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Increasing phosphorus concentration in seed of annual pasture legume species increases herbage and seed yields

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Abstract

In glasshouse experiments with low levels of soil applied phosphorus (P), yields of four annual pasture legumes (*Medicago polymorpha*, *Trifolium subterraneum*, *T. balansae*, *Ornithopus compressus*) increased with increasing P concentration in the seed.

In a further experiment, *M. polymorpha* cv. Serena was grown at the same plant density from seed of two P concentrations and two seed sizes when two levels of finely ground superphosphate were applied to the soil. Higher P concentrations in the seed increased yields of dried tops by about 30% for the first harvest (21 days), 20% for the second harvest (52 days), and 9% at maturity (103 days), and seed yields by 11%. Larger seeds increased yields of dried tops by between 6–46% for the first two harvests but at maturity yields of dried tops and seed were unaffected by seed size. None of the interactions were statistically significant ($P > 0.05$), except for the first harvest when two interactions (P concentration in the seed × seed size (*i.e.* P content in seed), and P applied to the soil × P concentration in the seed × seed size) were significant at $P < 0.05$ level.

In a field experiment, *Trifolium subterraneum* clover seed (two cultivars) of the same size but with two different P concentrations was sown at the same plant density and two levels of granulated (0.2–5 mm) superphosphate were applied to the soil surface. The higher level of superphosphate increased dried herbage yields of the dense clover swards by three- to four-fold 90 and 120 days after sowing. The higher P concentration in the seed increased yields of dried herbage by between 50 to 25%, depending on the level of P applied to the soil and the harvest date.

Introduction

Austin (1966) showed that when watercress (*Rorippa nasturtium aquaticum*) was grown in sand culture from seed with different phosphorus (P) concentrations, the dry matter (DM) yields of tops of plants increased with increasing P concentration in the seed. However, the effect of seed P concentration on DM yields of tops diminished with age and disappeared by maturity, but seed yields still increased with increasing P concentration in the seed (Austin, 1966).

We wished to test whether increasing P concentration in seed of annual legume species (*e.g. Medicago polymorpha*, *Trifolium subterraneum*, *T. balansae*, *Ornithopus compressus*) commonly used for pastures in south-western Australia increased DM herbage and seed yields of plants grown from the seed. In the Mediterranean-type climate common to much of southern Australia, pasture production during autumn (March-June) is critical for animal production (Purser, 1980). During autumn, paddock-grown feed for stock comprises the dried residues of last-year's pasture, together with emerging seedlings of annual plants which are small and probably mostly inaccessible to stock. The dry feed is usually becoming scarce and the quality deteriorates rapidly as it becomes wet from increasing rain fall. Thus increasing DM herbage yields of emerging seedlings during autumn due to increasing P concentration in the seed could be very significant practically.

Bolland and Baker (1988) showed in a glasshouse pot experiment (1.8 kg soil/pot) that seed of *Medicago polymorpha* cv. Serena of the same size but with increasing P concentration produced higher yields of dried herbage for up to 35 days after sowing. The benefit occurred both in the absence and presence of superphosphate applied to the soil. This paper presents the results of glasshouse box (13.5 kg soil/box) experiments to test whether higher P concentration in the seed of *Trifolium subterraneum*, *T. balansae* and *Ornithopus compressus* increase yields of dried herbage (experiments 1–5) or seed yield (experiment 5, *M. polymorpha* cv. Serena). A field experiment (experiment 6) with two cultivars of *Trifolium subterraneum* examined whether sowing clover seed of the same size but with increasing P concentration in the seed increased subsequent dried herbage yields of the plants. Two levels of superphosphate were applied to the soil.

Materials and methods

(*1*) *Glasshouse box experiments*

Soil The soil used for all the five box experiments was the <2 mm fraction of the surface 10 cm of newly cleared, lateritic, ironstone-gravel sand (KS-Uc 4.21, Northcote 1979), which is a dystric xerorthent (Soil Survey Staff, 1975). The soil was collected from North Bannister, 90 km south-east of Perth. The pH of the soil (1:5 soil:0.01 *M* $CaCl_2$) was 5.3; total P (after digestion in concentrated H_2SO_4) was 58 μg P g^{-1} soil; bicarbonate P extracted from the soil (Colwell, 1963) was 2 μg P g^{-1} soil; and the P adsorption capacity (Ozanne and Shaw, 1967) was 15.

Experiments 1–4 Each of these experiments measured the dry matter (DM) herbage production of a different pasture legume 49–70 days after sowing when only one level of superphosphate (1.5 g P/pot) was applied to the soil in all the boxes.

Subsamples of 13.5 kg of soil were weighed out into rectangular, undrained boxes 29 by 39 cm and 12 cm deep (depth of soil was 10 cm) in a glasshouse with a mean maximum temperature of 17.3°C (range 12–23°C). A basal nutrient solution was applied to the soil surface of all boxes to supply the following nutrients per box: 1.1 g nitrogen as ammonium nitrate, 1.1 g potassium and 1.6 g sulphur as potassium sulphate, 6.4 mg boron as boric acid, 530 mg manganese as manganese sulphate, 79 mg magnesium as magnesium sulphate, 158 mg calcium as calcium chloride, 32 mg copper as copper sulphate, 27 mg zinc as zinc sulphate, 1.4 mg molybdenum as sodium molybdate, and 1.4 mg cobalt as cobalt sulphate. A total of 675 mg of ammonium and 2.3 g nitrate and 2.7 g sulphate was added per box. When the soils had dried after application of the basal nutrients, 16.5 g of finely ground superphosphate (to pass a 250-μm sieve) was added to the soil and the soils and fertilizer were thoroughly mixed. The superphosphate contained 9.1% total P, 7.3% water-soluble P, 1.3% neutral ammonium citrate-soluble P, 0.5% acid soluble P, 22% calcium, 10.5% sulphur, 0.5%, iron and 0.4% aluminium, 19 $\mu g\ g^{-1}$ copper and 437 $\mu g\ g^{-1}$ zinc. The soils were then watered to field capacity (gravimetric field capacity was 16%) with deionised water and were retunred to field capacity by daily watering by weight. The boxes were re-randomized in the glasshouse every second day. Application of basal fertilizers and superphosphate, and wetting the soil to field capacity for the first time were done 7 days before sowing the seed. Further applications of basal nutrient solution containing 675 mg ammonium and 2.3 g nitrate were applied to the boxes 10, 20 and 40 days after sowing.

Twenty seven seeds of each species were sown per box at 1-cm depth. The plants were thinned to 21 uniform plants/box fifteen days after sowing.

The different P concentrations in the seed used for each of the six experiments described in this paper were obtained by applying different amounts of superphosphate to the seed crops in previous years.

Specific details for each of the experiments 1–4 are now provided.

Experiment 1 (on *M. polymorpha* cv. Serena) used a randomized complete block of three P concentrations in the seed (3.1, 4.6 and 5.4 g P kg^{-1}) with four replications. Plant tops were harvested 49 days after sowing. Seed of the same size (2.1 mg) was used for the experiment.

Experiment 2 (on *T. subterraneum* cv. Junee) used a randomized complete block of four P concentrations in the seed (4.8, 5.2, 6.5 and 7.5 g P kg^{-1}) with four replications. Plant tops were harvested 70 days after sowing. Seed of the same size (5.4 mg) was used for the experiment.

Experiment 3 (on *T. balansae* cv. Paradana) used a randomized complete block of three P concentrations in the seed (3.4, 4.5 and 5.2 g P kg^{-1}) with four replications. Yield of dried plant tops were measured 52 days after sowing. Seed of the same size (0.8 mg) was used for the experiment.

Experiment 4 (on *O. compressus* cv. Madeira). The design was a randomized complete block of four P concentrations in the seed (3.1, 4.7, 5.2 and 6.1 g P kg^{-1}) with four replications. Yield of dried tops were measured 52 days after sowing. Seed of the same size (2.1 mg) was used for the experiment.

Experiment 5. This experiment (on *M. polymorpha* cv. Serena) was to measure the effect of P concentration in seed and the effect of seed size on yields of dried herbage and seed yields. The treatments were a factorial combination of two levels of P applied to the soil, two P concentrations in the seed, two seed sizes and three harvests. There were three replicates. The two levels of superphosphate applied to the soil were 1.5 and 15.0 g P/box; the two P concentrations in the seed were 3.6 and 7.0 g P kg^{-1}; the two seed sizes were 2.6 and 3.8 mg/seed; the three harvest dates were 21, 52 and 103 days after sowing, the latter being at maturity when all the medic plants had dried.

The procedures used to fertilize, water, sow and manage the boxes were as described for experiments 1–4, except that basal nutrient solution containing 675 mg ammonium and 2.3 g nitrate was applied to the boxes 10, 20, 40 and 80 days after sowing the seed.

Measurements Dry matter (DM) yields of tops were measured by cutting plants at ground level and drying the tops for three days at 70°C in a forced-draught oven. Seed yields were measured by collecting all the pods after drying and weighing the total top yield (dried herbage plus pods and seed) and the seeds were removed from the pods by hand before weighing.

The P concentration in dried tops and in seed were measured by grinding the tops and seed and digesting the finely ground material first in nitric acid and then in a 1:1 mixture of nitric acid and perchloric acid and measuring the P concentration in the digests by the molybdovanado-phosphate method (AOAC, 1975).

Just before sowing the Serena medic seed in the main experiment, five random 10-g soil samples (0–10 cm) were collected from each box to measure bicarbonate-extractable soil P at 23°C using the procedures outlined by Colwell (1963).

(2) Field experiment (experiment 6)

The field experiment was at Dale, 100 km east of Perth, Western Australia, on newly cleared, lateritic, ironstone-gravel sand which was similar to the North Bannister soil used for the glasshouse box experiments. The average annual rainfall at Dale is 584 mm. The design of the field experiment was a randomized complete block with three replications of two subterranean clover cultivars, two P concentrations in the clover seed, and two levels of superphosphate applied to the soil. The clover cultivars were Northam and Dalkeith. Seed of the same size (5.1 ± 0.06 mg/seed) were used for the experiment. The two P concentrations in the seed were: Northam, 3.6 and 5.6 g P kg^{-1}; Dalkeith, 3.3 and 6.4 g P kg^{-1}. The levels of granulated (0.2–5 mm) superphosphate applied to the soil were 5 and 100 kg P ha^{-1}. Seed was sown at 70 kg ha^{-1}. The superphosphate and seed were applied (May 19, 1988) to the soil surface in 1.4 by 5.0 m plots. The plots were surrounded by 2 m of untreated soil. The seed and fertilizer were covered with soil using light harrows. Before sowing, the seed was inoculated with *Rhizobium trifolii* strain WU 95 and lime-pelleted. The following basal fertilizers were applied to the soil surface of each plot before sowing (kg ha^{-1}): 10 copper sulfate (27% copper), 2 zinc oxide (80% zinc), 0.2 molybdenum trioxide (67% molybdenum), 30 manganese sulfate (32% manganese), 50 magnesium sulfate (10% magnesium), 3 sodium borate (11% boron), 0.2 cobalt sulfate (21% cobalt), 100 potassium chloride (50% potassium) and 100 gypsum (18% sulfur, 22% calcium).

Emergence of clover plants was satisfactory, and was not affected by the cultivar, the level of P applied to the soil, or the P concentration in the seed. There was an average of 5 plants/dm^2 measured a month after sowing.

Yields of dried herbage were measured 90 and 120 days after sowing by cutting top growth at ground level within five random 10 by 100 cm quadrats per plot, using a new section of the plot for each harvest. The cut material was dried for three days at 70°C in a forced-draught oven before weighing.

Results

Experiments 1–4

Yields of dried tops increased with increasing concentration of P in the seed for all four species (Fig. 1). Increases in DM production per unit of seed P was estimated from the slope of linear regression in Figure 1. For the 50-day old plants the slope values varied with species, being least for *T. balansae* and largest for *M. polymorpha*.

P concentrations measured in dried tops were unaffected by the P concentration in seed used to grow the plants, being (mean g P kg^{-1} ± s.e.) 2.5 ± 0.1 for *M. polymorpha*, 2.7 ± 0.1 for subterranean clover, and 2.8 ± 0.1 for both *T. balansae* and *O. compressus*. For these pasture legumes, P deficiency does not usually occur unless P concentrations in dried herbage is less than 2 g P kg^{-1} (unpublished data of the Western Australian Department of Agriculture). P content (*i.e.* P concentration × yield) in the dried tops increased in response to increasing P concentration in seed used to grow the plants in an almost identical fashion as DM yield of the tops.

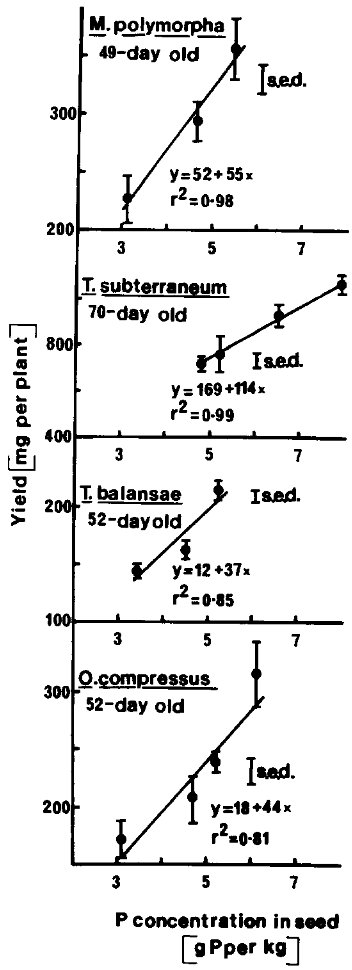

Fig. 1. Relationship between yield of dried tops and the P concentration in seed used to grow the tops measured for the four different annual pasture legume species in experiments 1–4. Lines are fits to linear regression, and the equation and coefficients are given in the figures. Bars for each point are s.e. of the mean (n = 4). The other bar in each figure are s.e.d. ($P < 0.05$) for all the data for each experiment calculated from analysis of variance.

Experiment 5

Differences in yields due to the main effects (*i.e.* level of P applied to the soil, P concentration in the seed and seed size) were statistically significant for all harvests except for seed size at the third harvest (Table 1). The only statistically significant interactions between the main effects were obtained for the two following interactions at the first harvest: [P concentration in the seed × seed size (*i.e.* P content in seed)], and [P

Table 1. Significance of the differences between yield results and P content (P concentration × yield) in plant tissue results for experiment 5 due to the main effects and the interactions of the main effects: ns = not significant, $P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.001$. Note there are no P concentration data, and therefore P content data for dried tops for harvest 3.

Source of variation	Harvest 1 (21 days) Dried tops	Harvest 2 (52 days) Dried tops	Harvest 3 (103 days)	
			Dried tops	Seed
P applied to the soil (soil P)	**	**	**	**
P concentration in the seed (seed P)	**	*	*	*
Seed size	**	*	ns	ns
Soil P × seed P	ns	ns	ns	ns
Soil P × seed size	ns	ns	ns	ns
Seed P × seed size	*	ns	ns	ns
Soil P × seed P × seed size	*	ns	ns	ns

applied to the soil × P concentration in the seed × seed size] (Table 1). Thus all the main effects significantly increased yields at each harvest, except for seed size at the third harvest. Though P content in seed (*i.e.* P concentration × seed size) in the seed increased yields, the only statistically significant effect was for the first harvest.

Dry matter (DM) yields of dried tops and seed yields of Serena medic plants were always significantly increased by the higher level of superphosphate applied to the soil (Fig. 2).

Medic seed of the same size, but with increasing P concentration in the seed, produced larger DM yields of tops for both levels of superphosphate applied to the soil (Fig. 2). However the relative increases in DM yields of tops decreased with time, being about 30% for the first harvest (21 days after sowing), 20% for the second harvest (52 days after sowing), and 9% at maturity (103 days after sowing). Higher P concentrations in seed increased seed yields by about 11% for both seed sizes and for both levels of superphosphate applied to the soil.

Larger seed with the same P concentration increased DM yields of plant tops grown from the seed for both levels of superphosphate applied to the soil for the first two harvests, but seed size had no effect on DM yields of tops or seed yields at maturity (Fig. 2). Relative increases in yield for the larger seed varied between 6–46%, depending on harvest, the P concentration in the seed and level of superphosphate applied to the soil.

At maturity, the harvest index (seed yield/total yield of tops including pods and seed) was unaffected by P concentration in the seed, seed

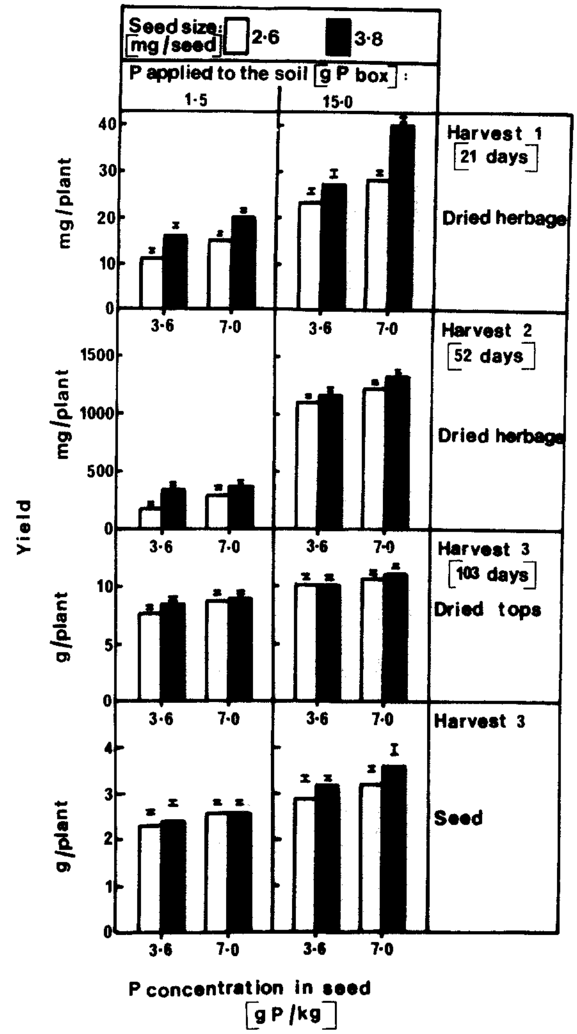

Fig. 2. Yield of dried tops and seed measured for *M. polymorpha* cv. Serena in experiment 5. Significance of the differences between the main effects and the interactions are given in Table 1. Bars are s.e. of the mean (n = 3).

size or the level of superphosphate applied to the soil, and was (mean ± s.e.) 0.30 ± 0.004 for all treatments.

The P concentration measured in dried tops and in seed increased as the level of superphosphate applied to the soil increased, but it was unaffected by either the P concentration in seed or the size of the seed used to grow the plants (Fig. 3). Differences in the P concentration values measured in dried tops or seed were only significant ($P < 0.01$) for the two levels of P applied to soil (Table 2). None of the other main effects, or any of the interactions between the main effects produced significant differences ($P > 0.05$) (Table 2).

The P content (P concentration × yield) in dried tops and seed was always significantly increased by the larger level of superphosphate applied to the soil, and by using seed with the higher P concentration (Fig. 4). However, seed size only significantly affected P content in dried tops for the first two harvests, and seed size had no effect on P content in seed produced at maturity. The significance of the differences between the P content data due to main effects and the interaction of the main effects are shown in Table 1.

Since there were only two levels of superphosphate applied to the soil, there were only two bicarbonate-extractable soil tests for P (Colwell P) values (mean ± s.e., n = 36): 41 ± 1 and 367 ± 7 μg P g^{-1} soil. Colwell P values were related to different yields, not only for the two levels of superphosphate applied to the soil, but also for plants grown from seed of different sizes, as is shown for 52 day-old dried tops in Figure 5a, and for plants grown from seed with different P concentrations in the seed, as is shown for seed yields in Figure 5b.

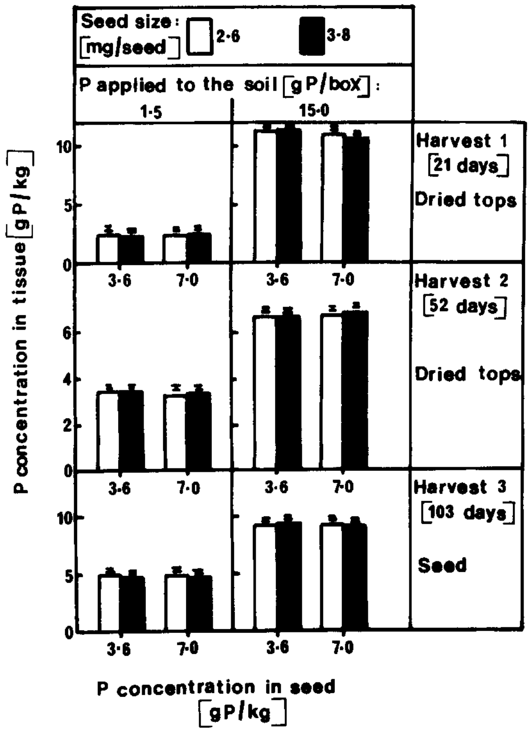

Fig. 3. P concentration measured in dried tops and seed in experiment 5. The only statistical differences ($P < 0.01$) in P concentration values measured in dried tops or seed were due to the level of P applied to the soil; none of the other main effects or interactions resulted in significant differences ($P > 0.05$) (Table 2). Bars are s.e. of the mean (n = 3).

Experiment 6 (field experiment)

Yields of dried *T. subterraneum* herbage were increased by about three to four-fold for the

Table 2. Significance of the differences between P concentration values measured in dried tops and seed of experiment 5 due to the main effects and the interactions of the main effects: ns = not significant, $P > 0.05$; $^{**}P < 0.01$

Source of variation	Harvest 1 (21 days) Dried tops	Harvest 2 (52 days) Dried tops	Harvest 3 (103 days) Seed
P applied to the soil (soil P)	**	**	**
P concentration in the seed (seed P)	ns	ns	ns
Seed size	ns	ns	ns
Soil P × seed P	ns	ns	ns
Soil P × seed size	ns	ns	ns
Seed P × seed size	ns	ns	ns
Soil P × seed P × seed size	ns	ns	ns

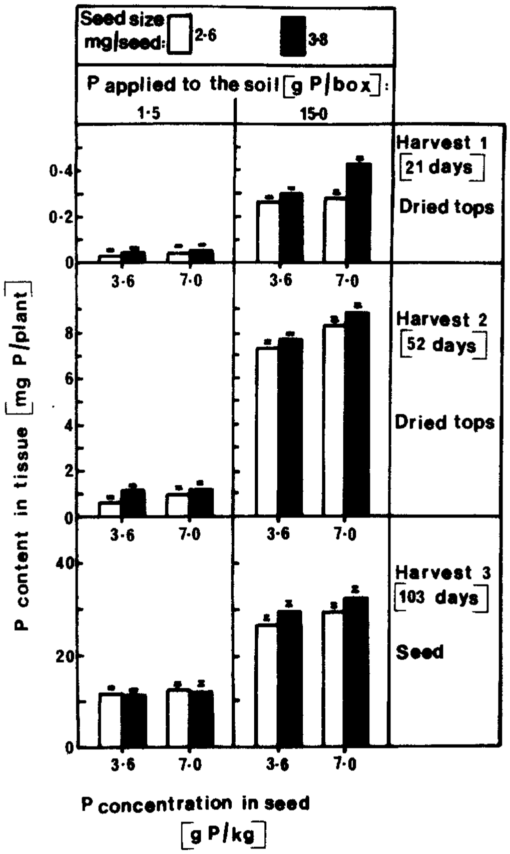

Fig. 4. P contents (P concentration × yield) measured in dried tops and seed in experiment 5. Significance of the differences between the main effects and the interactions are given in Table 1. Bars are s.e. of the mean (n = 3).

higher level of superphosphate applied to the soil (Fig. 6). The higher P concentration in the seed increased herbage yields by about 50% for the first harvest when the lowest level of P was applied to the soil, and by about 40% for the largest level of soil-applied P. Corresponding increases in yield for the second harvest were smaller, about 35 and 25%.

Discussion

Increasing P concentration in seeds of annual plants increases yields of plants grown from the seed (Austin, 1966; Birecka *et al.*, 1962; Bolland and Baker, 1988; Durrant, 1958; Szykalski, 1961, 1962; and Figs. 1, 2 and 6). Higher P concentration in the seed may increase root growth of

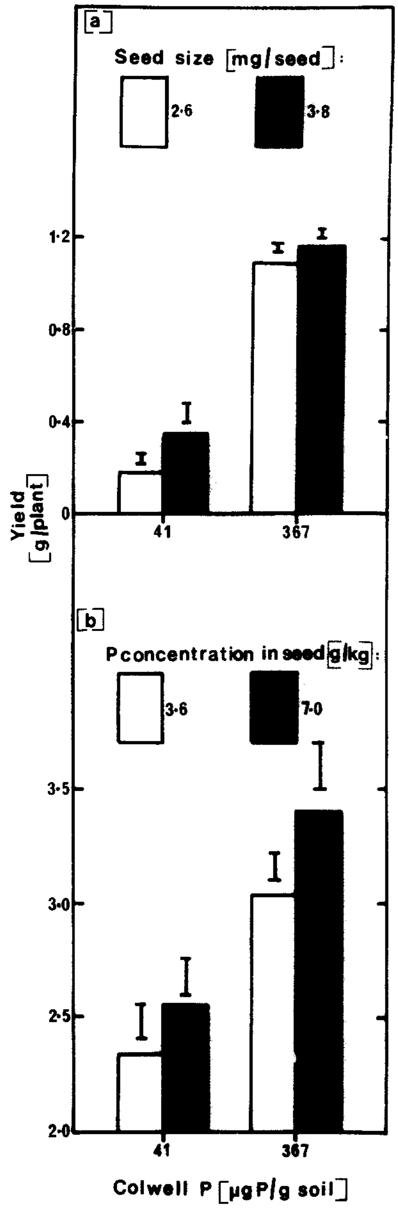

Fig. 5. Relationship between yield and bicarbonate-soluble P extracted from the soil (Colwell P) measured in experiment 5: (a) dried medic tops, harvested 52 days after sowing, grown from seed containing 2.0 g P kg^{-1} but with the two seed sizes shown in the Figure; (b) seed yields from seed containing two P concentration. Note that for (b) seed yields were unaffected by the size of the seeds sown (Table 1) so that mean data are presented for both sizes. Bars are s.e. of the mean (n = 3 in (a); n = 6 in (b)).

the embryo so that the seedlings start to take up nutrients (including soil P) and water from the soil from a greater volume of soil earlier than seedlings grown from P containing a lower P

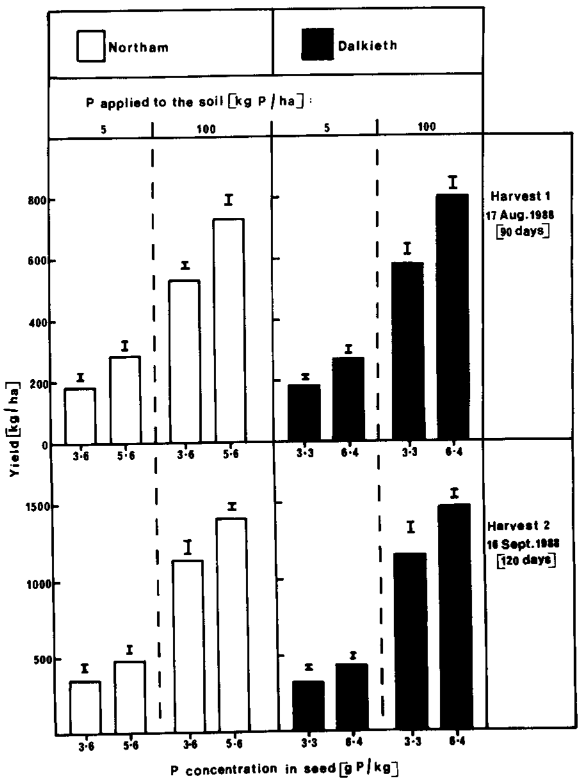

Fig. 6. Yield of dried *T. subterraneum* herbage measured for cvv. Northam and Dalkieth in experiment 6 (field experiment). Bars are s.e. of the mean (n = 3).

concentration. However, the relative increase in DM yields of tops due to higher seed P concentrations appears to decrease with increasing age of the plant (Austin, 1966; and Figs. 2 and 6), and also with increasing level of P applied to the soil (Austin, 1966; Fig. 6).

Increasing seed size (*i.e.* seed mass) also increased P content (P concentration × seed mass) in the seed. However, differences in yields due to seed size were not statistically significant ($P > 0.05$) at harvest 3 (*i.e.* at maturity), and differences in yields due to P content were only statistically significant ($P < 0.05$) at the first harvest. Thus differences in yields were always significantly different for the two levels of P applied to the soil and for the two P concentrations used in the seed.

Increasing DM yields of annual pasture legumes in autumn by increasing P concentration in the seed may allow important increases in stock-carrying capacity and production in the Mediterranean-type climate common to much of southern Australia. Furthermore, increases in seed yields of annual pasture legumes resulting from increasing P concentration in seed used to grow the plants may increase persistence of legumes in southern Australian pastures.

Increased growth of seedlings of annual legumes due to higher P levels in the seed may help the plant to cope with diseases and pests, such as root rot of subterranean clover in Western Australia, caused by various fungi including *Pythium irregulare* and *Fusarium oxysporum*, which can severely reduce emergence and survival of clover seedlings (Wong *et al.*, 1985).

The soil test for P (*i.e.* Colwell P) values were related to different yields not only due to the different levels of P applied to the soil but also due to P concentration in the seed and seed size. These results may in part explain some of the year-to-year variations encountered in soil test for P calibrations measured at the same site for the same cultivar of the same plant species in south-western Australia (Bolland *et al.*, 1988, 1989).

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