Putting the P in *Ptilotus*; a phosphorus-accumulating herb native to Australia

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### INTRODUCTION

As global reserves of phosphorus (P) suitable for manufacture of fertilizers become depleted there is an urgent need to develop crops where yield is less reliant on application of readily available fertilizer P. Lambers *et al.* (2006) suggest one approach to address this need is domestication of species native to environments impoverished in the labile inorganic forms of P that most crop plants require. Such species are likely to possess traits that allow uptake of P from other sources in the soil and/or allow more efficient use of P (Handreck, 1997). Alternatively, a greater understanding of the P nutrition of such species could eventually be used to target breeding of current crop species (Lambers *et al.*, 2006). In this paper we examine the nutritional response of a short-lived perennial herb native to the deficient soils of Australia, *Ptilotus polystachyus* (green mulla mulla).

Plants native to Australia possess a range of adaptations to maintain adequate P nutrition when growing in soil with low availability of labile inorganic P (Handreck, 1997). For instance, root-system adaptations to enhance P acquisition that are commonly found in Australia include symbiosis with mycorrhizal fungi (Brundrett and Abbott, 1991; Johnston and Ryan, 2000; O’Connor, *et al.*, 2001), formation of cluster and dauciform roots (Purnell, 1960; Shane *et al.*, 2005; Shane and Lambers, 2005) and alterations in root-system morphology and plasticity (Denton *et al.*, 2006). Adaptations to maximize internal P use efficiency include a low P requirement and an ability to store P for later use when P availability and growth are temporally separated (Handreck, 1997; Shane *et al.*, 2004; Denton *et al.*, 2007). These adaptations are sometimes coupled with an inability, particularly for some members of the *Proteaceae*, to downregulate P uptake when P is plentiful, resulting in P toxicity at relatively low P supply (Shane *et al.*, 2003, 2004). However, the response of Australian native species to added P is variable (Gikaara *et al.*, 2004). For instance, P toxicity at low P supply is most commonly reported for woody, relatively long-lived members of the *Proteaceae* native to the most P-deficient soils, but it is by no means a ubiquitous trait for the *Proteaceae* (Shane and Lambers, 2006; Standish *et al.*, 2007). Indeed, the highly seasonal and variable nature of many Australian environments may mean that a more flexible approach to P nutrition may be beneficial, particularly for shorter-lived species. Such an approach could include means to enhance P acquisition, coupled with an ability to store relatively large amounts of P for later use and an ability to grow rapidly and reproduce in response to temporary increases in P availability, as may occur after summer rain. However, relatively little is known

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about the P nutrition of short-lived and ephemeral grasses and forbs native to Australia. Their response to P fertilizer (Garden and Bolger, 2001) and adaptations to P-deficient soils may be variable.

The genus *Ptilotus* (family *Amaranthaceae*) includes approximately 100 species that are mostly endemic to Australia (Benl, 1971; Lee et al., 2007). Most species occur in arid and semi-arid regions (Lee et al., 2007). They range in growth form from prostrate to erect herbs and some may form small, woody shrubs (Lee et al., 2007). *Ptilotus* polystachyus has long been thought a promising candidate for domestication as a short-lived perennial forage herb for acid soils in the cropping zones of southern Australia (Gardner, 1934). The natural distribution of *P. polystachyus* encompasses the majority of mainland Australia, except for northern Queensland and the high-rainfall zones of New South Wales, Victoria and southern Western Australia (Fig. 1; CHAH, 2006). It is naturally abundant in uncultivated areas of fields and roadsides in the wheatbelt areas of Western Australia, which receive low winter-dominant rainfall and have deep, nutrient-deficient, acid sand soils. We examined the growth response of *P. polystachyus* (ptilotus) to P and N addition in a sandy soil of extremely low bicarbonate-extractable P and mineral N. *Chicorium intybus* (chicory) ‘Puna’, a commercially utilized perennial forage plant of Mediterranean origin, was included for comparison. Three hypotheses were tested: (1) *Ptilotus* will produce more biomass under low soil bicarbonate-extractable P or mineral N than chicory; (2) growth of *Ptilotus* will be less responsive to additions of N and P than chicory; and (3) *Ptilotus* will be less able to regulate uptake of nutrients than chicory and thus suffer toxicity to P, and perhaps N, at lower rates of addition than chicory.

**MATERIALS AND METHODS**

**Experiment 1**

A glasshouse experiment examined the impact of readily soluble P (seven levels) and N (seven levels) on growth and nutrient uptake of *Ptilotus* polystachyus (Gaudich.) F. Muell. and *Chicorium intybus* L. Seeds of *Ptilotus* were collected in December 2003 from a gravel pit north of the Department of Agriculture Research Station, Merredin, Western Australia (31°31’S, 118°10’E, 315 mm long-term mean annual rainfall). Seeds of *C. intybus* ‘Puna’ were obtained from a commercial seed supplier. All seeds were sown directly into a nutrient-deficient brown fluvisol soil locally known as a ‘Lancelin sand’ (UC-42; Northcote, 1979). The soil contained 2 mg kg⁻¹ of nitrate-N, 1 mg kg⁻¹ of ammonium-N, 2 mg kg⁻¹ of bicarbonate-extractable P (Colwell method; Rayment and Higginson, 1992), 28 mg kg⁻¹ of bicarbonate-extractable K (Colwell method; Rayment and Higginson, 1992), 0.93 % organic carbon (Walkley and Black, 1934) and had a pH of 6.1 (CaCl₂; Rayment and Higginson, 1992). Soil was steam-sterilized twice, 3 d apart, at 80°C for 3 h and air-dried. Pots were lined with a polyethylene bag and filled with 3 kg of soil.

Nutrient solutions were mixed into the soil. Seven levels of P as KH₂PO₄ were used (0, 15, 30, 60, 120, 180, 300 mg P pot⁻¹). KCl was added proportionally to maintain a constant cationic background, and to avoid N deficiency 100 mg pot⁻¹ of N as NH₄NO₃ was applied (30 mg pot⁻¹ with basal nutrients, and 35 mg pot⁻¹ 2 and 4 weeks after sowing). Seven levels of N as NH₄NO₃ were applied (0, 10, 20, 45, 90, 180, 270 mg N pot⁻¹) along with 85 mg pot⁻¹ of P as KH₂PO₄ to avoid P deficiency. Control pots with no P or N application were also established. All pots initially had basal nutrients mixed into the soil: 45 MnSO₄, 27 ZnSO₄, 9 CuSO₄, 2.1 H₂BO₃, 0.9 CoSO₄, 0.6 Na₂MoO₄, 240 MgSO₄, 520 K₂SO₄ and 450 CaCl₂ (mg pot⁻¹). The pots were arranged in five randomized blocks where all treatments were present once in each block.

Seed was sown at a depth of 1–2 mm for *Ptilotus* and 5 mm for *Chicorium* on 26 October, 2004. After 1 week, seedlings were thinned to two per pot. Pots were maintained at 80 % water-holding capacity with deionized water applied when required. Pots were re-randomized within blocks at each watering. Plants were sprayed on 18 November to control thrips and mites. Average temperatures in the glasshouse were 27 °C (range 21–30 °C) during the day and 19 °C (range 16–22 °C) during the night.

Plants were harvested after 5 weeks. Roots were washed and cut off and leaves were counted. Leaf surface area was measured using a leaf area meter and leaf thickness (two measurements per leaf) using electronic callipers. Shoot fresh weight (leaf plus stem) was recorded. After drying in a forced-draught oven at 70 °C for 72 h, shoot and root dry weights were recorded and shoot samples ground for further analyses. For measurement of P concentration, shoots were digested in 10 : 1 HNO₃ : HClO₄ and P was estimated using the standard colorimetric molybdovanadophosphate method (Boltz and Lueck, 1958). Shoot material from *Medicago sativa* with a known P concentration was used as a reference standard. Seed P concentration was also measured. Concentration of N in shoots was measured by dry combustion and thermal conductivity (LECO CHN – 1000; St. Joseph, MI). To confirm the unusually high P concentrations in shoots recorded in some treatments, plants from two treatments (120 mg pot⁻¹ P and 0 mg pot⁻¹ N) were sent to a commercial laboratory (CSBP Ltd, Bibra Lakes, Perth, Australia) for repeat analysis. At this laboratory, plant material was digested in nitric acid using a Milestone microwave and P was measured by inductively coupled plasma atomic emission spectroscopy.

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**FIG. 1.** Distribution map of *Ptilotus* polystachyus in Australia. Dots indicate sites where plants have been recorded to occur. Data from CHAH (2006).
(ICP-AES; McQuaker et al., 1979). Concentrations of other macronutrients and micronutrients in the samples were also obtained using this method. Analysis for P and N could not be carried out when sample size was too small or plants had died; hence these data are not available for the control pots and the 0 mg pot⁻¹ of P treatment in the P-addition series for both species.

Experiment 2

The purpose of Experiment 2 was to confirm the ability of ptilotus to accumulate high concentrations of P and to more closely examine where this P was stored in leaves using cryo-analytical scanning electron microscopy (SEM). The experiment was established and managed in an identical manner to Experiment 1 except only three rates of P as KH₂PO₄ were applied (15, 60, 300 mg P pot⁻¹). KCl was added as in Experiment 1 and 150 mg pot⁻¹ of N as NH₄NO₃ was applied (60 mg pot⁻¹ at sowing and 45 mg pot⁻¹ 2 and 4 weeks after sowing). The pots were arranged in four randomized blocks where all treatments were present once in each block. Pots were watered to 80% water-holding capacity twice each week. Experiment 2 was sown on 8 July, 2005 and harvested after 4 weeks. Average temperatures were 19°C (range 17–22°C) during the day and 13°C (range 9–17°C) during the night.

At harvest, the youngest fully emerged leaf and a subsample of roots were taken from each ptilotus plant in the 300 mg P pot⁻¹ treatment. These leaves were immediately cryo-fixed in liquid N₂ (LN₂), placed in vials and stored in a cryo-store. Remaining leaves and roots were dried, weighed and ground as for Experiment 1 and analysed for P by a commercial laboratory (Chemistry Centre Western Australia, East Perth, Perth). Samples were digested in a mixture of concentrated nitric and perchloric acids (final temperature 250°C). The solution was then diluted with water and presented to an inductively coupled plasma atomic emission spectrophotometer (ICP-AES; Varian, Melbourne, Australia) for determination of P by comparison with known standards. Due to the small size of many samples, pairs of replicates were sometimes combined, leaving three or two samples per treatment for analysis.

Samples from the cryo-store were prepared for cryo-SEM and microanalysis as described by Ryan et al. (2003). Cell vacuoles of individual cells (guard, palisade and spongy mesophyll) were analysed with a Link eX system (Oxford Instruments) using the Be window. Note that the cytoplasm presented an area too thin to be analysed using this method. Spectral data for K, P and Mg were converted to elemental concentrations using frozen standards. As the analysis does not distinguish between ions and elements in insoluble form, the concentrations of elements in all structures are expressed in mm. The lower limit of reliable quantitation of elements by the X-ray microanalysis was considered to be 10 mm. For further details of preparation methods for cryo-SEM and analysis see Huang et al. (1994) and McCully et al. (2000).

Field-grown plants

An established field experiment designed to assess agronomic performance of ptilotus was sampled on 12 May, 2005. The site was located at the Merredin Department of Agriculture Research Station, Western Australia (31°31’S, 118°10’E, 315 mm long-term mean annual rainfall), on a yellow sandy earth (Moore, 2004) also known as a yellow Kandosol (Isbell, 1996). The pH (CaCl₂) in the top 20 cm was 4.1. Eight plants of approximately 50 cm height and 50 cm diameter that had been sown in 2004 and had recently regenerated following summer were cut at ground level. All plants were flowering but their shoots still consisted primarily of vegetative material. Shoots were dried, weighed, ground and analysed for nutrient concentrations by CSBP as for Experiment 1.

Data analysis

Statistical analysis was performed using Genstat version 9.2 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK, 2007). For Experiment 1, the effect of P and N on each parameter for the two species was examined using general ANOVAs. Each ANOVA included the factors Species (chicory, ptilotus) and either P-addition (seven levels) or N-addition (seven levels) and Block. The Species × P-addition (or N-addition) interaction was always examined. Outliers were carefully checked and no more than two removed for any parameter. If a significant (P < 0.05) interaction was found, the estimated means from the interaction are presented along with the relevant l.s.d. at P = 0.05. If the interaction was not significant then the estimated means for both Species and P-addition (or N-addition) are presented with, if Species or P-addition (or N-addition) had a significant effect, the relevant l.s.d. at P = 0.05. In Experiment 2, the effect of P on shoot weight was similarly analysed with each ANOVA including the factors Species (chicory, ptilotus) and P-addition (three levels) and Block. For the concentration of P in shoots, where samples were bulked from four to two or three replicates per treatment, the mean and s.e.m. are presented for each treatment. All graphs were generated using SigmaPlot for windows version 10 (Systat Software Inc.).

RESULTS

Experiment 1

The 1000-seed weight was 1.27 g for ptilotus and 1.51 g for chicory. Seed P concentration was 6.1 mg g⁻¹ for ptilotus and 9.2 mg g⁻¹ for chicory.

Growth of the control plants that received no additional nutrients varied greatly between ptilotus and chicory (Table 1). The chicory plants that survived to the end of the experiment were small and stunted with a shoot dry weight of around 34% of the ptilotus plants.

<table>
<thead>
<tr>
<th>Survival ( %)</th>
<th>Number of leaves</th>
<th>Shoot d. wt (g pot⁻¹)</th>
<th>Root d. wt (g pot⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ptilotus</td>
<td>100</td>
<td>47 ± 4</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td>Chicory</td>
<td>40</td>
<td>6</td>
<td>0.15–0.17</td>
</tr>
</tbody>
</table>

Ptilotus: mean ± s.e.m, n = 5. Chicory: range, n = 2.
Shoot and root dry weights of ptilotus and chicory were affected differently by P addition (Figs 2 and 3). Ptilotus had a greater shoot dry weight than chicory at the lowest three levels of P addition and at the highest level. At the three intermediate levels of P addition there was little difference between the two species. Ptilotus shoot dry weight increased with P addition, but began to plateau at 15 mg P pot\(^{-1}\) and then decreased by around 28% between 180 and 300 mg P pot\(^{-1}\). Shoot dry weight increased with N addition in a similar manner for both species, with chicory consistently maintaining a slightly higher shoot dry weight.

At the highest level of P addition, the oldest leaves of ptilotus were yellowed and the leaf margins close to the leaf tip of some leaves were necrotic (Fig. 2). Similar symptoms were not evident for chicory. At the lowest rates of N addition, the oldest leaves of ptilotus were yellowed and there was also reddening and necrosis around the margins of the older leaves (Fig. 2). Chicory also had yellowing of the older leaves at the lowest rates of N addition and many leaves also exhibited brown spots. These symptoms were apparent up to 45 mg pot\(^{-1}\) of N. All plants appeared healthy at the highest rate of N addition (Fig. 2).

The effect of P addition on root dry weight was similar to the effect on shoot dry weight (Fig. 3B). However, chicory root dry weight showed a greater response than shoot dry weight at intermediate levels of P addition. Indeed, chicory had approximately twice the root dry mass of ptilotus at these P levels. In addition, for chicory the decrease in root dry mass between 180 and 300 mg P pot\(^{-1}\) (59%) was far greater than for shoot dry weight. Root dry weight responded similarly to N addition for ptilotus and chicory, increasing up to 90 mg pot\(^{-1}\) N and then declining sharply. Root dry weight in the N-addition series was consistently more than three times higher for chicory than ptilotus. The root mass ratio of ptilotus and chicory responded in a similar manner to addition of P and N, with no impact from P addition but a decrease from around 50% to 30% in response to N addition (Fig 3C). Root mass ratio was much higher for chicory than ptilotus.

Leaf area followed a similar trend to shoot dry weight in the P-addition series (Fig. 4). Leaf number increased with P addition for both species and was greater for ptilotus; a difference that decreased from ~10 times to ~6 times as P level increased. Leaf area and leaf number increased with N addition, with ptilotus showing a greater response. Leaf thickness for both species increased with P addition, but not N addition. Ptilotus leaves were more than twice the thickness of chicory leaves. Shoot water content responded differently to P addition for ptilotus and chicory; however, variation was slight with water content ranging from 87–90% for ptilotus and 89–92% for chicory (results not shown). Addition of N affected shoot water content in the same manner for both species, but variation was again slight, with water content ranging from 88–91% (results not shown).

Concentrations of P and N in shoots were higher for ptilotus than chicory in both the P-addition and N-addition series (Fig. 5). The concentration of P in shoots increased with P addition, but while it reached a plateau for chicory at around 6 mg g\(^{-1}\) with addition of 120 mg pot\(^{-1}\) of P, for ptilotus the concentration of P in shoots kept steadily increasing up to 38 mg g\(^{-1}\). In the N-addition series, the concentration of P in shoots varied little in chicory but was very high for ptilotus at 0 mg pot\(^{-1}\) of N and then decreased as N level increased. For both ptilotus and chicory, the concentration of N in shoots was highest at 0 mg pot\(^{-1}\) of P, dropping greatly at 15 mg pot\(^{-1}\) of P. As P addition then increased, N concentration then reached a plateau for chicory, and decreased slightly for ptilotus. The concentration of N in shoots increased with N addition, especially after 45 mg pot\(^{-1}\) of N; this trend was more marked for ptilotus than chicory.

The evaluation of nutrient concentrations in shoots from two treatments by a commercial laboratory showed P was the element that differed most between ptilotus and chicory, being much higher in ptilotus, while S, Ca and B were slightly higher in chicory (Table 2). The values for P concentration from the commercial laboratory were consistent with our own analyses for P concentrations up to 25 mg g\(^{-1}\). However, in the treatment with the highest P concentrations, the commercial laboratory reported P concentrations to be higher than our own measurements (Table 2).

**Experiment 2**

Compared to ptilotus, chicory accumulated a relatively much higher shoot dry weight in Experiment 2 than Experiment 1 (Fig. 6). In contrast to Experiment 1, where ptilotus had a greater shoot and root dry mass than chicory at 15 mg pot\(^{-1}\) of P, in Experiment 2 there was no difference between the two species at this low level of P. In addition, in Experiment 2 at 60 and 300 mg pot\(^{-1}\) of P, chicory shoot dry weight exceeded that of ptilotus to a far greater extent than in Experiment 1. However, the concentration of P in shoots was very consistent between Experiments 1 and 2. In Experiment 2, the concentration of P in shoots of ptilotus again greatly exceeded that of chicory at 300 mg pot\(^{-1}\) of P, reaching ~30 mg g\(^{-1}\).

Cryo-SEM of the transverse faces of planed leaves of ptilotus from the 300 mg pot\(^{-1}\) of P treatment showed two layers of palisade cells and a spongy mesophyll layer 4–5 cells thick (Fig. 7). Large multicellular trichomes ~100 µm wide at the base and 150–200 µm high were abundant. Cells generally had a swollen appearance and air spaces in both the palisade and spongy mesophyll were relatively uncommon. The X-ray micro-analysis of these leaves showed high P concentrations in the vacuoles of guard, epidermal, palisade and spongy mesophyll cells (Table 3). The guard cell vacuoles had substantially higher concentrations of P and K than the other cell types analysed. The upper and lower epidermal cells did not differ greatly and were therefore considered together. The epidermal cells had lower concentrations of P and Mg, but higher concentrations of K than the palisade and spongy mesophyll. For the palisade cells, the cells in the layer immediately below the upper epidermis ranged from 57–91 mm of P. However, cells in the second and third layers of palisade cells below the epidermis were more variable, with three of the seven cells analysed having a much higher P concentration (106, 129, 178 mm). Cells closer to vascular bundles, or in the trichomes, did not have higher...
Fig. 2. Ptilotus and chicory shoots from five treatments in Experiment 1. (A) Control (no added P or N); (B) 15 mg pot$^{-1}$ of P in the P-addition series; (C) 300 mg pot$^{-1}$ of P in the P-addition series; (D) 10 mg pot$^{-1}$ of N in the N-addition series; and (E) 270 mg pot$^{-1}$ of N in the N-addition series. In the P-addition series 100 mg pot$^{-1}$ of N was supplied; in the N-addition series 85 mg pot$^{-1}$ of P was supplied.
concentrations of P than the other cells analysed. Root cells had very variable P concentrations and an inadequate number of cells were analysed to draw conclusions about differences between cell types. However, P concentration did reach up to 153 mM; a similar concentration to that found in the leaf cells. The Mg : K : P molar ratio was 1 : 8 : 3 for the guard cells, 1 : 9 : 3 for the epidermal cells and 1 : 3 : 2 for both the palisade cells and spongy mesophyll cells.

Field data

The analysis of ptilotus shoots from plants growing in the field showed a P concentration of 2.2 mg g⁻¹; a value equivalent to the 15 mg pot⁻¹ of P treatment in the P-addition series in Experiment 1 (Table 4). The concentration of 36 mg kg⁻¹ of N in shoots is equivalent to the 90 mg pot⁻¹ of N treatment in the N-addition series in Experiment 1. Concentrations of K, S, Ca, Mg and Ca in the field-grown plants were similar to values for the treatments analysed in Experiment 1 (Table 2), while Na and B were higher, and Zn lower, in the field-grown plants.

DISCUSSION

Plant growth at low P and N availability

The hypothesis that ptilotus will produce more biomass than chicory in soil with low bicarbonate-extractable P or mineral N was largely supported by Experiment 1. Ptilotus grew much better than chicory when P and N were both limiting (controls) and when P was low and N adequate (P-addition series). There was little difference in the growth of ptilotus and chicory when N was low and P adequate (N-addition series). The mechanisms responsible for the ability of ptilotus to grow well under severe P limitation could not be determined.
by the experiment. Ptilotus seeds did not have greater P reserves, indeed individual chicory seeds had nearly double the P reserves of ptilotus seeds. Inspection of roots showed no formation of cluster roots and the sterilization of the soil should have precluded formation of mycorrhizas. Ptilotus consistently had a lower root mass ratio, including in the P-addition series at 0 mg pot\(^{-1}\) of added P (ptilotus 29%, chicory 53%); although not in the controls due to the extremely poor shoot growth of chicory (Table 1, Fig. 2). The concentrations of P in shoot data do not suggest that ptilotus had a lower P requirement than chicory. Uptake of P by ptilotus may have been enhanced by root adaptations such as longer, more numerous root hairs or release of root exudates such as carboxylates or phosphatases (Lambers et al., 2006). While further research is required to elucidate the mechanisms involved, it seems likely that ptilotus can access substantial amounts of P from pools of soil P not available to current commercial crop species.

**Growth response to P and N addition**

The hypothesis that growth of ptilotus will be less responsive to additions of N and P than chicory was generally not supported, except in relation to the root dry weight response to P addition. In the P-addition series in Experiment 1, shoot dry weight reached a similar maximum for both species, but ptilotus responded primarily between 0 and 15 mg pot\(^{-1}\) of added P (although it continued to increase leaf number until 60 mg pot\(^{-1}\), of P), while chicory responded between 0 and 60 mg pot\(^{-1}\) of added P. While chicory root dry weight showed a similar delay in response to P addition, overall it was far more responsive to P addition than ptilotus root dry weight.

**FIG. 4.** Response of ptilotus (Pt) and chicory (Ch) to addition of seven levels of P and N (Experiment 1): (A) leaf area; (B) leaf number; and (C) leaf thickness. In the P-addition series 100 mg pot\(^{-1}\) of N was supplied; in the N-addition series 85 mg pot\(^{-1}\) of P was supplied. If the interaction between Species and P- or N-addition was significant, the estimated means for the interaction are presented with the l.s.d. at \(P = 0.05\). If the interaction between Species and P- or N-addition was not significant, the estimated means for Species and P- or N-addition are both presented, along with the l.s.d. at \(P = 0.05\) if the factor had a significant effect (ns = not significant).
weight, reaching more than double the dry weight of ptilotus roots between 60 and 180 mg pot\(^{-1}\) of P. The response to N addition did not differ between ptilotus and chicory for shoot and root dry weight, root mass ratio and leaf thickness, while the response of leaf area and leaf number varied only slightly between the two species.

In Experiment 2, ptilotus did not exhibit a growth response to P addition. Experiment 2 was conducted in mid-winter while Experiment 1 was conducted in late spring. The relatively poor growth of ptilotus in Experiment 2 suggests that it was more constrained by the winter conditions than chicory. The natural distribution of ptilotus (Fig. 1) suggests it may be well adapted to hot, dry conditions.

The leaves of ptilotus were more than twice the thickness of the leaves of chicory, and ptilotus plants had many small leaves compared to the few large leaves of chicory. Chicory responded to abundant N and P by increasing individual leaf size, while ptilotus responded by increasing leaf number. It is interesting that in Experiment 1 a similar shoot dry weight was achieved by ptilotus and chicory at the higher levels of P addition, and throughout the N-addition series, even though ptilotus had less than half the leaf area of chicory.

**Table 2.** Shoot nutrient concentrations of plants in Experiment 1 from the 120 mg pot\(^{-1}\) of P treatment in the P-addition series (100 mg pot\(^{-1}\) of N supplied) and the 0 mg pot\(^{-1}\) of N treatment in the N-addition series (85 mg pot\(^{-1}\) of P supplied)

<table>
<thead>
<tr>
<th>Concentration (mg g(^{-1}))</th>
<th>Concentration (mg kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(^*-)</td>
<td>P</td>
</tr>
<tr>
<td>120 P</td>
<td></td>
</tr>
<tr>
<td>Ptilotus</td>
<td>25.1</td>
</tr>
<tr>
<td>Chicory</td>
<td>5.8</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0 N</td>
<td></td>
</tr>
<tr>
<td>Ptilotus</td>
<td>34.5</td>
</tr>
<tr>
<td>Chicory</td>
<td>4.7</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means. P-values from t-tests.

* Standard colorimetric molybdovanadophosphate method. All other values from inductively coupled plasma atomic emission spectroscopy.

Fig. 5. Response of ptilotus and chicory to addition of seven levels of P and N (Experiment 1): concentration in shoots of (A) P, and (B) N. In the P-addition series 100 mg pot\(^{-1}\) of N was supplied; in the N-addition series 85 mg pot\(^{-1}\) of P was supplied. As the interaction between Species and P- or N-addition was significant, the estimated means for the interaction are presented with the l.s.d. at P = 0.05.
Regulation of N and P uptake

The hypothesis that ptilotus will be less able to regulate uptake of nutrients than chicory and thus suffer toxicity to P, and perhaps N, at lower rates of addition than chicory was supported in part and in relation only to P addition. Chicory and perhaps N, at lower rates of addition than chicory was able to both grow well on soil with low bicarbonate-extractable P and respond well to addition of P in spite of poor downregulation of P uptake, although at 200 kg ha\(^{-1}\) of P, shoot dry weight increased throughout the P-addition series. At the low rates of N addition, the low shoot dry weights and similar leaf yellowing of ptilotus and chicory suggests the poor growth of ptilotus was simply due to N deficiency.

Several studies support our finding that Ptilotus species are able to both grow well on soil with low bicarbonate-extractable P and respond well to addition of P in spite of poor downregulation of P uptake when P is plentiful (e.g. Brennan et al., 2000). Islam et al. (1999) examined a subtropical, semi-arid Australian grassland on soil with low P availability, dominated in winter by forbs from the Amaranthaceae (P. exaltatus, P. macrocephalus, P. aerovoides), Chenopodiaceae and Malvaceae. Concentration of P in shoots was greatest in the Ptilotus species, ranging up to 1-75 mg g\(^{-1}\). In an accompanying glasshouse experiment, growth of P. macrocephalus responded to P additions up to 200 kg ha\(^{-1}\) of P (\(\sim300\) mg P pot\(^{-1}\) equivalent) and showed no symptoms of P toxicity, while P. exaltatus responded up to 100 kg ha\(^{-1}\) and then decreased in growth. For both species, uptake of P increased with P addition, suggesting a poor ability to downregulate P uptake, although at 200 kg ha\(^{-1}\) of P addition leaf P reached only around 4–5 mg g\(^{-1}\).

In our experiments, the extraordinarily high P concentrations in ptilotus shoots, up to around 4% of dry matter, were confirmed by the re-analysis of samples in a second laboratory and by Experiment 2. To our knowledge these are the highest concentrations recorded for a herbaceous plant. In a glasshouse experiment, Sharma et al. (2007) examined growth of more than 40 crop and weed species likely to be adapted to high-P soils in a soil with low available P. The majority of species accumulated 3.5–6.5 mg g\(^{-1}\) of P in shoots. The highest concentration of P
after 8 weeks was ~20 mg g⁻¹ in the stems of cucumbers (*Cucumis sativus*) grown with addition of 114 mg pot⁻¹ of P. Whilst comparisons must be made with care due to differences in soil type, this concentration is only slightly lower than found for *ptilotus* shoots in the 120 mg pot⁻¹ of P treatment in the current experiment.

The causative mechanism for the over-accumulation of P in *ptilotus* is unknown. Sharma *et al.* (2007) found that P-accumulating cucumber and yellow squash plants had increased activity of acid phosphomonoesterase and, in particular, 6-phytase in root extracts when plants were grown in P-enriched soils. They did not investigate whether these plants also had an enhanced ability to grow in soils with low bicarbonate-extractable P. In *Arabidopsis thaliana*, the inorganic phosphate over-accumulator *pho2* (Delaize and Randall, 1995) was found by Aung *et al.* (2006) to be caused by a single nucleotide mutation of the *UBC24* gene.

For *ptilotus* grown at the high P levels, the lack of any symptoms of P-toxicity is surprising, especially given the adaptation of this species to Australian soils low in bicarbonate-extractable P. Other studies generally show P toxicity developing once whole-shoot P concentrations reach only 10–20 mg g⁻¹ (see Shane *et al.*, 2004, and references therein); although toxicity has been reported at concentrations of only 3.4–7.0 mg g⁻¹ in two tropical food legumes (Bell *et al.*, 1990). In the current experiment, leaf cell vacuoles in the youngest fully emerged leaf contained P at concentrations up to ~200 nm. Concentrations of P in the vacuole are generally reported to change in response to P availability to a far greater extent that cytoplasmic P, but still tend to be maintained at less than 25 nm (Lee and Ratcliffe, 1993; Schachtman *et al.*, 1998). The form of the vacuolar P in *ptilotus* is unknown, although the high concentrations of both K and Mg suggest that these two cations could be involved. Strong relationships have been found for P with K and Mg concentration for intraradical mycorrhizal hyphae containing high P concentrations (Ryan *et al.*, 2003). Interestingly, in both Shane *et al.* (2004) and the present study, leaves from plants grown with abundant P, when examined under the cryo-SEM, consisted of cells with a swollen appearance and contained very little intercellular space. This finding may indicate that the cells were encountering difficulties maintaining osmotic balance.

In the current experiment, P in the tissue of the youngest fully emerged leaf did not appear to be stored preferentially in any cell type. This contrasts with the results of Shane *et al.* (2004) who found P concentration to be higher in palisade cells than in bundle sheath or epidermal cells in mature leaves of *Hakea prostrata* showing symptoms of P toxicity. Shane *et al.* (2004) speculate that this preferential allocation of P to palisade cells may be a means to maintain photosynthesis under P-limiting conditions. In our experiment it is possible that stores of P were present in other shoot components such as stems (e.g. Sharma *et al.*, 2007) or in older leaves.

The poor down-regulation of P uptake by *ptilotus* may reflect a natural environment with soils low in labile inorganic P, coupled in Mediterranean climates with a preference for growth in the warmer summer months when the relatively P-rich top soil will mostly be dry. Ability to accumulate high concentrations of P in the wet, cold winter months or during any brief periods when rain moistens the top soil following summer rains could therefore be beneficial.

### Ptilotus warrants further development

The ability of *ptilotus* to grow well in soils with low labile inorganic P may make it especially well suited for introduction to farming systems where productivity is constrained by this factor, such as pastures on organic or low-input farms in Australia or other regions of the world with similar soils and systems with low inputs of fertilizer P (Kirchmann *et al.*, 2007). The presence of *ptilotus* in a mixed-species pasture could provide a pathway for poorly labile inorganic P to be utilized and perhaps re-enter the P cycle and later be accessed by more traditional pasture plants (Nuruzzaman *et al.*, 2005). In contrast, there could also be a role for *ptilotus* in land management when plentiful P in soil poses an environmental threat and needs to be removed from the system (Novak and Chan, 2002; Tibbett and Diaz, 2005; Sharma *et al.*, 2007; Diaz *et al.*, 2008). The ability of *ptilotus* to accumulate high concentrations of P in its shoot tissues whilst maintaining biomass production makes it eminently suitable for such a phytoremediation role.

### Conclusions

In comparison to chicory, *ptilotus* demonstrated a remarkable ability to grow well under low and high P availability. Plant growth and health appeared good at high P levels, in spite of the plants being unable to downregulate P uptake and therefore accumulating extremely high concentrations of P in their tissues. This plant merits further study as no obvious mechanism was found to account for its ability to access P from a soil low in bicarbonate-extractable P. In addition, the ability of *ptilotus* to accumulate and tolerate high concentrations of P is relevant to phytoremediation of P contaminated soils, such as are increasingly present under intensive confined livestock operations (Novak and Chan, 2002).

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LITERATURE CITED


