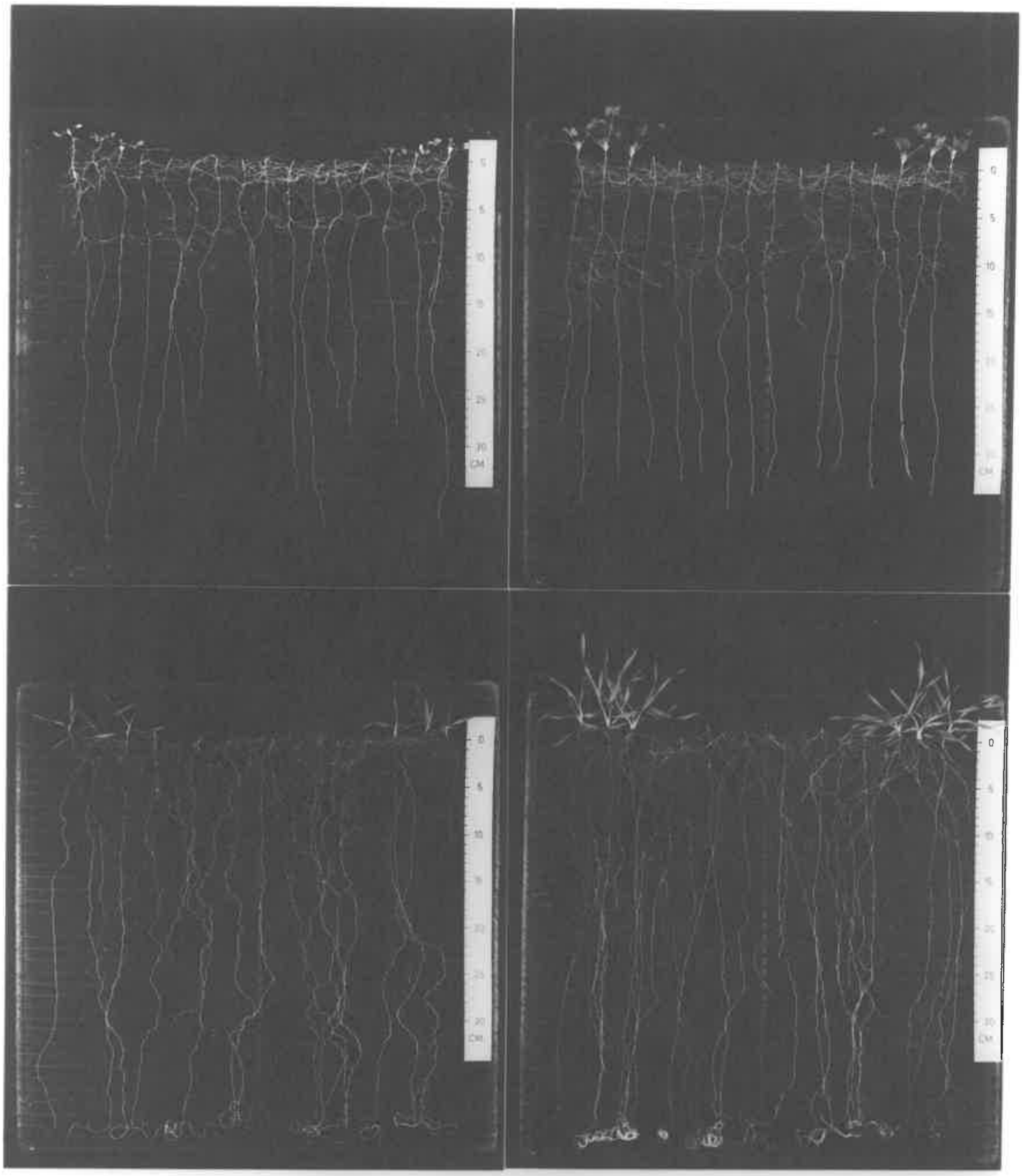
Table 7.5

Root length per plant in the 0 to 8 and
12 to 14 cm layers (cm)

Treatment	L_{0-8}		L_{12-14}	
	Brome	Clover	Brome	Clover
Harvest 2				
P0 Monoculture	214	166	31	34
Shoots mixed	211	201	26	16
Shoots and roots mixed*	243		22	
P1 Monoculture	347	298	35	25
Shoots mixed	382	336	27	38
Shoots and roots mixed*	420		23	
P2 Monoculture	432	305	28	21
Shoots mixed	595	281	57	23
Shoots and roots mixed*	379		26	
LSD 5%	203	64	21	13
1%	281	87	29	18
Harvest 4				
P0 Monoculture	464	369	124	99
Shoots mixed	461	420	113	103
Shoots and roots mixed	405	374	139	124
P1 Monoculture	900	582	148	103
Shoots mixed	759	654	136	104
Shoots and roots mixed	667	561	149	139
P2 Monoculture	1095	772	147	127
Shoots mixed	1131	923	155	128
Shoots and roots mixed	672	1296	129	280
LSD 5%	342	264	90	78
1%	449	346	118	102

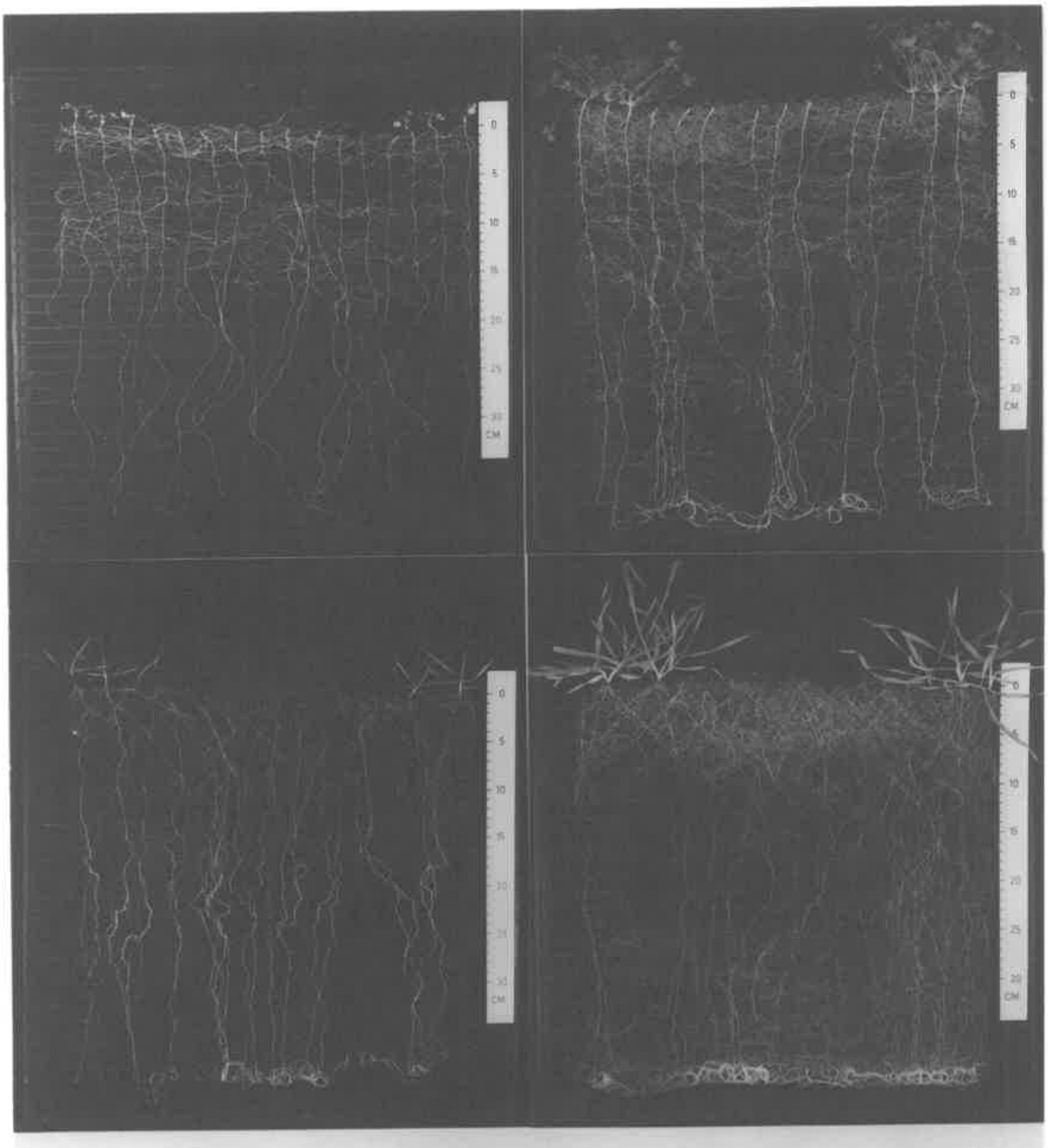
* Components not labelled at the second harvest and data represents total root length per plant in mixture.

Figure 7.6. The intact roots washed with a 2.5 cm thick slab of soil from monocultures of subterranean clover (above) and brome grass (below) at P0 and P2 from harvest 1 and harvest 2 (overleaf).



P0 **P2**

HARVEST 1



HARVEST 2

in each layer for each treatment is shown in Figure 7.7.

iv) Phosphorus in the shoot

Figure 7.8 shows the phosphorus concentration in the shoot (%) and Figure 7.9 shows the total phosphorus per shoot for each rate of phosphorus and composition. At the first harvest in both species there were large differences in the concentration of P in the shoot between phosphate rates. Relatively little phosphorus entered the shoots after the second harvest, and the amount of phosphorus sometimes declined after the third harvest. Detailed data on phosphorus concentration in the shoot are given in Appendix Table A12.

7.3.2 Mixtures

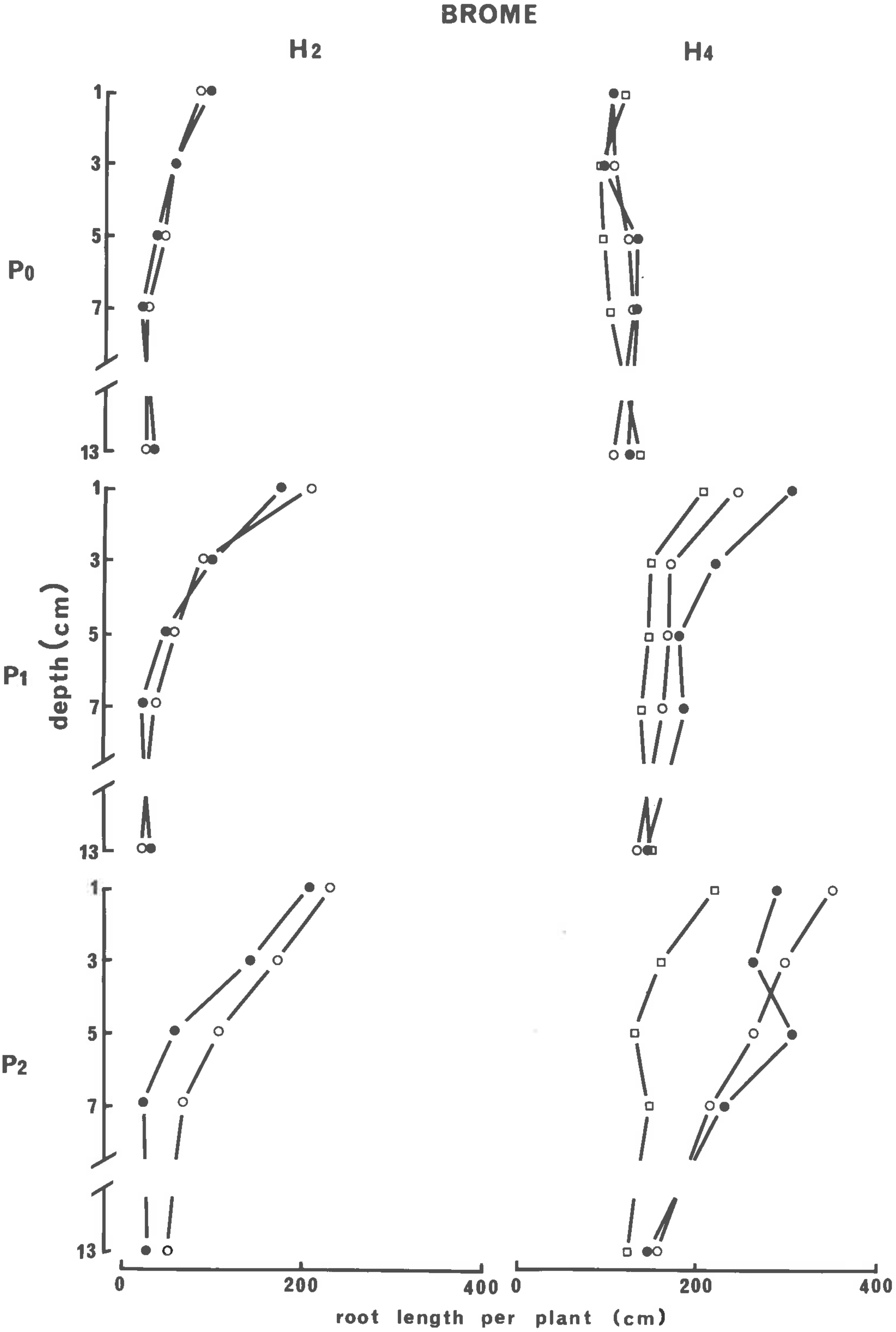
i) Shoot dry weight

Interspecific mixing of the roots and shoots or the shoots alone caused significant changes in the dry weight of the shoot per plant of both species from the values found in monoculture. Data were fitted to either the 3 or 4 parameter models. The results of this analysis are given in Appendix Table A13 and summarized in Table 7.6. Generally the hypothesis $k_b = 1/k_c$ was tenable. In ten instances indicated in Appendix Table A1~~1~~3 the variance of the two species were significantly different, precluding the application of the between models test. In three instances the between models

Figure 7.7. The root length per plant (cm) as a function of depth at three rates of applied phosphate and at two harvests for brome grass and subterranean clover (overleaf).

Key:

	Brome grass	Subterranean clover
Monocultures	○	△
Shoots mixed	●	▲
Shoots and roots mixed	□	■


BROME
H2
H4
P0
P1
P2
depth (cm)
1
3
5
7
13
0
200
400
root length per plant (cm)

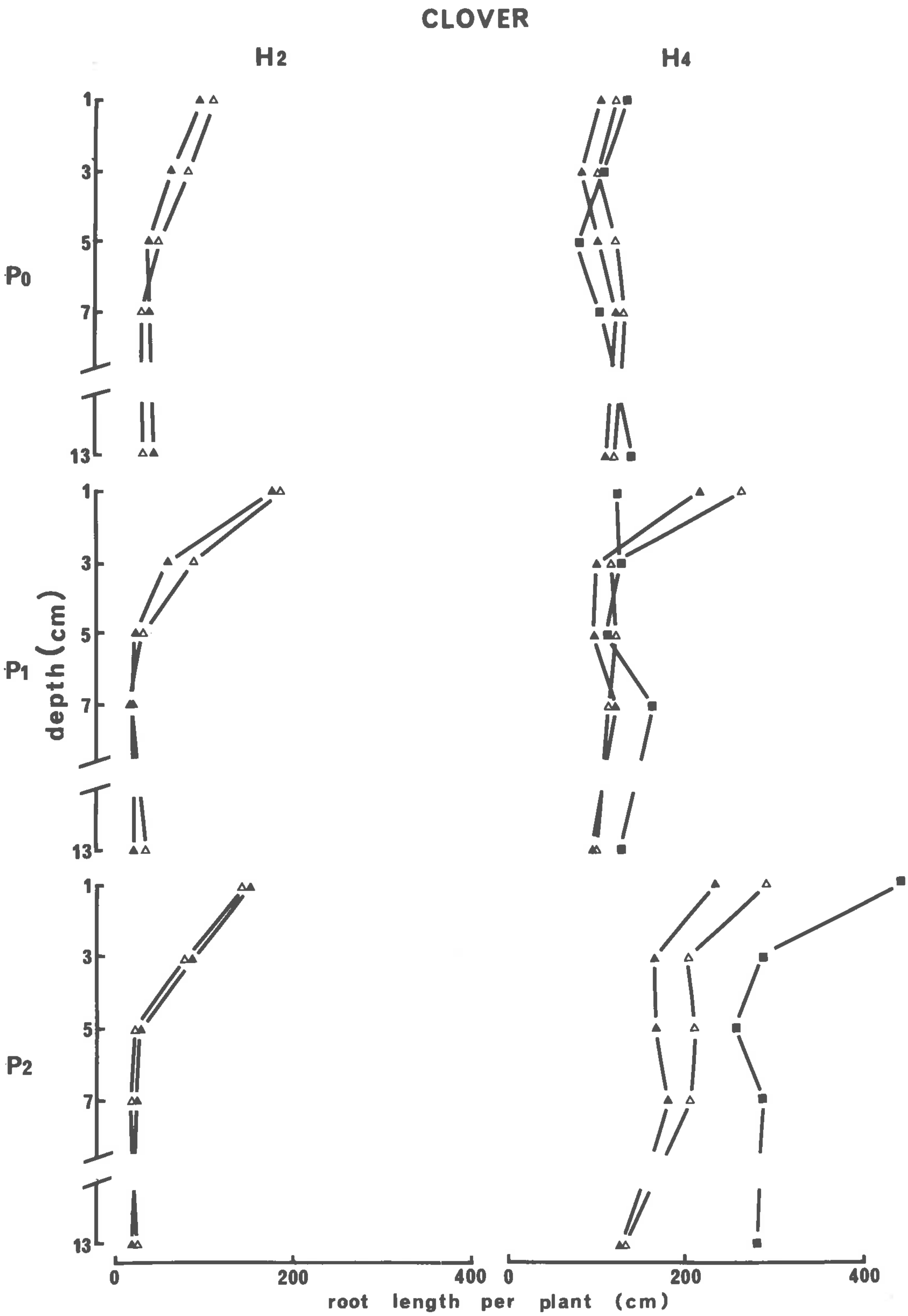

CLOVER
H2
H4
P0
P1
P2
depth (cm)
1
3
5
7
13
0
200
400
root length per plant (cm)

Figure 7.8. The phosphorus concentration in the shoots (%) of brome grass (o) and subterranean clover (Δ) as a function of time at three rates of applied phosphorus for each mixture composition.

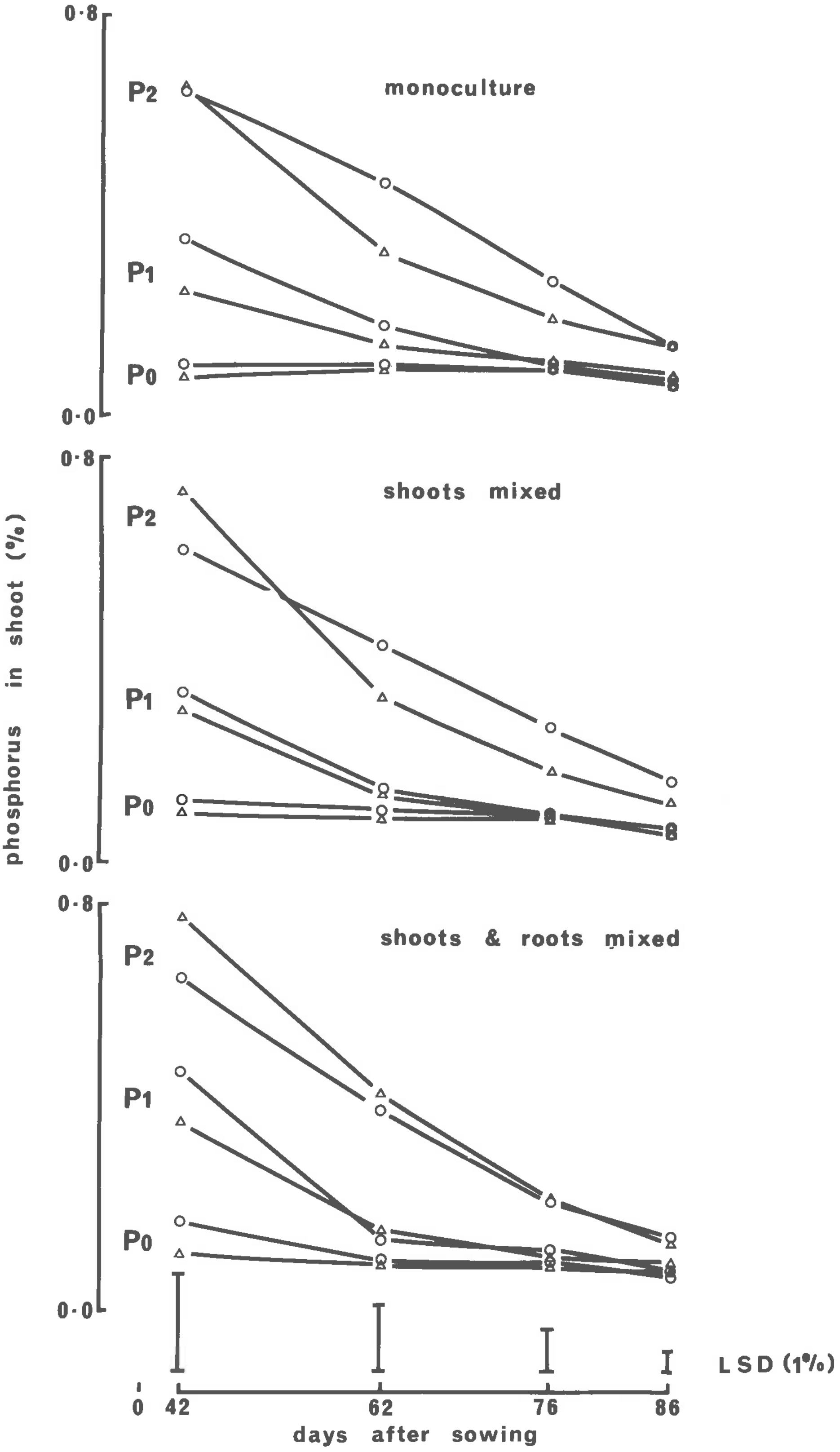

0·8
0·0
P2
P1
P0
monoculture
shoots mixed
shoots & roots mixed
phosphorus in shoot (%)
LSD (1%)
0
42
62
76
86
days after sowing

Figure 7.9. The total phosphorus in the shoot per plant (μg) of brome grass (o) and subterranean clover (Δ) as a function of time at three rates of applied phosphorus for each mixture composition.

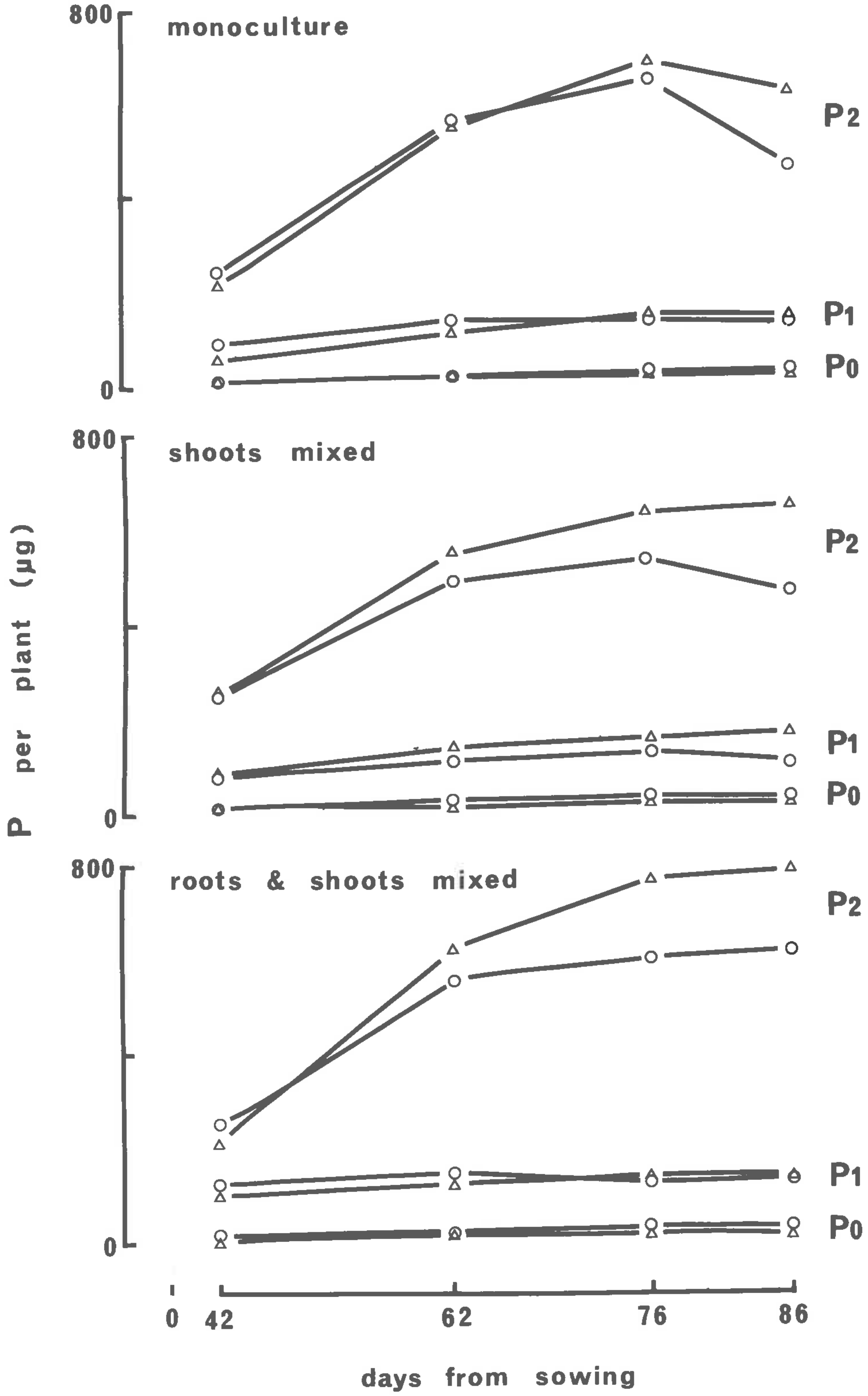

800
monoculture
P2
P1
P0
0
800
shoots mixed
P2
P1
P0
0
800
roots & shoots mixed
P2
P1
P0
0
P per plant (µg)
0
42
62
76
86
days from sowing

Table 7.6

Calculated crowding coefficients, k, from shoot dry weight after de Wit (1960) and Thomas (1970)(1)

		Shoots only mixed		Root and shoots mixed	
		k_b	k_c	k_b	k_c
P0	H1	0.98	0.94	0.72	0.79
	H2	1.48	1.03	1.23	$1/k_b$
	H3	1.07	$1/k_b$	0.85	0.85
	H4	1.07	$1/k_b$	1.21	$1/k_b$
P1	H1	0.78	1.20	0.97	1.11
	H2	0.91	1.67	2.33	1.04
	H3	0.91	$1/k_b$	1.08	$1/k_b$
	H4	0.65	$1/k_b$	1.10	$1/k_b$
P2	H1	1.02	$1/k_b$	1.27	$1/k_b$
	H2	0.96	$1/k_b$	1.37	$1/k_b$
	H3	0.71	$1/k_b$	1.10	$1/k_b$
	H4	0.84	1.76	2.08	2.20

(1) Comprehensive data given in Appendix Table A13.

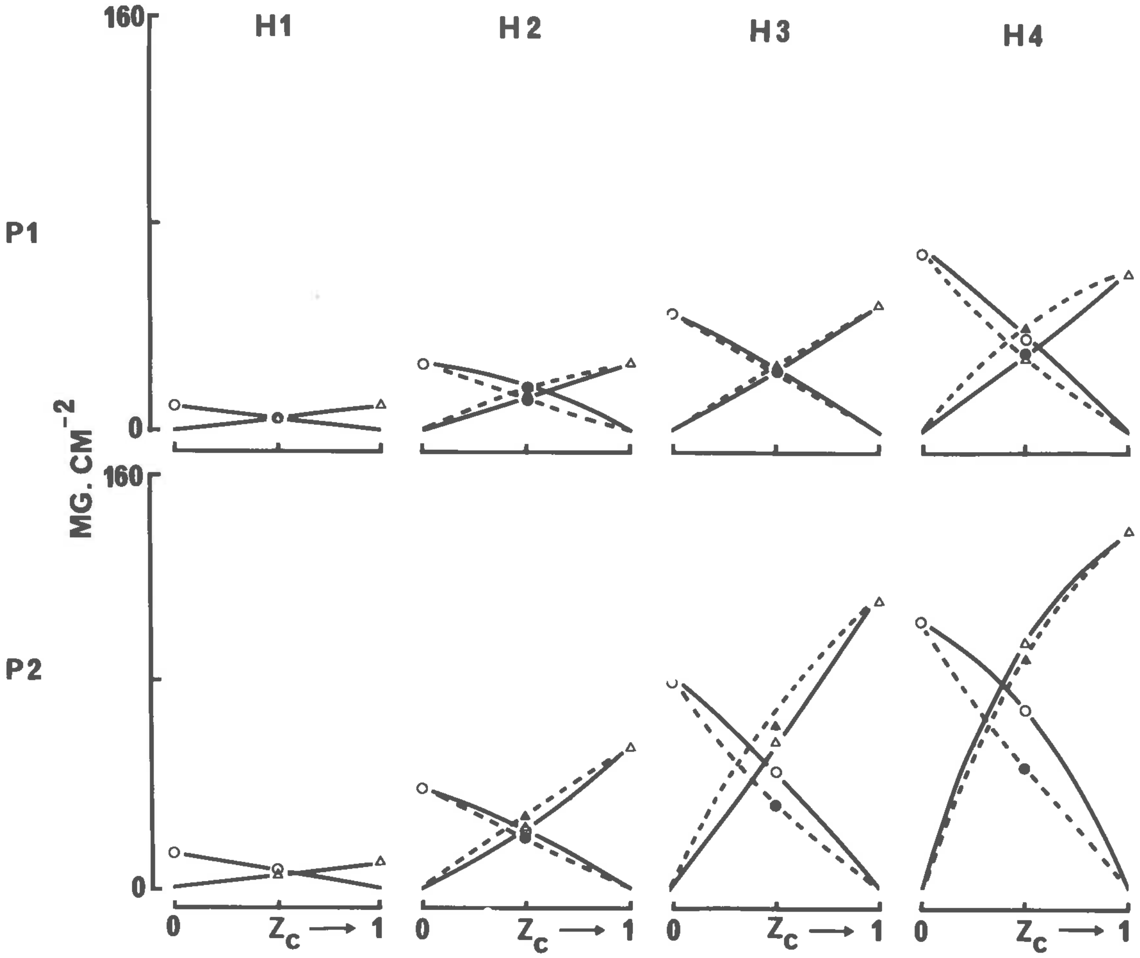
F ratio was significant, the differences being associated with overyielding ($k_b.k_c \gg 1$). Replacement diagrams were drawn, using the crowding coefficients in Table 7.6, for P1 and P2 rates (Figure 7.10). Diagrams were not constructed for P0 because the two species caused little if any interference when grown together.

When both roots and shoots were mixed the crowding coefficients for the P1 and P2 rates were close to 1, except when overyielding occurred. However when the roots of the two species were separated the competitive ability of the clover at P1 and P2 was greater than when the roots and shoots were mixed. Although k_c appeared to increase with successive harvests, statistically significant interaction of composition and time effects was not found when the data were transformed logarithmically (Table 7.1). This was because the two species had similar relative growth rates after the first harvest in the two kinds of mixture at each rate of P.

ii) Leaf area

The leaf area of the grass when shoots alone were mixed was similar to that in monoculture at all rates of P. However when the roots and shoots were mixed A for the grass increased at P1 and P2. In both mixtures the grass showed a decline in A from the 3rd to 4th harvests similar to that observed in

Figure 7.10. Replacement diagrams for shoot dry weight for brome grass(o) and subterranean clover (Δ) with fitted lines using the crowding coefficients given in Table 7.7. The open symbols and continuous lines represent the root and shoot mixture and the solid symbols and hatched line represent the shoot only mixture.


160
H1
H2
H3
H4
P1
MG. CM^{-2}
0
160
P2
0
0
Z_c ⟶ 1
0
Z_c ⟶ 1
0
Z_c ⟶ 1
0
Z_c ⟶ 1

monoculture. Clover increased in A when grown in mixture at the P1 and P2 rates, and the leaf area of the clover in mixture was increasing up to the final harvest.

iii) Root length

Tables 7.4 and 7.5 show that there were no significant responses to mixing at the second harvest. By harvest 4 the grass showed a decrease in L_{0-8} when roots were mixed compared to the monoculture or shoot only mixture. The clover L_{0-8} at harvest 4 showed no effect of mixing at the P0 and P1 rates but showed an increase at the P2 rate, giving rise to an interaction of phosphorus and composition. Clover L_{12-14} showed an increase when the roots were mixed over the monoculture and when shoots only were mixed.

iv) Phosphorus in the shoot

The phosphorus concentration in the grass shoots was not altered significantly when the grass was grown in either kind of mixture. Clover showed a significant response ($P \leqslant 0.01$) to the composition treatments, having a higher phosphorus content in either mixture by the first harvest (Figure 7.8). The difference between the composition treatments decreased with time, and had disappeared by the final harvest. Clover in mixtures had a higher uptake of phosphorus to the shoot over the four harvests than in monoculture (Figure 7.9). This effect was greater when both shoots and roots were mixed.

7.3.3 Depletion of phosphorus supply

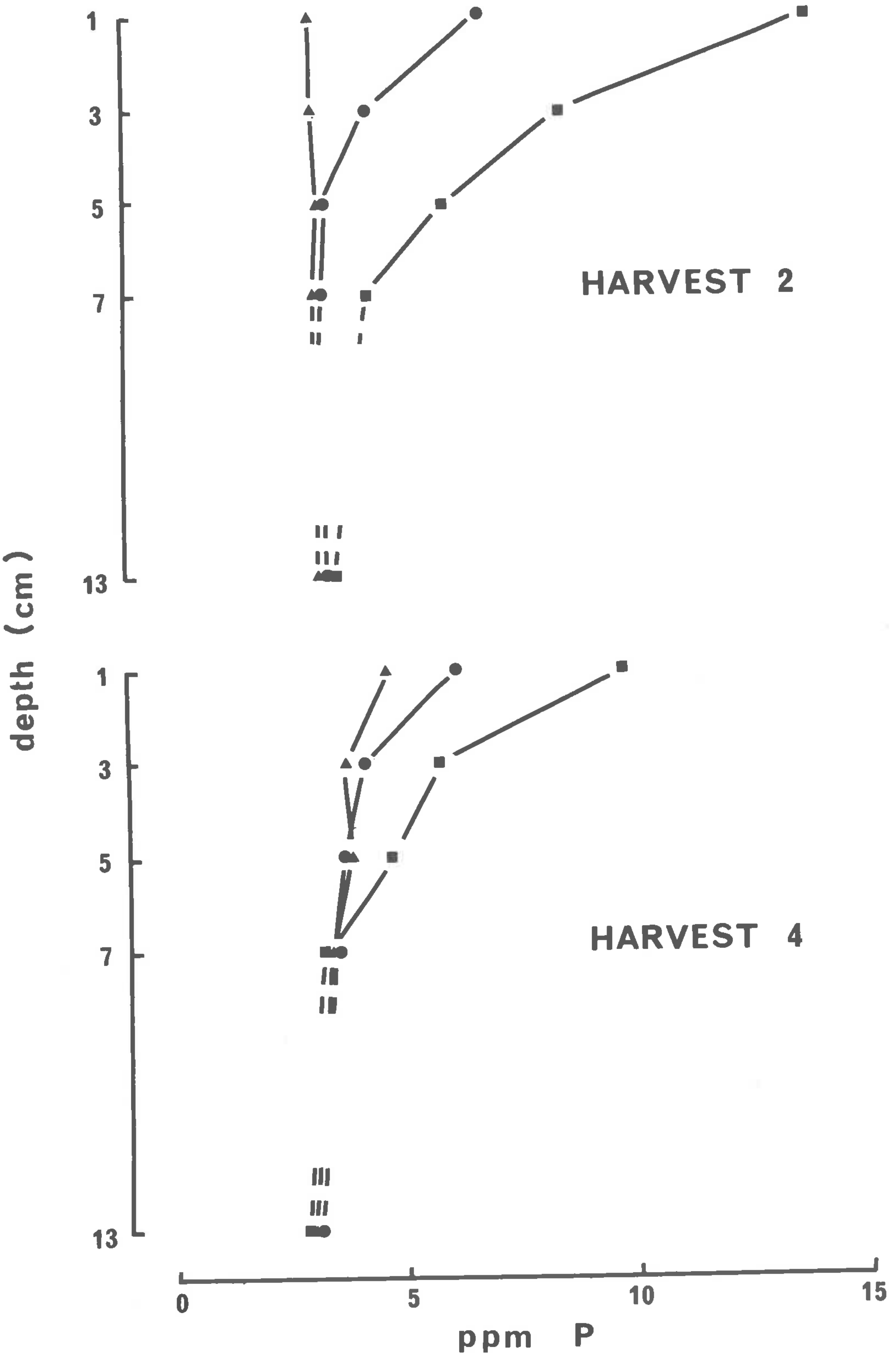
Composition treatments did not cause any difference in acid extractable phosphate concentration in the soil (Appendix Table A14). Pooled mean values of the concentration of phosphorus extractable by 1N H_2SO_4 are given in Figure 7.11. The added phosphate did not appear to have moved below the 6 to 8 cm layer. Phosphate present at harvest 2 had been depleted by the final harvest, the greatest depletion occurring in the layer with the highest L_v. The extractable phosphate in the upper layers at PO had increased by 1 to 2 ppm by the final harvest.

7.4 Discussion

The crowding coefficients of the two species at PO suggest that little interference occurred. The small values of F (< 0.7) and L_v (< 0.8) support this conclusion.

Earlier experiments showed a general pattern of increasing competitive ability of the grass component at higher phosphate levels. In this experiment, however, when the roots and shoots were mixed, the crowding coefficients of the grass or clover differed little at either rate of P except when overyielding occurred.

Figure 7.11. Amounts of phosphate extractable from the soil with 1N H_2SO_4 (ppm air dried soil) as a function of depth at harvest 2 and 4 for P0 (▲), P1 (●) and P2 (■). Means of amounts for all compositions determined on soil samples bulked from all replicates (comprehensive data in Appendix A12). Values for 2 cm layers are plotted at the depth corresponding to the mid-point of each layer.


HARVEST 2
HARVEST 4
depth (cm)
1
3
5
7
13
1
3
5
7
13
0
5
10
15
ppm P

The most interesting result was the change in crowding coefficient found when the roots of the two species were kept separate. The grass generally became more competitive when both the roots and shoots were mixed. As hypothesized initially this result is consistent with direct competition between the two species for scarce supplies of phosphorus. Careful interpretation is needed, however, because of the occasional occurrence of overyielding.

Overyielding in a mixture implies an enlargement of the environment. At the second harvest and at P1 when both roots and shoots were mixed, the grass component showed a marked increase in yield per plant over the monoculture. However this was not repeated in subsequent harvests and may be regarded as an artifact. Both kinds of mixture overyielded in the P2 treatment at the final harvest. Additional nitrogen, derived from the activity of the nitrogen fixing Rhizobium, possibly could have accounted for an increase in response by the grass component when both roots and shoots were mixed. Alternatively the aerial environment of the mixture may have been enlarged by overlapping the edges of the containers. This would only occur if the borders around the treatments had proved to be inadequate. The borders in this experiment were similar to those shown by Black (1961) to be adequate for subterranean swards. The most likely cause of overyielding in this experiment was a difference in temporal growth pattern. It should be noted that the leaf area of the grass fell from the 3rd to the 4th harvest while clover A increased over this period (Table 7.3). The decrease in grass A did not cause a decrease in dry weight and probably represents a change from the vegetative to reproductive phase of growth. The clover, on the other hand, exploited the aerial environment over this period, resulting in overyielding.

The apparent recovery of phosphorus, C, was calculated using Equation (16) for each rate of P using the mean phosphorus uptake for all compositions. By the second harvest the value of C for P1 and P2 was 16 and 27 per cent respectively. These values increased to 17 and 32 per cent by the final harvest. The proportion of applied phosphorus remaining in an acid extractable form in the soil, D, is given by:

$$D = 100(R_h - R_o)/H \tag{17}$$

where H is the amount of P added as fertilizer, and R is the amount of acid extractable phosphorus in the soil when fertilizer was applied (R_h) or when no fertilizer was applied (R_o). Pooling for all compositions, the values for D by the second harvest at P1 and P2 were 10 and 14 per cent respectively. These values fell to 4 and 5 per cent by the final harvest. Thus by the second harvest 74 per cent of the applied phosphorus at P1 and 59 per cent at P2 had been converted to an insoluble form. In sandy soils similar to the experimental soil, Powrie (1961) found that half of the phosphorus applied as superphosphate remaining in the top 10 cm was insoluble in 1N H_2SO_4.

While the difference between ~~results of~~ the effects of the two kinds of mixture was ~~are~~ consistent with direct competition between the two species for scarce supplies of phosphorus there is circumstantial evidence against the competition having been exclusively for supplies of phosphorus. The effect of direct competition for supply of a scarce nutrient on yield would be expected to be less at a high level of supply and greatest at an intermediate level (Welbank, 1961). Yet comparable differences in k_b between the two kinds of mixture occurred at P1 and at P2. Figure 7.9 shows that over half of the phosphorus uptake to the shoot occurred before the first harvest. Quantitative root measurements were not made at this harvest. However it is clear that the greater part of phosphorus uptake occurred during a period of relatively low root density. Thirdly, the rates of phosphorus uptake to the shoots in both species were similar for the two kinds of mixture (Figure 7.9).

The increased competitive ability of the grass in mixtures where the roots as well as the shoots were mixed could be attributed to allelopathy; however chemical suppression of one species by secretions from another is rare (Borner, 1960). Nevertheless, an effect of this kind cannot be ruled out. Although 160 kg/ha of N was applied during the experiment, it is possible that, when the grass roots were mixed with nodulated clover roots, the grass benefit~~t~~ed from the extra nitrogen.

8. General Discussion

This series of experiments has shown that, during the vegetative stages of growth, the competitive ability of brome grass and subterranean clover may be altered by the level of phosphate supply. In the first three experiments the competitive ability of brome grass was increased at the higher phosphate levels. This effect was not repeated in Experiment IV. The season in 1970 (Experiment IV) was in no way remarkable, although it was warmer in the latter part than in the corresponding period during the earlier experiments (see Appendix Figure A1). This probably favoured the clover, as did the increase in soil temperature in Experiment III.

The results of the first three experiments are consistent with the conclusions of Rossiter (1964), who assessed the effect of varying rates of phosphate supply on the botanical composition of a sown pasture over a period of ten years. Rossiter found a pattern of subterranean clover dominance at intermediate phosphate levels but grass dominance at higher phosphate levels. It is, of course, hazardous to extrapolate from dry weight changes during the vegetative phase of growth in short term experiments conducted in the glasshouse to long term experiments and observations in the field. The effects of seasonal fluctuations, seedling establishment, mortality, grazing and nutrient recycling would greatly influence natural populations in the field.

The differences observed in growth during these experiments may be accentuated at high density. If the density is sufficiently high seedling mortality occurs, and a competitive advantage conferred on one component may lead to greater mortality of the other. Mortality during the season has been reported at seedling densities in excess of 1.5 plants per cm^2 (Rossiter and Pack, in Rossiter, 1966). Willoughby (1954) observed plant mortality during a growing season in pasture comprising subterranean clover and wimmera ryegrass and having a maximum plant density during the season of 0.6 plants per cm^2.

An increase in plant density from 0.08 to 0.29 plants per cm^2 in Experiment II did not affect the competitive ability of the two species. This result is surprising as there are many plant attributes which are affected by changes in plant densities and some of these attributes would influence the competitive ability of the components in mixture. Harper (1964) sowed mixtures and monocultures of flax and linseed (*Linum usitatissiMum* L.) at two densities. The seed yield in mixture of flax, a monoculm, increased and the seed yield of the linseed decreased at high densities. However the seed yield of linseed, with its capacity for basal branching, was increased and that of flax decreased at low densities. Cocks (1969) found that the larger seed size and rapid early growth of *Hordeum leporinum* gave it an early competitive advantage over *Lolium rigidum* at high densities. At lower densities interspecific competition did not begin until later and the competitive advantage of *H. leporinum* was lost.

The main alternative processes whereby the response to mixing may be affected by the level of phosphorus supply have been considered when discussing individual experiments. Although these experiments were designed less to indicate the mechanisms involved in affecting the growth of the individual than to measure changes in competitive ability in populations, some general suggestions can be made on the events that may have occurred.

The likelihood of direct competition for scarce supplies of phosphorus depends on the mobility of the phosphate ion in the particular soil and on the rooting density of the genotype concerned. Bray (1954) suggested that neighbouring plants might not compete for phosphorus, because, even though their roots might intermingle, each would exploit only a zone of soil of extremely small radius due to the relative immobility of phosphate in many soils. Evidence that phosphorus uptake is related closely to root weight (Cornforth, 1968) or root length (Andrews and Newman, 1970) rather than to the volume of soil per plant supports Bray's hypothesis that uptake is likely to be localised.

However, in the sandy soils used in the present experiments phosphate was relatively mobile, as indicated by the downwards movement of phosphate applied to the top of the soil in solution (Figures 6.6 and 7.11), and the apparent diffusivity of phosphate in the soils concerned is likely to have been of the order of 1.10^{-7} $cm^{2}.sec^{-1}$ (Barley, private communication). Following Philip (1957) the radial half-distance between roots b (cm) may be defined as:

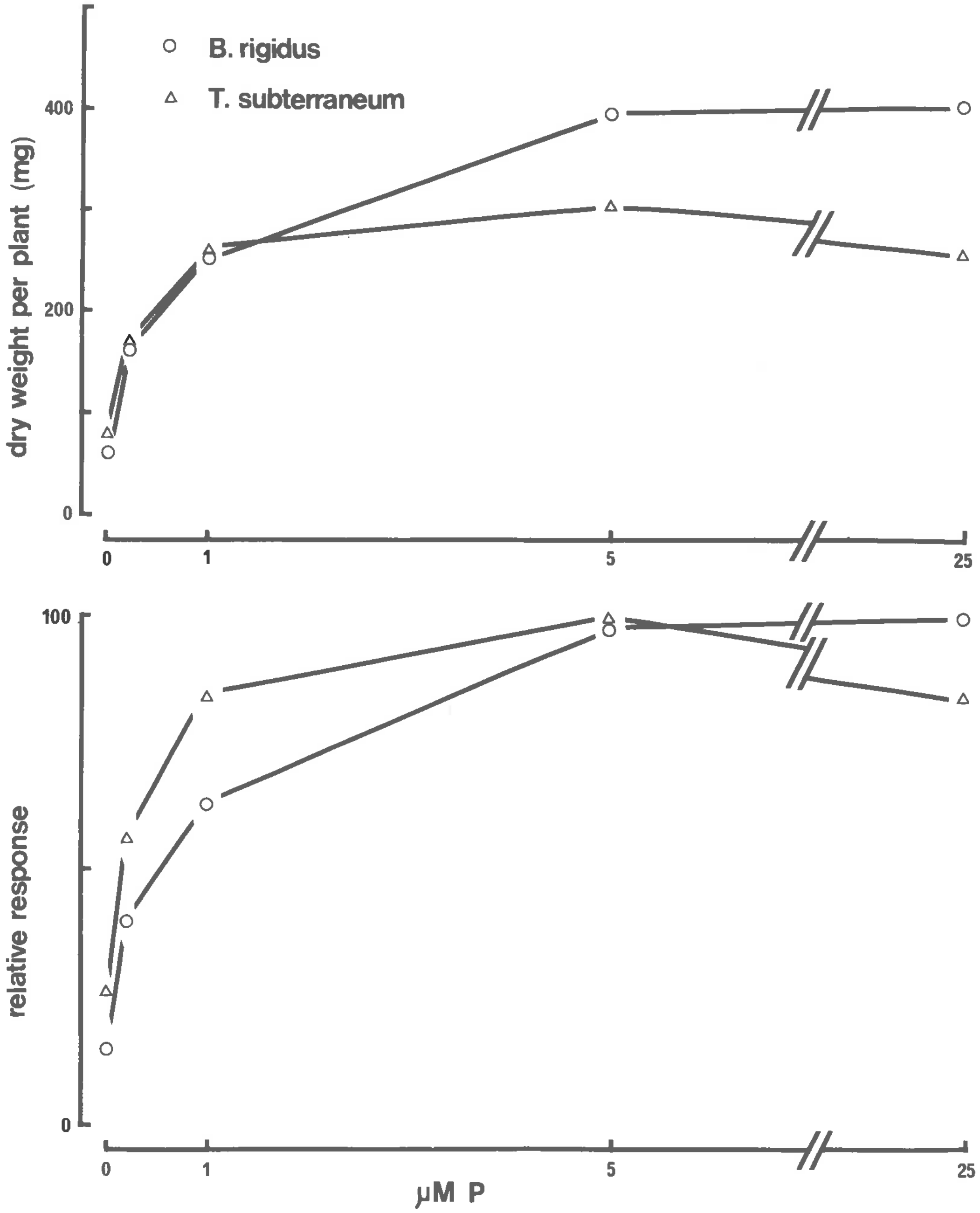
$$b \quad = \quad 1/(\pi L_{v})^{\frac{1}{2}} \qquad (18)$$

The L_{v} value of 30 cm^{-2} found towards the end of the growing period in the 0 to 2 cm layer corresponds to b = 0.1 cm. Olsen and Kemper (1968) give the radius of the depletion zone as a function of time. For example, assuming an apparent diffusivity of 1.10^{-7} $cm^{2}.sec^{-1}$, and adopting the boundary condition $c_{\cancel{v}r} = 0$, $t \geqslant 0$, $r = 0.05$ cm, where c is the solution concentration ($g.cm^{-3}$), t is the uptake time (sec), r is the radius of the axial part of the root, the outer boundary corresponding to ⩾ 50 per cent depletion would occur at a radial distance of 0.2 cm around the axis of the root after 20 days. The presence of root hairs would cause this zone to spread through the soil to an even greater distance. We conclude that direct competition could have occurred for phosphate.

On the other hand L_v was not so high during the early vegetative stages. During this time the plants would be influenced by the level of available phosphate. Asher and Loneragan (1967) measured the response of brome grass and subterranean clover cv. Mount Barker to phosphorus concentrations in flowing solution culture (Figure 8.1). It is interesting to note that the yield response of the two species, in solution culture, ran parallel to the changes in competitive ability found at different rates of phosphate when the plants were grown in mixture in the present experiments. Asher and Loneragan found that clover and grass yields were similar at low phosphate concentrations ($\leqslant 1\ \mu M$) while the grass yielded more than the clover at high phosphate concentrations ($\geqslant 5\ \mu M$). Bradshaw et al. (1960), Snaydon and Bradshaw (1962), Rorison (1969) and van den Bergh (1969) give instances where the nutritional characteristics of a genotype are correlated with the natural occurrence of that genotype.

However the response curves observed in soil differ from those found in solution culture. Ozanne, Keay and Biddiscombe (1969) grew a variety of pasture plants in a sandy loam over a wide range of phosphate levels. The response curve of the subterranean clover was sigmoidal, reaching a maximum yield at

Figure 8.1. The response of *Bromus rigidus* and *Trifolium subterraneum* to phosphorus concentration in flowing culture solution.
Source: Asher and Loneragan (1967).



ASHER & LONERAGAN (1967)

100 ppm P in dry soil. Wimmera ryegrass and silver grass responded to low levels of applied phosphate and reached a yield plateau at 50 ppm P in dry soil.

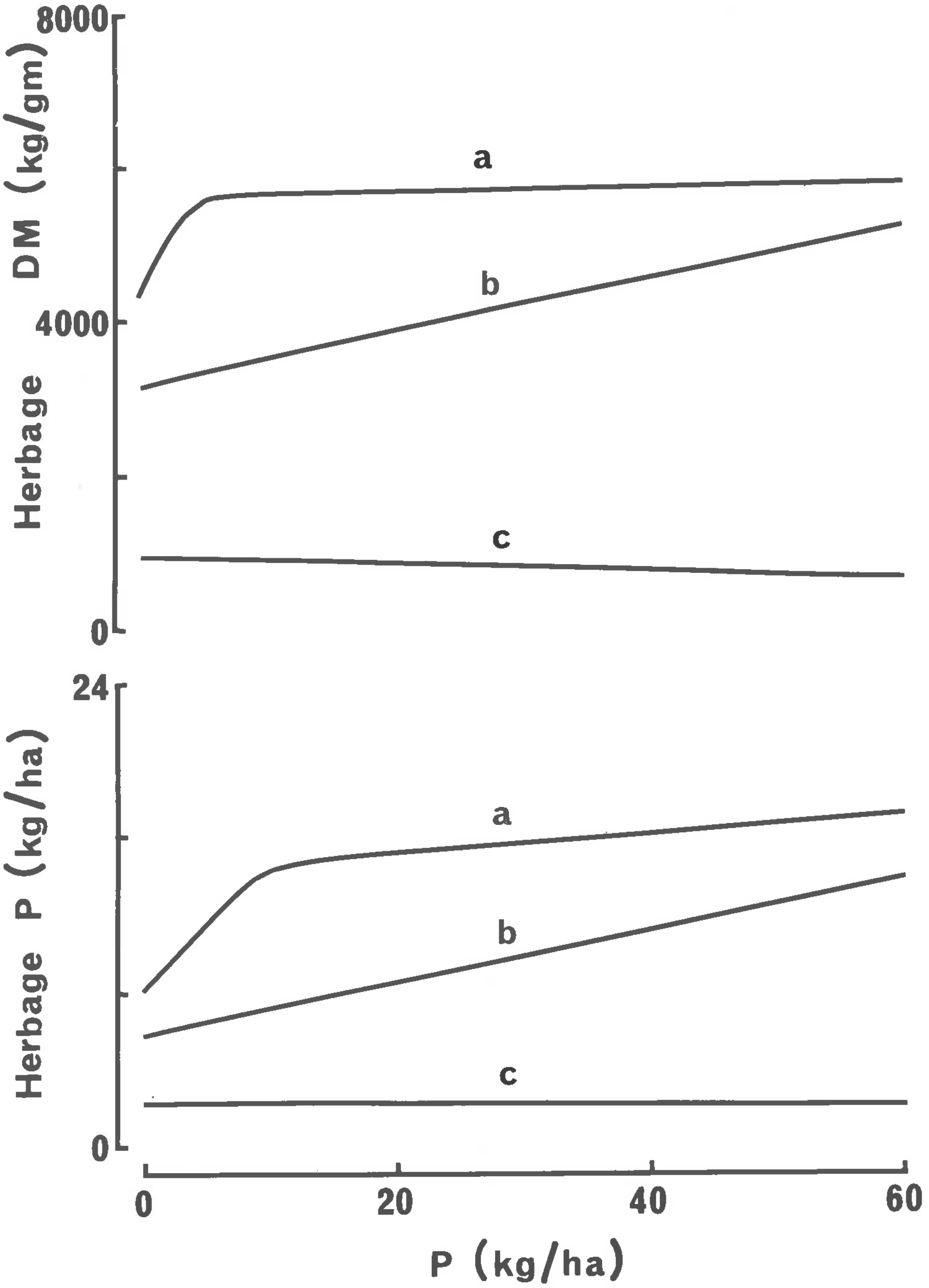
The most likely sequence of events influencing the performance of the components in mixture at different rates of phosphorus supply would have been (i) the immediate effect of phosphorus rate on growth, independent of competition for phosphorus itself, followed by (ii) direct competition for phosphorus at a later stage when L_v had become high.

An interesting contrast in the influence of phosphate status on grass/clover competition occurs between the annual pastures of southern Australia and the perennial pastures of New Zealand. After the initial equilibrium of the indigenous species has been disturbed by the addition of fertilizers and the introduction of new species, the New Zealand pastures consist largely of *Agrostis tenuis* Sibth (brown top) and *Trifolium repens* L. (white clover). As in the southern Australian pastures the legume is the only source of nitrogen. However, unlike the subterranean clover, white clover is the 'high fertility' species, and the ratio of clover to brown top is reduced if the phosphorus supplies are not maintained at a high level. From Jackman and Mouat (1970) Figure 8.2 shows that accumulated dressings of 60 kg/ha of phosphorus are required for the pasture to reach the same yield

Figure 8.2. The response to rates of applied phosphate of *Trifolium repens* (white clover) in monoculture (a) and as a component of a mixture with *Agrostis tenuis* (b). The *A. tenuis* (brown top) component in the mixture is given as (c).

Source: Jackman and Mo~~n~~uat (1970).

SOURCE : JACKMAN & MOUAT (1970)



level as a monoculture of the legume. Yet, as shown by the phosphorus uptake in Figure 8.2, there is no indication of competition for phosphorus by brown top. The grass appears to be able to suppress the legume at all but very high levels of phosphorus supply without itself being able to respond to additional phosphorus.

The effect of soil phosphate on the competitive ability of annual pasture species has important agronomic implications. Until, or unless, nitrogen can be supplied economically as fertilizer, a necessary aim of pasture management in southern Australia is to maintain a stable high yielding mixture of grass and legume, with an appreciable legume component. The short term effects of climatic variation on botanical composition are largely countered by grazing management. There is considerable scope for controlling the longer term trends in botanical composition by adjusting the rate at which phosphorus is supplied. On the sandy siliceous soil of south-east South Australia superphosphate is generally applied at rates in excess of pasture requirements. As Powrie (1963) pointed out, this practice is not only uneconomic, but it also removes the possibility of manipulating the botanical composition of the pasture by controlling the nutrient supply.

The rates of phosphorus applied as treatments in these experiments were derived from results of Powrie and Jennings (in press). They found little herbage dry matter response above a soil phosphorus level of 30 ppm acid extractable phosphorus in sandy soils similar to that used in the experiments. An equivalent amount of phosphorus was applied as the highest rate in these experiments. Although Experiment IV showed that part of the applied phosphorus may rapidly be converted to an acid-insoluble form, the relatively small response between P1 and P2 observed in Experiment III suggests that the supply would be approaching a non-responsive level at P2.

There are few examples of fertilizers being used deliberately on pastures simply to regulate botanical composition. Fertilizer nitrogen, for example, is applied to highly productive Dutch pastures to reduce the legume component, chiefly _T. repens_ L., to below 10 per cent and thereby reduce the incidence of bloat in cattle (t'Hart, 1956). More commonly the change in botanical composition brought about by the application of fertilizers is combined with an increase in productivity.

There are numerous reports of overyielding having occurred in grass/legume mixtures. De Wit, Tow and Ennick (1966) demonstrated that nitrogen fixed by _Rhizobium_ associated with the legume could cause overyielding when nitrogen was in scarce supply. Verhagen, Wilson and Britten (1963) hypothesized that, for most efficient light utilization, the 'ideal' canopy structure called for a high vertical component in the top leaves and a high horizontal component in the lower leaves. The canopy of a grass/legume mixture is close to this 'ideal' structure, and may outyield monocultures with predominantly either vertical or horizontal leaves. Although these mechanisms may have been involved in the overyielding that occurred in Experiment IV at the last harvest, the more likely explanation is the shift of the grass from the vegetative to reproductive phase. Overyielding has been observed in grass/legume mixtures in the field (Chamblee, 1958; Bakhuis and Kleter, 1965).

Further work on the effect of phosphorus on grass and clover populations under different grazing and management conditions is required to explore the feasibility of control with fertilizers of botanical composition and pasture quality. It would be of general scientific interest to further assess the relative importance of direct competition for phosphorus and the indirect effects of a growth response to phosphorus on competitive ability of a component in a mixture. It would also be of interest to measure the genetic variability of nutrient response within the species used, and to correlate differences found with the effects of phosphorus supply on competitive ability.

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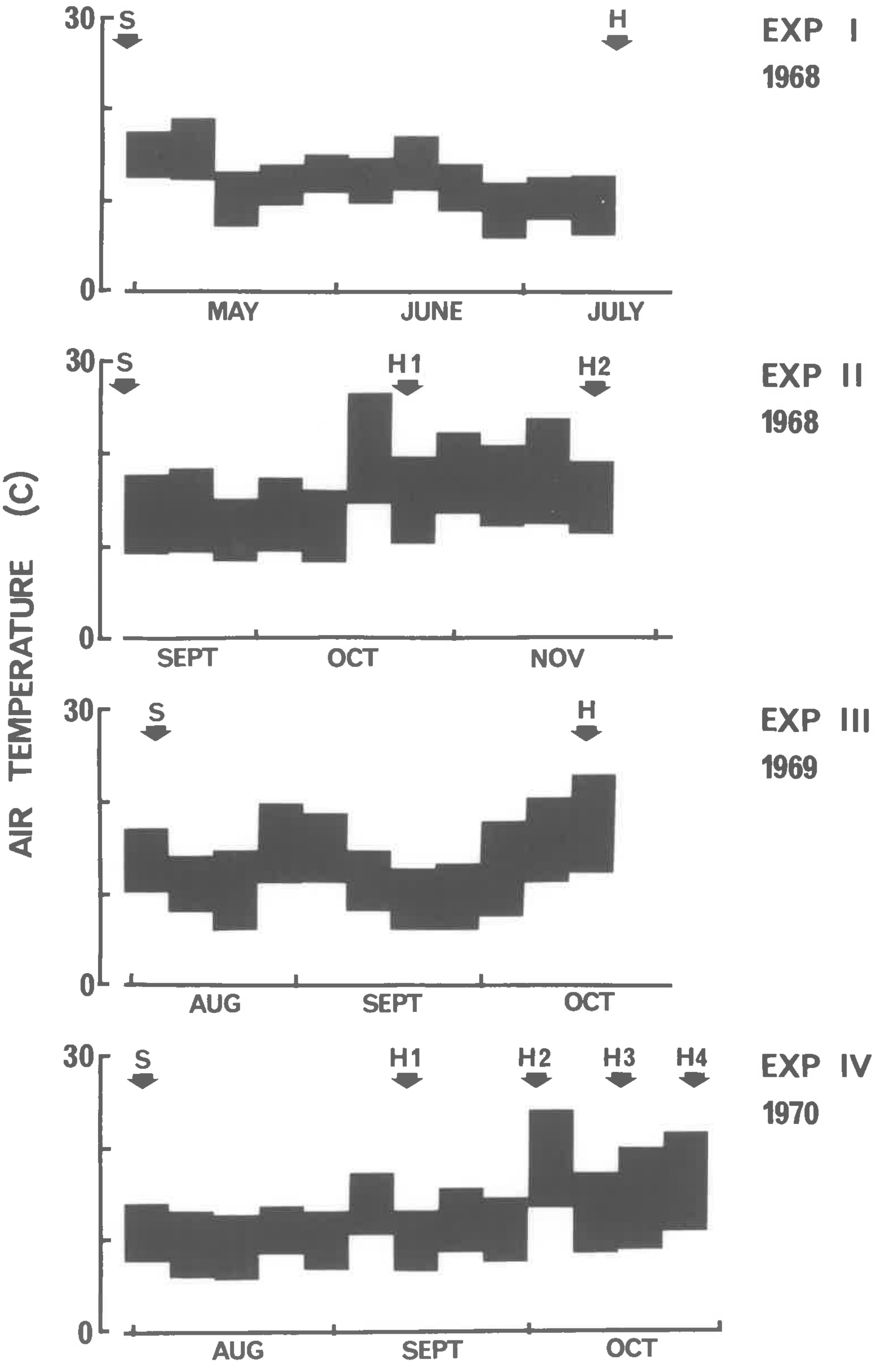
Vitis 5, 345-350.

<u>Appendix Figure A1</u>

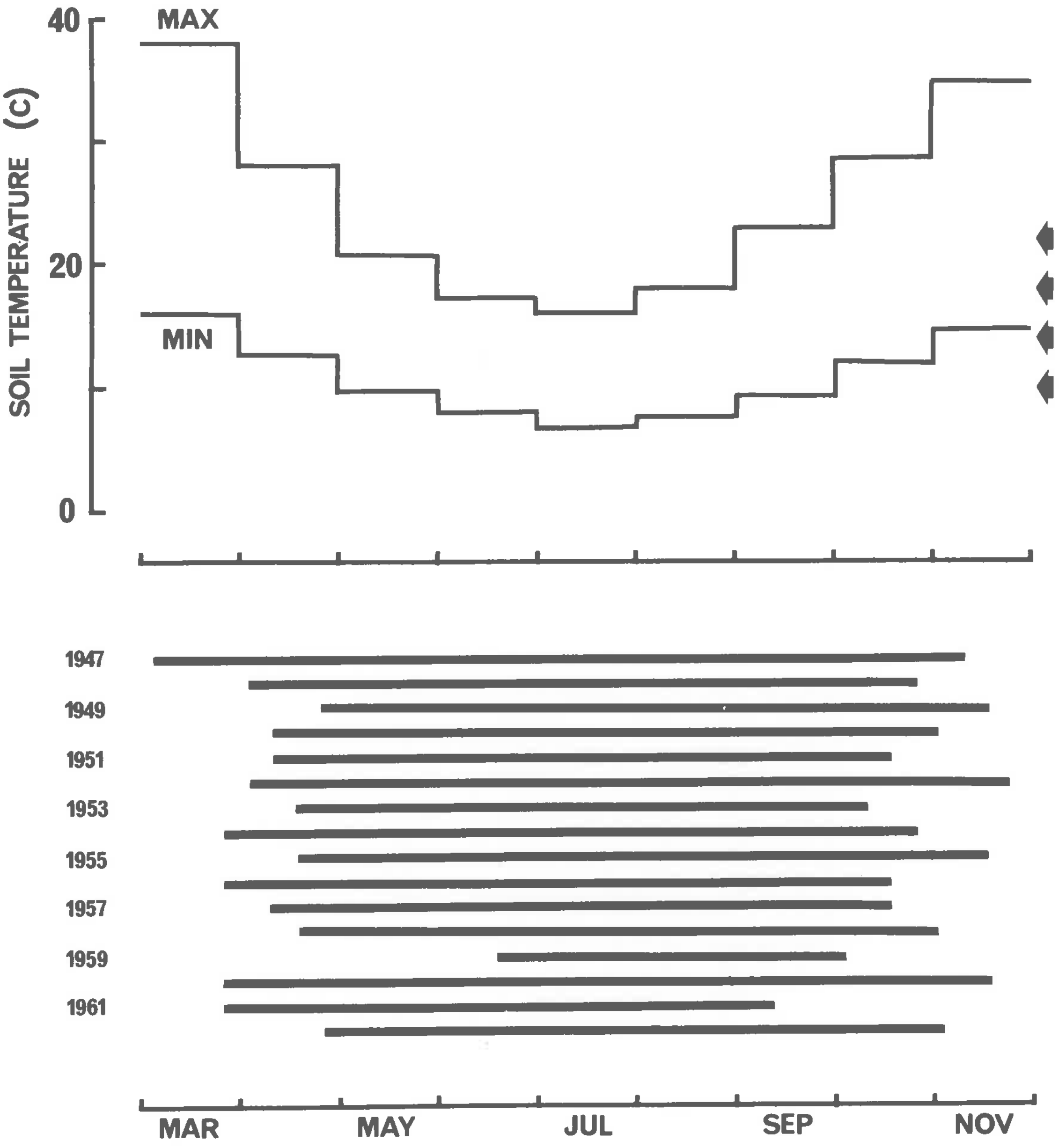
Bar chart showing range between mean weekly maximum and minimum air temperature (°C) recorded at the Waite Institute Meteorological Station over the period of Experiments I, II, III and IV. The sowing dates (S) and harvesting dates (H) are indicated by arrows.



AIR TEMPERATURE (C)
30
0
S
H
EXP I
1968
MAY
JUNE
JULY
30
0
S
H1
H2
EXP II
1968
SEPT
OCT
NOV
30
0
S
H
EXP III
1969
AUG
SEPT
OCT
30
0
S
H1
H2
H3
H4
EXP IV
1970
AUG
SEPT
OCT

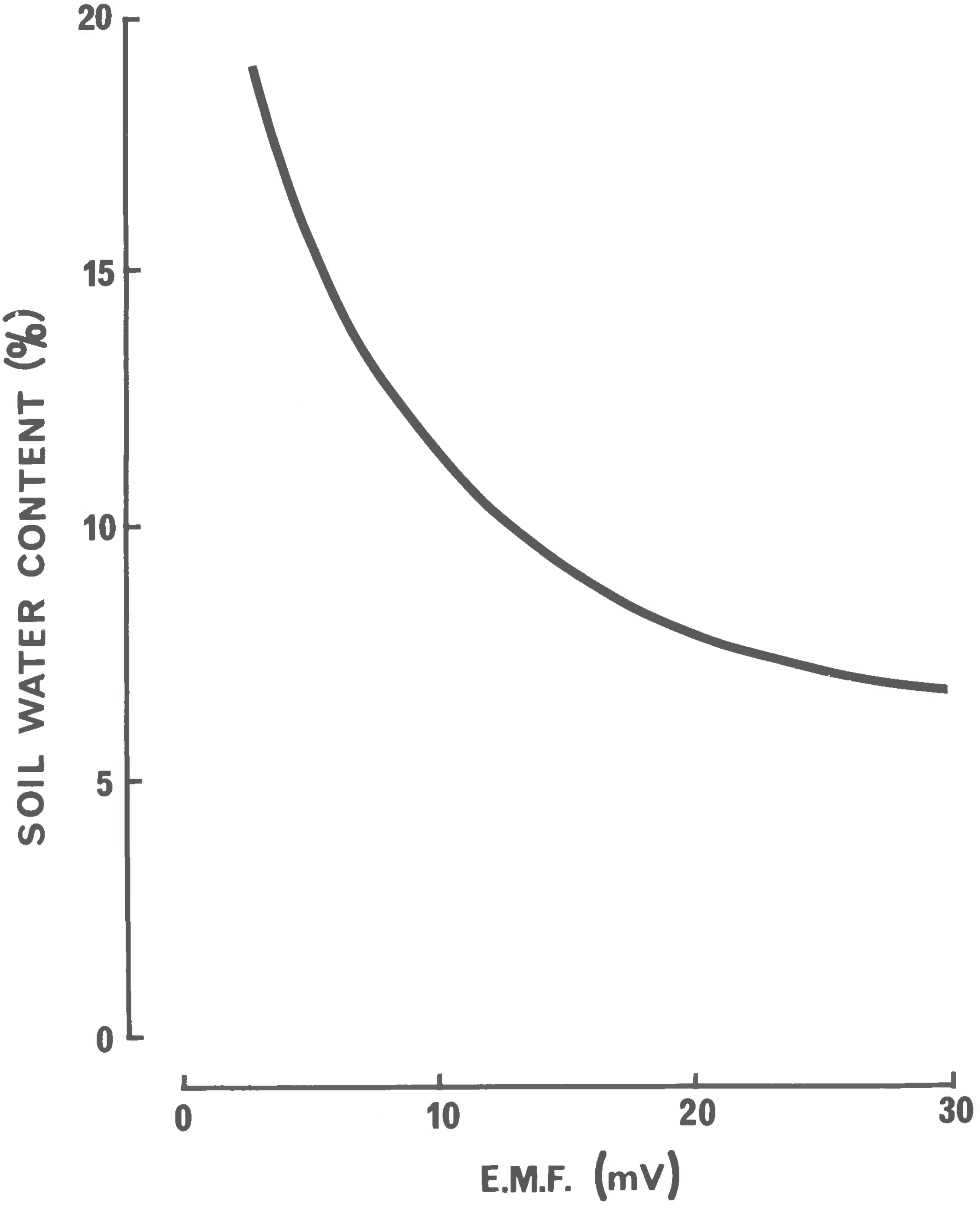
Appendix Figure A2

Monthly mean maximum and minimum soil temperature (°C) at a depth of 2.5 cm from the Waite Institute Meteorological Station. The arrows indicate the water temperature in the tanks in Experiment III. Temperatures prevailing during the growing season are indicated by reference to the lower figure, which shows the duration of the season estimated by the method of Trumble (1937) for 16 seasons.


40
20
0
SOIL TEMPERATURE (C)
MAX
MIN
1947
1949
1951
1953
1955
1957
1959
1961
MAR
MAY
JUL
SEP
NOV

Appendix Figure A3.

Mean calibration curve of the thermal conductivity probes. Water content of soil varied by mixing different amounts of water with soil.


SOIL WATER CONTENT (%)
20
15
10
5
0
0
10
20
30
E.M.F. (mV)

APPENDIX TABLE A1

Experiment I

Shoot dry weight per plant (mg)

Plant frequency	Treatment Nil	 P	 K	 S
		Brome		
1.0	321	325	336	450
0.75	356	385	372	400
0.50	423	416	448	552
0.25	601	637	577	779
			L.S.D. 1%	58
		Clover		
1.0	356	381	350	415
0.75	328	292	276	289
0.50	298	240	260	266
0.25	270	225	317	342
			L.S.D. 1%	92

APPENDIX TABLE A2

Experiment I

Leaf area per plant (cm^2)

Plant frequency	Treatment Nil	P	K	S
	Brome			
1.00	17.4	18.1	19.4	23.3
0.75	19.1	23.8	24.9	17.3
0.50	18.5	27.6	22.9	22.0
0.25	21.8	30.4	25.9	28.6
			L.S.D. 1%	11.0
	Clover			
1.00	10.9	11.2	10.3	11.5
0.75	7.8	9.0	8.0	8.1
0.50	6.9	7.1	6.9	6.1
0.25	6.0	7.7	6.8	6.4
			L.S.D. 1%	2.8

APPENDIX TABLE A3

Experiment I

Phosphorus concentration of shoots (%)

Plant frequency	Treatment			
	Nil	P	K	S
		Brome		
1.00	.194	.478	.220	.192
0.75	.180	.510	.212	.204
0.50	.173	.516	.238	.172
0.25	.194	.470	.206	.166
			L.S.D. 1%	.110
		Clover		
1.00	.206	.446	.117	.210
0.75	.166	.378	.164	.142
0.50	.168	.448	.149	.140
0.25	.170	.448	.140	.150
			L.S.D. 1%	.086

APPENDIX TABLE A4

Experiment I

Potassium concentration of shoots (%)

Plant frequency	Treatment			
	Nil	P	K	S
		Brome		
1.00	2.25	1.75	2.76	2.00
0.75	2.15	1.88	2.76	2.20
0.50	2.00	2.00	2.60	1.90
0.25	2.10	1.87	2.75	2.20
			L.S.D. 1%	0.83
		Clover		
1.00	1.45	1.60	2.00	1.65
0.75	1.05	0.90	1.87	1.10
0.50	0.75	0.75	1.60	0.90
0.25	0.75	0.75	1.45	0.75
			L.S.D. 1%	0.75

APPENDIX TABLE A5

Experiment I

Sulphur concentration of shoots (%)

Plant frequency	Treatment			
	Nil	P	K	S
		Brome		
1.00	.096	.110	.107	.310
0.75	.090	.095	.110	.310
0.50	.081	.105	.115	.290
0.25	.077	.103	.105	.295
			L.S.D. 1%	.075
		Clover		
1.00	.153	.160	.177	.297
0.75	.155	.137	.120	.310
0.50	.144	.127	.120	.275
0.25	.103	.115	.130	.247
			L.S.D. 1%	.060

APPENDIX TABLE A6

Experiment II

Calculations of the crowding coefficient, k, for the 4- and 3-parameter models after de Wit (1960) and Thomas (1970)

Treatment		No. of parameters in model	Crowding coefficients k_b	k_c	kxk	Between species F ratio	Between models F ratio
Low Density							
Harvest 1	P1	4	0.66	0.72	0.48	1.01(NS)	.196(NS)
		3	0.92	1.09	1.00		
	P2	4	1.42	0.48	0.69	1.01(NS)	.038(NS)
		3	1.95	0.51	1.00		
Harvest 2	P1	4	1.21	0.80	0.97	1.02(NS)	.001(NS)
		3	1.23	0.81	1.00		
	P2	4	1.73	0.64	1.10	1.00(NS)	.002(NS)
		3	1.61	0.62	1.00		
High Density							
Harvest 1	P1	4	1.51	0.75	1.13	1.01(NS)	.006(NS)
		3	1.39	0.72	1.00		
	P2	4	2.09	0.54	1.12	1.00(NS)	.004(NS)
		3	1.90	0.53	1.00		
Harvest 2	P1	4	1.60	0.90	1.44	1.02(NS)	.049(NS)
		3	1.26	0.79	1.00		
	P2	4	1.46	0.72	1.05	1.00(NS)	.009(NS)
		3	1.41	0.71	1.00		

APPENDIX TABLE A7

Experiment II

Root density, L_V, in each of three soil layers for brome grass (B) and clover (C), (cm^{-2})

Soil layer		P1			P2		
		0-2	6-8	12-14	0-2	6-8	12-14
				Harvest 1			
Treatment							
Low D	B mono	11.8	2.5	3.0	11.8	3.7	2.2
	C mono	7.6	1.4	1.9	6.8	1.4	1.5
	B mix	4.3	0.6	0.4	4.6	0.3	1.0
	C mix	4.8	0.7	0.2	4.5	0.2	0.2
	Mix total	9.1	1.3	0.6	9.1	0.5	1.2
High D	B mono	19.1	4.9	6.6	23.5	9.8	7.4
	C mono	16.5	4.8	2.5	12.3	2.0	1.4
	B mix	8.6	1.1	1.0	7.9	1.6	2.1
	C mix	7.0	1.8	0.9	5.8	1.8	1.9
	Mix total	15.6	2.9	1.9	13.7	3.4	4.0
				Harvest 2			
Low D	B mono	21.0	4.0	2.1	19.3	11.6	3.4
	C mono	14.1	2.3	0.8	12.6	4.4	1.7
	B mix	9.9	3.3	1.7	9.6	5.6	1.9
	C mix	9.3	1.3	0.3	12.3	2.4	0.4
	Mix total	19.3	4.6	2.0	21.9	8.0	2.3
High D	B mono	29.9	12.8	9.4	29.7	16.6	8.2
	C mono	20.6	7.9	6.2	16.5	10.2	9.1
	B mix	12.9	7.4	6.9	13.9	8.6	5.1
	C mix	15.7	7.3	4.1	16.7	6.4	3.4
	Mix total	28.6	14.7	11.0	30.6	15.0	8.5

APPENDIX TABLE A8

Table of mean squares from the analysis of variance of the dry weight, leaf area (A), and shoot-P expressed per plant

Treatment	df	Dry weight	Fresh weight	A	Shoot-P	
					%	Total
					$\times 10^{-5}$	$\times 10^{2}$
Brome						
Temperature (T)	3	20031	319.5	1411	214	2719
Reps within temp	8	359	6.7	148	87	800
Phosphate (P)	1	3239	31.5	431	10906	6460
Frequency (z)	1	5377	1385.3	4580	1920	2141
T x P	1	105	28.4	183	18	76
T x z	3	399	13.3	134	57	256
P x z	3	2647	92.3	153	149	312
T x P x z	3	162	4.6	27	22	27
Residual	24	233	6.0	54	117	54
Total	47					
Clover						
Temperature (T)	3	67027	2161.3	8814	2222	5103
Reps within temp	8	621	21.0	48	109	70
Phosphate (P)	1	329	115.5	275	130280	45828
Frequency (z)	1	4939	738.6	1415	166	427
T x P	1	83	21.5	1	476	1
T x z	3	1917	65.8	86	71	3196
P x z	3	894	22.1	193	59	224
T x P x z	3	111	28.2	61	119	145
Residual	24	331	7.7	25	169	78
Total	47					

APPENDIX TABLE A9

Table of mean squares from the analysis of variance of the L_{0-8} and L_{12-14} (cm per plant)

Treatment	df	Variate			
		Brome		Clover	
		L_{0-8}	L_{12-14}	L_{0-8}	L_{12-14}
Temperature (T)	3	41607	794	3692	326
Reps within temp	8	4199	39	3428	41
Phosphate (P)	1	10414	173	21358	280
Frequency (z)	1	2303	245	12031	28
T x P	1	4958	91	4605	1
T x z	3	8465	95	4752	1
P x z	3	5512	245	2772	1
T x P x z	3	407	229	3579	24
Residual	24	2853	68	2509	75
Total	47				

APPENDIX TABLE A10

Experiment III

Calculated crowding coefficient from dry weight data for the 4 and 3 parameter models after de Wit (1960) and Thomas (1970)

Treatment	No. of parameters in model	Crowding coefficients k_b	k_c	$k_b x k_c$	Between species F ratio	Between models F ratio
10°C P1	4	2.16	0.71	1.52	4.64(NS)	1.63(NS)
	3	1.55	0.64	1.00		
10°C P2	4	3.51	0.52	1.82	3.13(NS)	1.54(NS)
	3	2.06	0.49	1.00		
14°C P1	4	1.53	0.74	1.13	25.8**	
	3	1.40	0.72	1.00		
14°C P2	4	2.48	0.70	1.73	3.47(NS)	3.24(NS)
	3	1.60	0.62	1.00		
18°C P1	4	1.32	0.80	1.06	1.28(NS)	0.07(NS)
	3	1.27	0.78	1.00		
18°C P2	4	1.17	0.79	0.93	1.44(NS)	0.14(NS)
	3	1.23	0.81	1.00		
22°C P1	4	0.82	1.11	0.91	2.23(NS)	0.22(NS)
	3	0.85	1.18	1.00		
22°C P2	4	0.92	0.99	0.90	1.20(NS)	0.35(NS)
	3	0.96	1.04	1.00		

APPENDIX TABLE A11

Soil phosphate extractable by 1N H_2SO_4 (ppm P)

	P1					P2				
Depth (cm)	0-2	2-4	4-6	6-8	12-14	0-2	2-4	4-6	6-8	12-14
Treatment										
10°C										
Brome	8.3	4.1	3.2	3.3	3.2	16.8	5.0	5.0	3.9	3.7
Clover	6.8	4.1	3.1	3.1	3.2	17.9	7.2	4.6	3.2	3.1
Mixture	7.4	4.2	4.1	3.5	3.2	15.4	7.0	4.1	3.3	3.2
14°C										
Brome	6.7	4.1	4.1	3.5	3.3	16.2	8.9	5.1	4.4	3.5
Clover	6.8	4.2	4.0	3.5	3.1	14.8	6.9	4.8	4.2	3.1
Mixture	6.6	5.0	4.2	3.7	3.3	14.0	7.1	4.8	3.9	3.2
18°C										
Brome	6.2	4.3	4.1	3.7	3.2	13.5	7.2	4.7	3.8	3.1
Clover	5.9	4.3	4.2	3.9	3.4	14.0	6.3	4.5	3.8	3.3
Mixture	6.3	4.5	4.0	3.3	3.1	12.8	7.8	5.2	4.0	3.3
22°C										
Brome	7.2	4.1	3.8	4.0	3.4	11.2	6.4	4.1	3.2	3.3
Clover	6.3	4.8	3.9	3.4	3.2	12.1	6.6	5.1	4.0	3.4
Mixture	6.1	4.5	4.1	3.1	3.3	11.6	6.7	4.8	3.7	3.4

APPENDIX TABLE A12

Experiment IV

Concentration of phosphorus in shoots (percent)

	Harvest			
	1	2	3	4
Brome				
P0				
Monoculture	.104	.103	.095	.079
Shoots mixed	.125	.105	.101	.076
Roots & shoots mixed	.173	.099	.120	.079
P1				
Monoculture	.351	.183	.104	.066
Shoots mixed	.337	.148	.099	.064
Roots & shoots mixed	.475	.138	.092	.065
P2				
Monoculture	.645	.463	.271	.147
Shoots mixed	.617	.430	.270	.163
Roots & shoots mixed	.658	.398	.211	.144
Clover				
P0				
Monoculture	.078	.093	.096	.071
Shoots mixed	.102	.088	.093	.077
Roots & shoots mixed	.110	.093	.087	.077
P1				
Monoculture	.245	.143	.108	.084
Shoots mixed	.302	.141	.101	.073
Roots & shoots mixed	.368	.157	.103	.089
P2				
Monoculture	.655	.325	.198	.145
Shoots mixed	.730	.325	.189	.120
Roots & shoots mixed	.772	.429	.217	.134
L.S.D. 1%	.192	.136	.079	.046

APPENDIX TABLE A13

Experiment IV

Crowding coefficients calculated from dry weight data for the 4 and 3 parameter models after de Wit (1960) and Thomas (1970)

Treatments	No. of parameters in model	Crowding coefficients			Between species F ratio df 6, 6	Between models F ratio df 1, 12
		k_b	k_c	kxk		
Shoots only mixed						
Harvest 1 P0	4	0.98	0.94	0.93	5.63*	
	3	1.02	0.98	1.00		
P1	4	0.78	1.20	0.93	14.4**	
	3	0.80	1.25	1.00		
P2	4	1.16	1.10	1.27	2.04(NS)	1.19(NS)
	3	1.02	0.98	1.00		
Harvest 2 P0	4	1.48	1.03	1.53	9.94**	
	3	1.15	0.87	1.00		
P1	4	0.91	1.67	1.52	5.67*	
	3	0.79	1.26	1.00		
P2	4	0.91	0.97	0.88	1.94(NS)	0.53(NS)
	3	0.96	1.04	1.00		
Harvest 3 P0	4	1.41	1.16	1.64	2.23(NS)	1.44(NS)
	3	1.07	0.93	1.00		
P1	4	0.93	1.14	1.06	2.24(NS)	0.04(NS)
	3	0.91	1.10	1.00		
P2	4	0.69	1.31	0.90	3.78(NS)	0.28(NS)
	3	0.71	1.40	1.00		
Harvest 4 P0	4	1.23	1.06	1.30	3.84(NS)	0.75(NS)
	3	1.07	0.94	1.00		
P1	4	0.69	1.93	1.33	1.90(NS)	1.19(NS)
	3	0.65	1.55	1.00		
P2	4	0.84	1.76	1.49	1.23	11.5**
	3	0.75	1.33	1.00		

APPENDIX TABLE A13 (continued)

Treatments	No. of parameters in model	Crowding coefficients			Between species F ratio df 6, 6	Between models F ratio df 1, 12
		k_b	k_c	kxk		
Root and shoots mixed						
Harvest 1 P0	4	0.72	0.79	0.57	11.00**	
	3	0.94	1.07	1.00		
P1	4	0.97	1.11	1.08	5.90*	
	3	0.94	1.07	1.00		
P2	4	1.06	0.71	0.75	3.29(NS)	1.77(NS)
	3	1.27	0.79	1.00		
Harvest 2 P0	4	1.06	0.74	0.79	2.21(NS)	0.60(NS)
	3	1.23	0.81	1.00		
P1	4	2.33	1.04	2.33	1.16(NS)	20.0***
	3	1.26	0.79	1.00		
P2	4	1.34	0.72	0.97	1.24(NS)	0.04(NS)
	3					
Harvest 3 P0	4	0.85	0.85	0.72	5.03*	
	3	1.01	0.99	1.00		
P1	4	1.13	0.96	1.09	2.58(NS)	0.08(NS)
	3	1.08	0.93	1.00		
P2	4	1.36	1.06	1.44	2.34(NS)	3.30(NS)
	3	1.10	0.91	1.00		
Harvest 4 P0	4	1.03	0.75	0.78	1.24(NS)	0.32(NS)
	3	1.21	0.83	1.00		
P1	4	1.12	0.92	1.03	2.43(NS)	0.01(NS)
	3	1.10	0.91	1.00		
P2	4	2.08	2.20	4.57	2.19(NS)	44.5***
	3	0.99	1.01	1.00		

APPENDIX TABLE A14

Soil phosphate extractable in 1N H_2SO_4 (ppm P)

	P0					P1					P2				
	0-2	2-4	4-6	6-8	12-14	0-2	2-4	4-6	6-8	12-14	0-2	2-4	4-6	6-8	12-14
						Harvest 2									
Monoculture															
Brome	2.9	2.9	3.2	3.1	3.3	6.7	4.5	3.3	3.4	3.1	16.7	10.4	6.8	5.1	3.8
Clover	3.3	3.1	3.0	3.1	3.1	6.6	4.0	3.5	3.3	3.6	13.5	7.2	5.3	3.9	3.6
Shoots mixed															
Brome	2.9	2.9	3.0	3.1	3.3	6.3	4.1	3.5	3.1	3.0	11.7	7.9	5.5	4.3	3.5
Clover	3.6	3.5	3.7	3.4	3.3	7.3	4.4	3.3	3.3	3.6	11.2	7.7	5.9	4.2	3.1
Roots & shoots mixed	2.8	3.3	2.9	2.9	3.1	6.3	4.4	3.0	3.1	3.2	16.1	8.8	6.2	3.7	3.6
						Harvest 4									
Monoculture															
Brome	5.0	3.9	3.8	3.8	2.9	5.6	4.2	3.9	4.3	3.1	9.0	5.6	4.7	3.7	2.9
Clover	5.9	3.5	4.0	3.5	3.1	7.3	4.1	3.5	3.1	3.2	11.1	5.2	4.1	3.3	2.9
Shoots mixed															
Brome	5.1	3.9	3.7	3.4	2.9	5.5	3.7	3.5	3.1	2.8	9.4	6.1	5.3	3.4	3.0
Clover	3.4	3.8	3.9	3.6	3.3	6.4	4.3	3.7	3.3	3.1	8.7	5.7	4.6	3.0	3.0
Roots & shoots mixed	3.7	3.4	3.8	2.9	3.0	5.5	4.2	3.8	3.3	3.2	10.3	5.7	5.1	3.3	3.0